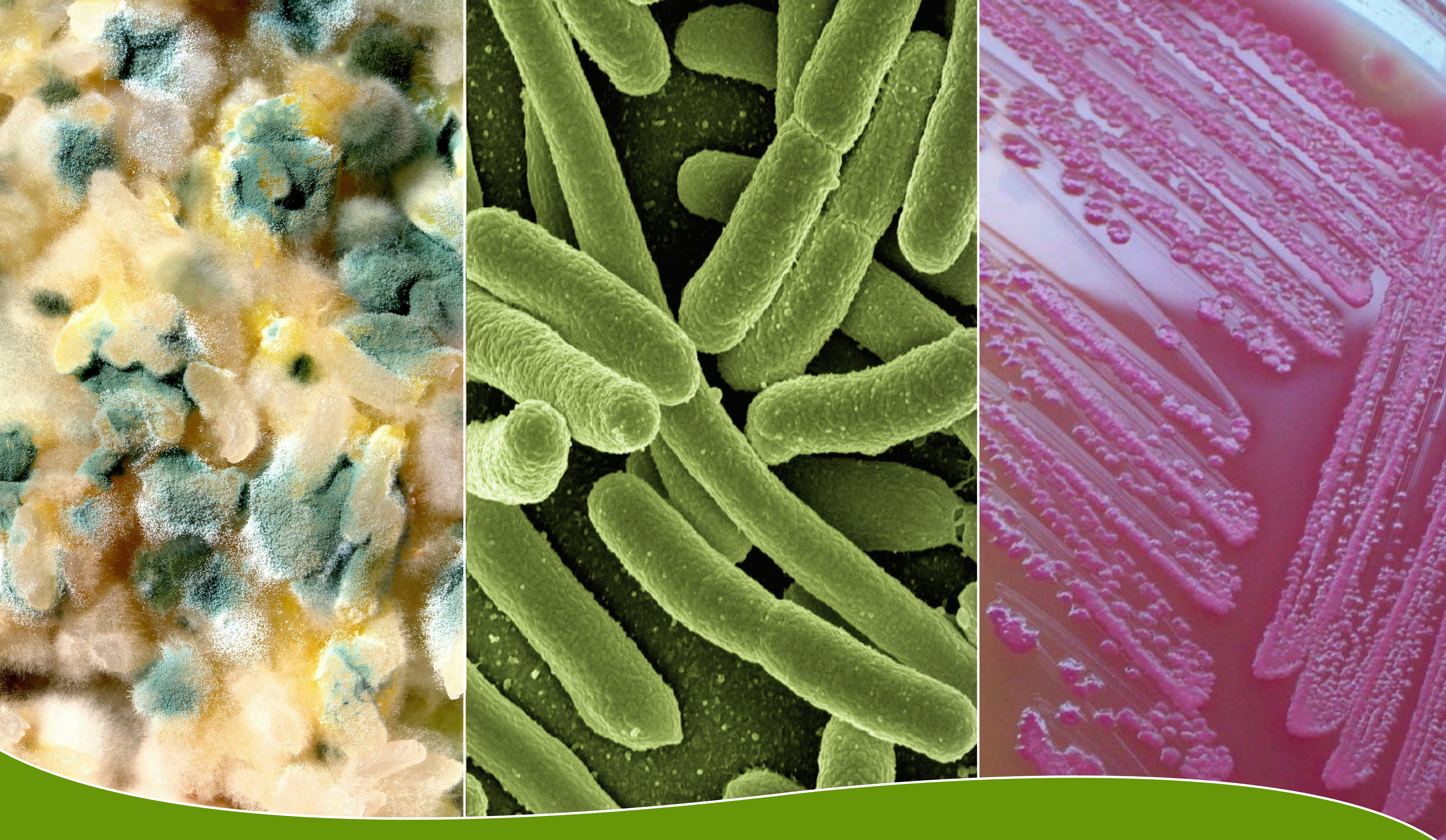

# Introduction to Food Microbiology

A Province of British Columbia
FOODSAFE™ program open textbook

BRITISH COLUMBIA

## Acknowledgments

The Introduction to Food Microbiology online course and open textbook were produced by the B.C. FOODSAFE Secretariat in collaboration with the B.C. Ministry of Health, Food Safety Branch; the BC Centre for Disease Control, Environmental Health Services and Open School BC (OSBC).

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**ISBN:** 978-0-7726-8076-1
**QP material number:** 7960003987

### Version History

Corrected, April 2025



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# Lesson A: Food Microbiology—An Introduction

## Introduction

In this first introductory lesson, we will begin to explore the field of microbiology generally and food microbiology specifically. We will look at different types of cells and at the types of microbes that impact the food industry. We'll also learn a little bit about how microbiologists investigate living things that, although invisible to the naked eye, have a tremendous impact on the production, health, and safety of the food industry.

Now watch this video introduction by microbiologist Dr. Michael Suits:

**Introduction to Food Microbiology**
https://www.youtube.com/watch?v=2liyVZ6OtWQ

### Learning outcomes

Upon completion of this lesson, learners will be able to:

- Describe the field of food microbiology
- Describe where microbes are found in our environment
- Describe the basic types of microbes
- Identify how microbes of interest to food professionals may be grouped or categorized
- Describe tools microbiologists use to identify bacteria, in particular using the following:
    - ◊ Gram staining
    - ◊ Shape (cocci, bacilli, spiral-shaped)
    - ◊ Spore-forming
    - ◊ Toxin-producing

## Terminology

Key terms given in bold:

**aerobic**
active
algae
**anaerobic**
antibiotic
**bacteria**
bacilli
carbohydrate
carrageenan
cestode
cocci-
cyanobacteria
dormant
endospore
**facultative anaerobe**
flatworm
fluke
fermentation
**foodborne illness**
**food microbiology**
**fungi**
genus
Gram staining
habitat
helminth
**mesophile**
**microbe**
micro-aerophilic
**microbiology**
**microbiome**
microcystin
**microorganism**
**mould**
Monera
nematode
**parasite**
**pathogenic**
prion
probiotics
**protozoa**
protein
**psychrophile**
**psychrotroph**
rods
roundworm
species
spiral-shaped
**spore**
tapeworm
**thermophile**
trematode
**toxin**
**vegetative**
**virus**
**yeast**

# Imagine this scenario...

*You've just finished the introduction to Food Microbiology course, and now you are looking forward to applying your knowledge and skills in a real situation. But first, it's time to get some sleep.*

It's your first day of work in the kitchen of a local restaurant. You notice that most of the employees are wearing street clothes—only the chefs wear aprons. The supervisor wipes his hands on his pants, shakes your hand, and asks you to start by helping to bring in a delivery of food. You see that all the fish, meat, chicken, vegetables, and dairy products that were delivered were left stacked outside the back door in the hot sun. You move everything onto a large counter in the work area. Many of the boxes are unlabelled and some of the fruits and vegetables are not even boxed.

You stop into the restroom by the back door, where a dog naps in the corner. After washing your hands, you realize there are no paper towels in the dispenser. You dry your hands on your pants and on the friendly dog. Your next assignment is to put the food away. Following the lead of other employees, you put the food away wherever you can find space on the shelves in the refrigerator. As you pack the broccoli into a corner underneath a shelf with raw beef, the sous chef comes by to collect ingredients for the house speciality: chicken with various vegetables. She asks for your help, so you jump at the opportunity. She asks you to cut some vegetables, and points you to a cutting board where she has just deboned several chickens. Looking around, you notice that people are wiping their knives on their clothes, even though someone is telling you how important it is not to use the same knife for meat and vegetables. One employee is constantly sniffling and wiping her nose with her sleeves. When a chef asks if you would like to taste his pasta sauce, you accept. He hands you the same spoon that he had just tasted the sauce with. The sauce is delicious. You begin to think you are going to like this job. The people are friendly, the atmosphere is relaxed, and the food is good.

*You wake up with a start, realizing that this was only a dream. As you relax again, you think about what you just dreamed and replay it through the lens of what you now know about food microbiology. You think about this restaurant and all the living things you cannot see as you drift back off to sleep, slowly re-entering the dream.*

Fecal bacteria on the dog hair was transferred from your unwashed hands to the broccoli. *Escherichia coli* O157:H7, a toxin-producing strain of fecal bacteria, is growing in the unpasteurized apple juice from a local farm that you brought in from the food delivery. *Salmonella* and *Campylobacter* are growing on the uncooked chicken the sous chef took to create the house specialty. You know these microbes are there, because in your dream you saw the dog rolling around outside, the apples being picked up from the pavement or from an orchard shared with cows, and chicken carcasses going through bloody processing wash-water. There is fungus and mould growing in the cracks between surfaces around the room, and the black bits in the corner were left by a mouse running alongside the wall. Does the beef from the delivery truck contain harmful bacteria or prions? Could the fish contain toxins that would trigger a public-health disaster?

*As your alarm clock wakes you for work, you realize how much you have learned and how much more aware you are now of the invisible world of microorganisms.*

All around you, there are an uncountable number of unseen microbes, some of which may be pathogenic (disease-causing). These microbes will grow quickly if the environmental conditions become optimal (best) for them. Everywhere, there are microbes you cannot see: on the countertops, the floor, the ceiling, your hands and clothes, the utensils in the room, and in the water you drink and the air you breathe.

## Why study food microbiology?

**Microbiology** is the study of microorganisms. **Microorganisms** (also called **microbes**) are organisms that are too small to see without a microscope (as you might have guessed). Microorganisms include algae, **bacteria**, **fungi, parasites** (made up of **protozoa** and worms), and **viruses**.

For some perspective of how the size of microbes compares to other objects and organisms, see the short simulation at the following link. Enter the site and use the scroll bar along the bottom to zoom in and out—caution: some users report getting dizzy when watching.

**Scale of the Universe**
https://www.youtube.com/watch?v=uaGEjrADGPA

In the human body, there are over 10 trillion human cells and approximately ten times that many, 100 trillion, bacteria. These bacteria share the food we eat, help us to digest it and recycle nutrients from our waste. Some of these bacteria protect us, but others can make us sick (are **pathogenic**). We have bacteria on our hands, in our mouth, our nose, and in our gut. Wherever they find an inviting environment, bacteria will make themselves at home. As well as being home to trillions of bacteria, our bodies also house various viruses and fungi. The community of microbes in a particular environment is known as a **microbiome**.

**Did you know?**
Just as each of us has a distinct fingerprint, each person has a unique microbiome. Those who live in close contact with one another, such as parents and their children, will have more similar microbiomes, but they will not be identical. The makeup of our microbiomes is affected not only by the food we eat, but also the people with whom we come into contact, the environments we pass through, and the environments the food itself has passed through as it makes its way from farm to fork.

The differences among people's microbiomes may explain why some people are more susceptible to disease than others and why people react differently to drug treatments. Although there are many microbes capable of causing human illness and food spoilage, there are even more that are beneficial and, in fact, essential to the health of our bodies and the environment.

The Human Microbiome Project was started in 2008 and completed in 2013. For this project, scientists took advantage of technological advances to catalogue the microorganisms that inhabit our bodies. This work taught us more about the communities of microorganisms that live in and on us. For example, these microorganisms add greater than 1000 more genes to the estimated 19 000 human protein-coding genes in the human body (University of Maryland School of Medicine, 2017). These findings have changed the way we view ourselves in the world.

Currently, we often fight harmful bacteria with antibiotics. But, like spraying a garden with insecticides that eliminate beneficial insects, antibiotics can kill off the good bacteria in your body as well as the bad. Scientists do not yet know if the microbiome is capable of restoring itself. Some bacteria, often referred to as opportunistic pathogens, move in once the antibiotics have done their job; these bacteria can cause severe illness. For example, *Clostridium difficile* bacteria can invade the intestinal tract after antibiotic treatment. *C. difficile* is difficult to get rid of and can cause severe intestinal problems.

Probiotics are bacteria that are helpful to the health of the body. Probiotic foods contain these helpful bacteria, such as yogurt containing live *Lactobacillus* and *Bifidobacterium* bacteria. It is far better for our health to have a microbiome containing bacteria that aid in digestion, such as these, than to have bacteria that cause illness, such as *C. difficile*.

Microbiology is an ever-evolving field, where discoveries not only help us to understand the invisible world around us, but may one day point to ways to manipulate that world to the betterment of human health. One day, scientists may understand how to improve health, fight disease, and even combat obesity by altering an individual's microbiome. At the University of British Columbia, researchers have found relationships between the composition of the microbiome of developing infants and diseases such as obesity and asthma (Arrieta, Stiemsma, Amenyogbe, Brown, & Finlay, 2014). They have further linked microbiome composition to several neurological diseases, including multiple sclerosis, Parkinson, and Alzheimers (Tremlett, Bauer, Appel-Cresswell, Finlay, & Waubant, 2017).

## Food microbiology

Food microbiology is the study of the specific microorganisms in food (food microbes), and their beneficial and harmful effects on the quality and safety of raw and processed food.

Food microbiologists provide us with knowledge and tools to understand the nature and characteristics of food microbes. Food microbes can be beneficial, neutral, or harmful to humans.

By understanding the fundamental principles of food microbiology and how these translate into both positive and negative effects for consumers, we can make our food establishments safer. We can focus on prevention and on promotion of a culture of food safety, to ensure the opening scenario in the lesson would never really happen.

Figure 1 describes topics important to food quality, safety, and processing that food microbiologists and food quality assurance / quality control technicians should understand, and where these topics are located in this course

| Areas of interest | Lesson |
|---|---|
| Microbial growth limits for pathogens<br>Basic techniques for counting and identifying microbes in food samples | B |
| Food preservation | C |
| Food spoilage | |
| Foodborne illness | D |
| Personal hygiene affecting foods | E |
| Sanitation in the food-processing environment | |
| Cross-contamination | |
| Barriers and microbial hurdles to making food safer | F |
| Interaction of microbes with packaging | |
| Critical limits for microbe survival | G |

**Figure 1** — Main areas of interest to food microbiologists and lessons where covered

# Microbes of importance to the food microbiologist

Microbes of interest to food microbiologists include microscopic representatives from six major groups: bacteria, fungi (includes **yeasts** and **moulds**), viruses, parasites (protists and even some worms), algae, and prions. Although we will look at each of these, of particular interest to this course are bacteria and fungi. We will briefly discuss a small number of viruses, algae, and parasites that are important in the food industry. We will also touch on the prion that causes variant Creutzfeldt-Jakob disease (the human form of mad cow disease).

In the opening scenario, we saw some ways that many different microbes can potentially affect our food. Before we learn how to prevent transmission and growth of dangerous microbes, we first need to learn a little more about them, such as what they need to live in their environment. But even before that, we need to identify the microbes we are dealing with.

## Beneficial aspects of microbes to the food industry

The actions of microbes can transform foods. Fermentation is the breakdown of substances in food by microbes. Fermentation occurs in a variety of foods and environments, and is essential in the production of some foods. Fermentation is essential in the production of some foods. It can give foods unique and wonderful tastes. For example, bread rises through the fermentation action of yeasts, sausages and cheeses are fermented with a mixture of moulds and lactic acid bacteria, and grape juice is transformed into wine through the fermentation of sugars by malo-lactic bacteria and specific types of yeast.

Fermented foods also last longer. Without fermentation, people in some countries with short growing seasons would not be able to preserve their fruits, vegetables, grains, milks, or meats. Fermentation gives us sauerkraut from cabbage, miso soup from soy beans, salami from meats, beer from barley, and tastier, longer-lasting olives. A table of all the types of fermented foods and their countries of origin would fill pages and pages of text!

Microbes also have other indirect benefits to the food industry. Microbes can be used as vehicles to produce and synthesize various ingredients and food additives, such as enzymes, vitamins, amino acids, and even bio-fuels, that would be otherwise more difficult and expensive to manufacture.

## Harmful aspects of microbes to the food industry

Food spoilage and **foodborne illness** are the two major harms of microbes to food businesses.

Food-safety microbiologists may worry most about pathogenic microbes causing foodborne illness. However, another big concern is that of spoilage microbes, which do not cause illness but when not properly controlled may cause huge, costly problems for the food industry. Note that some types of food spoilage are non-microbial; for example, freezer burn occurs when foods are poorly wrapped; evaporation and oxidation destroy the quality and flavor.

*Guests, like fish, begin to smell after three days.*

Benjamin Franklin

Spoilage microbes (sometimes referred to in the industry as objectionable bacteria) are casually called "stinkies" or "slimies." Spoilage microbes cause undesirable deterioration of food quality that may affect the appearance (colour), odour, and/or taste of the final food product. Rotten-egg smells may arise from putrefaction and breakdown of proteins in foods by specific bacteria that produce sulfurous compounds and odours. Other microbes can produce a surface slime on aging foods, or excessive gas that will cause bulging cans or exploding packets of ketchup. Damaged and bulging food cans, such as the can received by the Vancouver Food Bank shown in Figure 2, are not safe for consumption. The Vancouver Food Bank and other businesses suffer significant monetary losses dealing with damaged food goods, because these goods cannot be thrown directly into the garbage. Special companies must be paid to safely decant and depackage the waste foods prior to their disposal in the organic compost stream.

**Figure 2** — This can, obviously under pressure, was received by the Vancouver Food Bank.

Foods that are drier, acidic, salted or stored under refrigeration tend to be less susceptible to spoilage. We will further cover harmful spoilage issues and the benefits of food preservation in Lesson C.

Foodborne illnesses (also called food poisoning) can arise from contaminated ingredients, improper cooking and processing, and unsanitary food handling. What if the bulging of the can in Figure 1 was due to the growth of *Clostridium botulinum*, the bacteria that causes botulism, because the canning process was flawed? No company wants their product to cause foodborne illness. Foodborne illnesses cause direct harm to the food industry through potential lawsuits, costly recalls, loss of reputation, and negative consumer confidence related to brand recognition.

One in eight Canadians gets ill from food poisoning every year. This leads to loss of work and productivity, sick-time payments, and even death. Food poisoning affects everyone. In Canada, common culprits of foodborne illness and the numbers of illnesses they cause per year are:

- Norovirus; 1 million illnesses
- *Campylobacter*; 145 000 illnesses
- *Salmonella*; 88 000 illnesses
- *Listeria*; 178 illnesses and an estimated thirty-five deaths

For more information, refer to *Food-Related Illnesses, Hospitalizations & Deaths In Canada* from the Public Health Agency of Canada, available at the following link.

**Food-Related Illnesses, Hospitalizations, & Deaths in Canada**
https://www.canada.ca/content/dam/hc-sc/healthy-canadians/migration/publications/eating-nutrition/foodborne-illness-infographic-maladies-origine-alimentaire-infographie/alt/pub-eng.pdf

While many types of bacteria are beneficial, as you have just read, many are capable of causing illness. In Lesson D, we will cover the bacteria most commonly associated with foodborne illness, their sources, the symptoms of the illnesses they cause, and how these illnesses can be prevented.

## Where are microbes found?

Microbes are found everywhere in our environment—including us! (Figure 3).


**Figure 3** — Microbes can be found everywhere.

## Classifying microbes—an overview

Before we begin to look at the characteristics of different microbes, let's first look at how microbes (and all other life) are named by scientists.

### Naming conventions: What's in a name?

Microbiologists would have trouble distinguishing all the different bacteria if they did not give them names. As you may have noticed, these names are often difficult to spell and to pronounce. Take *Escherichia coli* for example. This bacteria was named after the German-Austrian pediatrician who discovered *E. coli* in 1857—Theodor Escherich. The first part of the name, *Escherichia*, refers to the genus, and the second part, coli, refers to the species. These binomial (two-part) labels are written in italics and the genus is capitalized. The same system is used for classifying plants and animals. For example, the scientific name for the wolf is *Canis lupus*, for the coyote it is *Canis latrans*, while for your pet dog it is *Canis domesticus* (Figure 4). If you wanted to refer to the entire genus, you could use *Canis* spp., where the abbreviation "spp." means all the species.

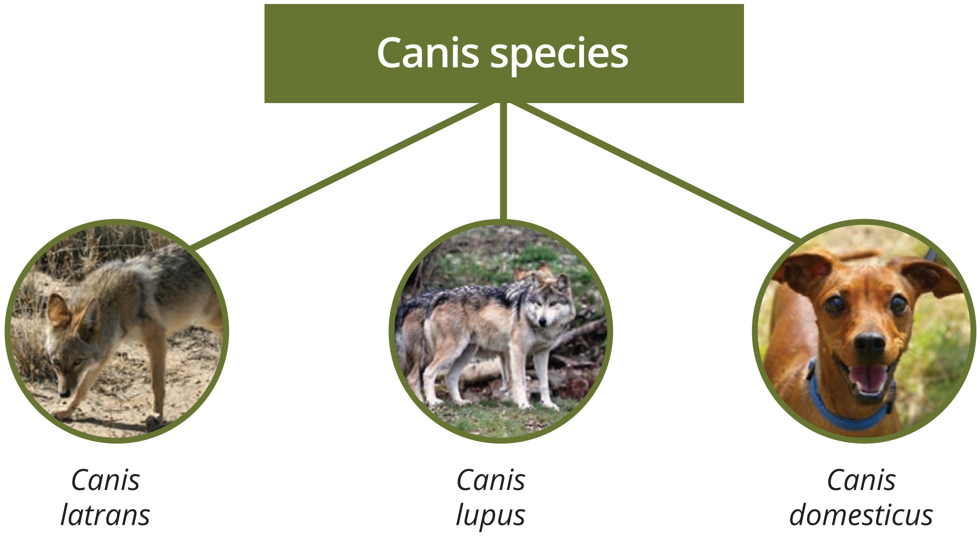



**Figure 4** — The three species *Canis domesticus, Canis lupus,* and *Canis latrans* are all members of the genus *Canis.*

At times, the first name (the genus name) may be abbreviated. Thus, we know *Escherichia coli* as *E. coli* (Figure 5). Another naming convention you will see involves extra letters, numbers, and/or names following the name of the bacteria, such as *E. coli* O157:H7 or *Salmonella* Newport. These designations are used to describe the specific strain or variant within the bacterial species. This further description refines the group within a particular genus and species. Referring back to the genus of *Canis*, this is similar to how you will find Rottweilers, Dachshunds, and many other breeds within the population of domestic dogs (i.e., species of *Canis domesticus*). You may also sometimes see the species name left out; for example, some reports refer to *Salmonella* Enteritidis, a pathogenic bacteria in the *Salmonella* genus. However, the full scientific name for *Salmonella* Enteritidis is *Salmonella enterica* var. Enteritidis, where the "var." stands for variant. If we were to apply this same naming convention to Dachshunds, we might write *Canis domesticus* var. Dachshund or just *Canis* Dachshund.

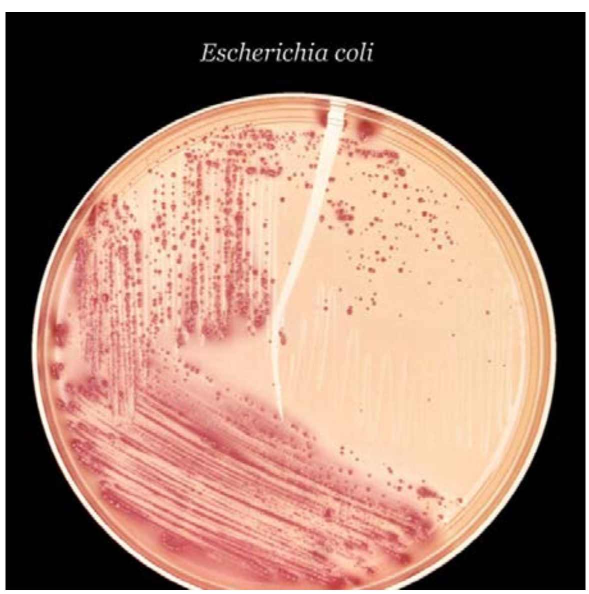



**Figure 5** — Colonies of *Escherichia coli* bacteria grown for 24 hours.

Figure 6 provides a snap-shot of the differences among categories of microbes. To fully understand the differences between these microorganisms, further reading and research would be required.

| | Bacteria | Virus | Fungi: Moulds and yeasts | Parasite | Algae | Prion |
|---|---|---|---|---|---|---|
| **Living or non-living** | Living | Non-living | Living | Living | Living | Non-living |
| **Single or multi-celled** | Single | Single | Multi | Multi | Multi | Not a cell |
| **Produces spores or eggs or cysts** | Some spores | No | Spores | Eggs | Some spores | No |
| **Asexual or sexual reproduction** | Asexual | Not applicable | Both asexual and sexual | Sexual | Both asexual and sexual | Not applicable |
| **Size range** | 0.3–300 µm | 17–440 nm | Yeast: 4–40 µm<br>Fungi: many km! | 0.5 cm–1 m | 1 µm–45 m | 10 nm |

**Figure 6** — Basic facts for different categories of microbes

Microbes are diverse. They can be living or non-living, and they may reproduce sexually or asexually (dividing and making identical copies). They can be very small or quite large—a prion is 10 nm or 0.00000001 of a metre, while some very large parasite worms are 1 metre long! See Figure 7 for some other unit conversions. Some algae, such as some kelp species, can be up to 45 metres long, while some fungi can grow underground throughout an entire forest!

| Unit | Name | Length in metres |
|---|---|---|
| 1 nm | Nanometre | 0.000000001 |
| 1 µm | Micrometre | 0.000001 |
| 1 mm | Millimetre | 0.001 |
| 1 cm | Centimetre | 0.01 |
| 1 m | Metre | 1.0 |
| 1 km | Kilometre | 1000 |

**Figure 7** — Conversion of various units of length to metres

In this course, we will not focus on the structural or biochemical characteristics of microbes, unless they convey a property or survival characteristic to the microbe that you would need to know about as a food microbiologist. For example, we will not cover which microbes have an outer cell wall or a cell membrane. But it is worth mentioning that many microbes have a stage in their life-cycle where they form a tough **spore**, or cyst, or deposit eggs or larval forms into foods. This information is important for a food microbiologist to know, as these microbes will have two or more types of bodies or structural forms. Each requires different control methods to manage their risk in food. For example, spore-forming bacteria such as *Clostridium* spp. are much harder to eliminate from canned foods than bacteria that do not form spores, such as *Salmonella* spp. It is also worth mentioning that viruses are different from most other microbes, because they cannot reproduce on their own. And although small, many viruses are quite tough and able to persist in many harsh environments.

## Traditional methods of identifying microbes

Microbes are often identified by specific traits or characteristics. These traits may include:

- Colour (e.g., blue-green or brown algae)
- Size and shape (e.g., bread yeasts are oval and up to 10 μm in diameter)
- How they appear when mixed with dyes (e.g., bacterial Gram staining)
- Whether they can move (motility)
- How they move (e.g., swimming straight or tumbling about)
- Whether they can form spores (tough, environmentally resistant forms of a microbe)
- Whether they can produce **toxins** (chemical poisons), and
- What they consume (e.g., the types of sugars and other nutrients)

We will provide an overview of traditional methods of identifying microbes, using bacteria as an example. Microbiologists identify bacteria by their shape, size, whether they form spores and toxins, their motility, and whether they are able to eat certain types of sugars, other carbohydrates, and proteins. This is also important to the food microbiologist, as it provides clues as to what types of bacteria grow in foods.

### Growing bacteria on nutrient agar

Agar plates are used to grow bacteria. Agar looks and behaves like gelatin desserts. Agar is made from seaweed and it will solidify after it is dissolved in hot water and cooled, just like gelatin. Food microbiologists add different types of sugars and nutrients to the agar mix before pouring into a round plate called a petri dish (Figure 8).

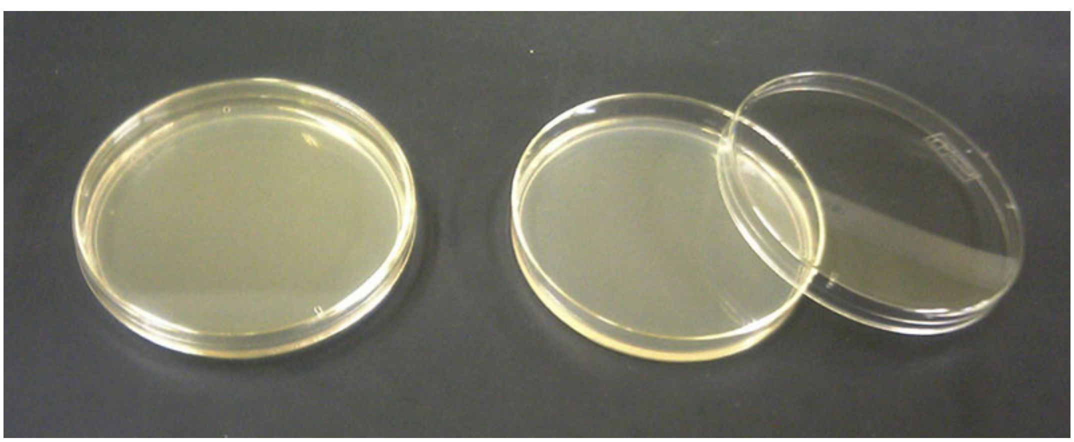


**Figure 8** — Nutrient agar plates

To find out what types of bacteria are present in a food, a sample of the food is added to the agar plate. Details on how this is done are provided in Lesson B. The colour and growth of the bacteria on the plate can indicate the type of bacteria present in the food (Figure 9).

**Figure 9** — Bacteria of different sizes and colours growing on a nutrient agar plate.

To be really sure what type of bacteria is in the food, the food microbiologist may look at a sample of it under a microscope, test how the bacteria reacts to a specific dye, test for motility by adding some of the bacteria to a liquid, and determine what the bacteria can digest by mixing it with different sugars and nutrients.

## Gram staining of bacteria

One way to make bacteria stand out from their environment is a technique known as **Gram staining**. This staining technique was invented by Hans Gram over 100 years ago. Bacteria are dried onto a clear glass slide then two different coloured dyes are added to stain the bacteria. The dyes are rinsed off with alcohol and water before the bacteria are observed under a microscope. If a sample of bacteria appears purple after, staining, the bacteria is a Gram-positive bacteria. If the sample it appears red after staining, it is a Gram-negative bacteria. The deeper colour of Gram-positive bacteria is due to their having thicker outer walls that are stained by the dye.

## Shapes of bacteria

One way to classify bacteria is by their shape, which can be round or oval (cocci), rods (bacilli), or spiral-shaped. Figure 10 is an example of cocci, *Staphylococcus aureus*, a spherical Gram-positive bacteria. Figure 11 shows an example of bacilli, *Salmonella spp*., rod-shaped Gram-negative bacteria.

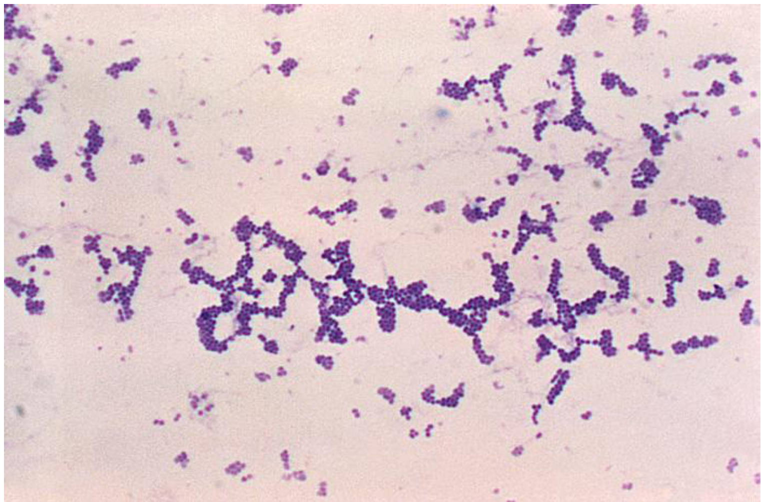


**Figure 10 —** *Staphylococcus aureus*: Gram-positive cocci

**Figure 11 —** *Salmonella*: Gram-negative rods or bacilli

*Vibrio cholerae* (Figure 12) is an example of a Gram-negative spiral-shaped bacteria, which are curved and look something like a fat comma or spiral. Bacteria sometimes appear in clusters, pairs, or chains.

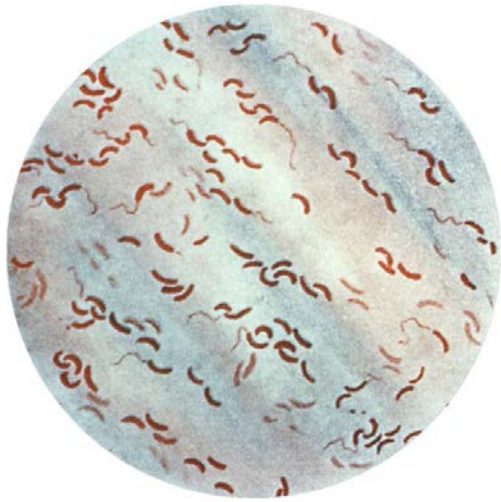


**Figure 12** — *V. cholera*: Gram-negative spiral-shaped bacteria

## Spore-forming bacteria

Some Gram-positive bacteria can form spores, also called **endospores**. A spore-forming bacterium encapsulates its vital structures in a tough outer coat when environmental conditions become harsh. These spores are extremely resistant to heat, chemicals, and other environmental conditions, and so help these bacteria to survive harsh environmental conditions.

Bacteria don't multiply in the spore form, but if conditions become favourable again they can start to grow again. This is the **vegetative** form of the bacteria, in which the bacteria can multiply. Lesson B will describe microbial growth in more detail. Examples of spore-forming bacteria of interest to the food industry are *Bacillus* spp. and *Clostridium* spp.

## Toxin-producing bacteria

A toxin is a chemical poison. Bacteria are also classified by whether they produce **toxins** that cause foodborne illness. The infamous bacteria *Clostridium botulinum* produces the deadly botulinum toxin that causes botulism. One teaspoon (5 mL) of botulinum can kill up to 100 000 people.

Toxins can be produced by many species of bacteria and other microbes, such as moulds and algae. Some toxins are released directly into foods, and their effects are immediate once the food is consumed. For example, *S. aureus* produces a toxin that causes vomiting. Other bacteria, such as *V. cholera*, release a toxin once they are ingested. These toxins are called enterotoxins; *entero* is Greek for "intestine." Enterotoxins often cause diarrhea, as they directly affect the gut function.

Toxins are also problematic in the food industry, because some are very resistant to heat. For example, histamine is resistant to heat and can sometimes be found in canned foods. Some microbes can make chemical changes and toxins in foods when temperatures are high enough to allow them to grow. That is why temperature control is so important to the food industry: once a toxin is formed, there may be no way to remove it from foods.

## Bacterial motility

Bacteria that can swim or move within a food can create a lot of problems. Unfortunately, most pathogenic bacteria do have this ability, and so are able to travel through high-moisture foods and other environments quite easily. This is one reason why microbiologists will look at sample bacteria on a slide under a microscope (Figure 13 and Figure 14). For example, *Listeria* is a problem in soft cheese.

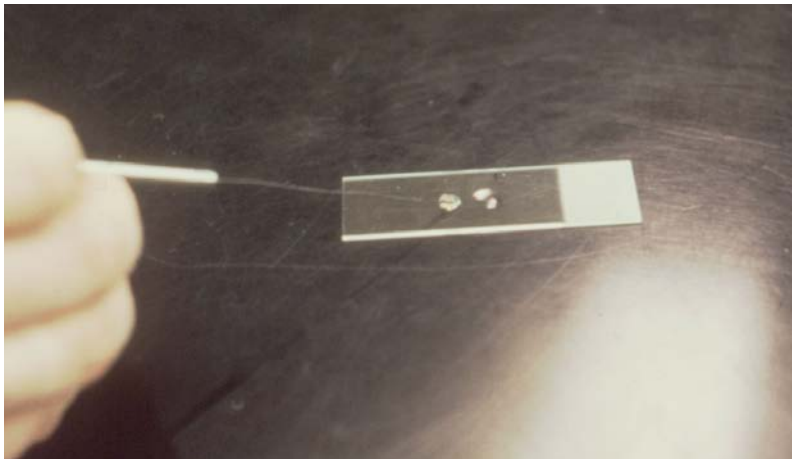


**Figure 13** — Placing bacteria on a glass slide

**Figure 14** — Viewing bacteria under a light microscope

Take a look at the video in the following link to see *Listeria* bacteria swimming and tumbling in a drop of water—they move fast for tiny microbes!

***Listeria* Monocytogenes Tumbling Motility**
https://www.youtube.com/watch?v=bV_Wd7JCo6A

## Microbes from a food industry and food microbiologist perspective

If you are responsible for food production, you must be very concerned about any microbes that affect your final product. These include microbes that are invisible to the eye but can be seen on a microscope. They may arrive in your food ingredients or on an employees' hands. They may be carried in on clothing or jewelry, or released into a food production area when someone coughs or sneezes. Microbes may be difficult to remove from equipment, or they might reside on overhead lighting fixtures, blow out from dirty filters in refrigeration units, or be found in drains. This is much more important to you than whether the bacteria have a cocci or spiral-shaped form. Here, we will describe three ways of differentiating microbes that are important to the food microbiologist:

1. Habitat or preferred environment. Where does the microbe live?
2. Function. This is what the microbe does. What food does the microbe eat, and how does it infect or reproduce?
3. Survival. This is also related to the microbe function, but is a very important from a food perspective—does the microbe survive in high salt or acid conditions?

### Habitats of microbes

Bacteria are also classified with reference to their habitat or preferred environments. Some bacteria thrive in the presence of the levels of oxygen normally in air, while others do not. Some bacteria like hot temperatures, while others survive in the freezer or in high salt conditions. Habitats can have alkaline or acidic pH. Consider, for example, the differences between a salmon and steer (cattle). These are very different looking animals that live in very different environments, so the microbes that live in and on them also prefer very different environments. Salmon live in salty, cold marine water, and (if you can remember your grade 8 biology class) they are cold-blooded. This means that a salmon adjusts its body temperature to the temperature of its environment. Wild salmon eat small marine organisms and other small fish. A steer, on the other hand, lives on land, near soil, and is warm-blooded. Steers have a vegetarian diet of grains and grasses. If we were to look at a table of microbe limits—the pH, amount of salt, and temperature range bacteria can tolerate—you might be able to guess which microbes are more commonly found in fish or cattle. *Listeria* is well-known for being able to grow at low temperatures, such as in a refrigerator, while *Staphylococcus* tolerates high acid, high salt conditions, and low water activity. Moulds and fungi are well adapted to cold, moist, and fairly acidic environments, which is why you often see mould growing on the tops of food in the refrigerator.

In Lesson B we will focus on microbial growth characteristics, and explain what we mean by water activity, pH, and acidity. By taking a closer look at preferred environments of microbes of importance to the food industry, we will be better placed to intervene in the growth of harmful bacteria and encourage growth when this is to our benefit, as in fermentation.

### Functions of microbes

For the industrial food microbiologist, finding the right microbe to do the job will make a better food product, save time, ensure consistency in the product, and make the company money. Microbes in starter cultures are one common example. Lactic acid bacteria are used to make yogurt. These bacteria digest lactose sugars in milk to provide taste and texture, and may even confer good digestive health. The starter cultures used for yogurt include strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

For the clinical food microbiologist investigating foodborne illness, finding out what the bacteria digests helps to identify the pathogen causing the illness. In the next lesson (B) you will see how regular *E. coli* is differentiated from *E. coli* O157:H7, the strain that causes diarrhea.

## Survival of microbes and environmental conditions

Bacteria are very versatile microbes. You can find them in many environments, from frozen water to thermal hot springs. They are found in plants, animals, humans, soils, and air. As described earlier, some bacteria are capable of forming a tough outer cell wall known as a spore while others have adaptive strategies to survive in harsh environments.

Food microbiologists use a lot of terminology to describe whether microbes can survive in environments with different levels of oxygen and temperature. A few important such terms are described in Figure 15 and in your glossary.

| Oxygen levels they need | Temperatures they like (*philes*) |
|---|---|
| **Aerobic**<br>Grows only in oxygen | **Thermophiles**<br>From 40°C to 80°C |
| **Anaerobic**<br>Grows only without oxygen | **Mesophiles**<br>From 20°C to 40°C |
| **Micro-aerophilic**<br>Tolerates small amounts of oxygen | **Psychrophiles**<br>From 0°C to 10°C |
| **Facultative anaerobe**<br>Grows in environments with or without oxygen | |

**Figure 15** — Classes of microbes based on their survival in different oxygen and temperature levels

In this introduction, we want you to think about classifying bacteria and other microbes using the terms previously described.

## Activity: Classifying bacteria

Figure 16 shows three examples of foodborne illnesses caused by bacteria you have likely heard of already: *Clostridium botulinum*, Escherichia coli, and *Campylobacter*.

**Botulism**
Caused by *Clostridium botulinum* bacteria

**Hamburger disease**
Caused by *Escherichia coli* O157:H7

**Septicemia**
Can be caused by *Campylobacter*

**Figure 16** — *C. botulinum, E. coli* and *Campylobacter*: identify their characteristics.

Three sets of characteristics are found in the tables that follow, one for each of the three bacteria in Figure 16. Even though we haven't told you very much about these organisms, try to select the bacteria that corresponds to each set of characteristics.

1.

| Habitat | Function | Survival |
|---|---|---|
| Warm blooded, mammals, especially cattle | Intestinal | Excellent stress response |
| | Form toxins in gut | Facultative anaerobe |

a. *Escherichia coli* 0157:H7
b. *Clostridium botulinum*
c. *Campylobacter*

2.

| Habitat | Function | Survival |
|---|---|---|
| Soils terrestrial and marine | Digest proteins | Spore formers |
| | Form toxins in foods | Anaerobe |
| | | Tolerates salt |

a. *Escherichia coli* 0157:H7
b. *Clostridium botulinum*
c. *Campylobacter*

3.

| Habitat | Function | Survival |
|---|---|---|
| Warm blooded mammals, especially poultry | Intestinal | Persists in water environments |
| | No toxins | microaerophilic |

a. *Escherichia coli* 0157:H7
b. *Clostridium botulinum*
c. *Campylobacter*

Check your answers with the answer key provided at the end of this textbook.

We've discussed bacteria within the context of how microbes are named and how food microbiologists might categorize them. The remainder of this lesson will introduce the other microbial groups listed in Figure 6: Basic facts for different categories of microbes.

## Viruses and prions

Viruses and prions are the smallest microbes affecting foods and both are considered non-living, as they cannot reproduce or make copies of themselves without a host.

### Viruses

Although viruses are not cells, they can still damage living organisms. Viruses lack the ability to reproduce on their own (they need a host cell) and are unable to metabolize substances to feed themselves. Viruses are essentially nucleic acid (i.e., DNA or RNA) enclosed in a protein shell (Figure 17).

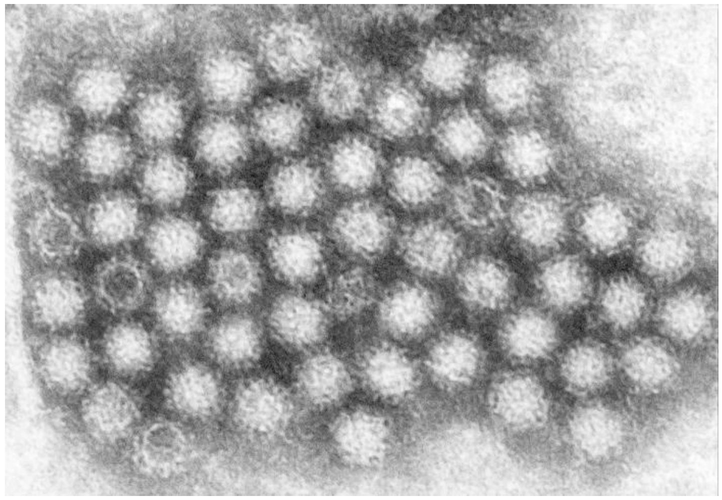


**Figure 17** — Transmission electron microscopic image of norovirus particles composed of RNA and protein

Viruses exist in two distinct states, **dormant** and **active**. Viruses can remain in their dormant state for extended periods of time, waiting to come in contact with appropriate host cells to hijack. When a virus comes into contact with an appropriate host, it becomes active.

The virus tricks the host cell into letting it through the cell membrane where it takes over the cell's genetic material and replaces part of it with its own. Viruses use the metabolic and reproductive capabilities of the host cell to reproduce. Viral infections are not affected by treatment with antibiotics, and viruses cannot reproduce outside of a a host, i.e. a person's or animal's body. A virus can make hundreds of thousands of copies of itself in this way. Of particular interest to us ae norovirus (family *Caliciviridae*) and hepatitis A virus (family *Picornaviridae)*.

## Prions

Prions are even stranger than viruses. They are pathogenic agents believed to be misfolded proteins that have the capacity to cause specific cellular proteins to also become misfolded. The susceptible proteins occur most abundantly in the brain. Prions are smaller than viruses and do not have any genetic material.

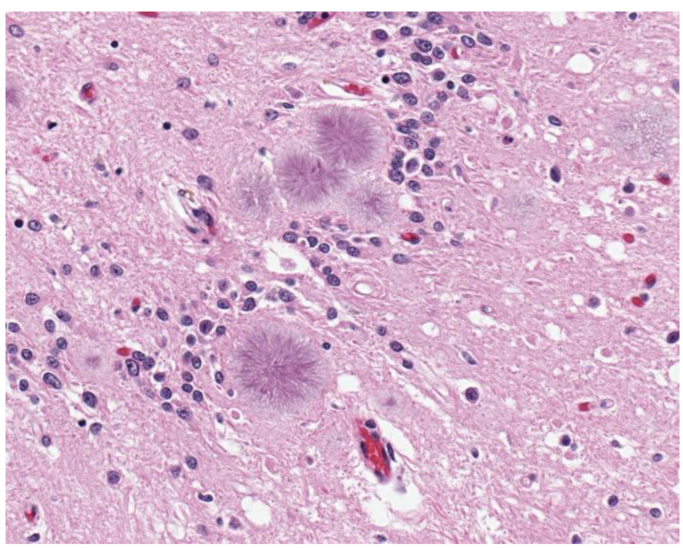


**Figure 18** — Brain tissue of person with variant Creutzfeldt-Jakob disease (vCJD)

Protein misfolding in the brain due to prions causes a group of diseases called spongiform encephalopathies. In cows, the spongiform encephalopathy disease is called mad cow disease; in humans, Creutzfeldt-Jakob disease or variant Creutzfeldt-Jakob disease (Figure 18); and in deer, chronic wasting disease.

Prions are still not well understood and although spongiform encephalopathies are rare, these diseases are progressive and always fatal.

## Microbial parasites

Microbial parasites of interest in the food industry generally fall into the categories of protozoans and worms (Figure 19).

| **Protozoans** | | ***Cyclospora cayetanensis*** | ***Cryptosporidium parvum*** | ***Giardia duodenalis*** | ***Toxoplasma gondii*** |
|---|---|---|---|---|---|
| Helminths (parasitic worms) | Cestodes (tapeworms) | *Diphyllobothrium latum* | *Taenia* spp. | | |
| | Trematodes (flatworms or flukes) | *Paragonimus westermani* | | | |
| | Nematodes (roundworms) | *Pseudoterranova decipiens* | *Anisakis simplex* | *Trichinella* spp. | |

**Figure 19** — Examples of microbial parasites found in foods

All foodborne parasites have complicated lifecycles and several structural forms. Protozoans and worms are multi-celled. These parasites generally infest the gastrointestinal tract of humans, with two exceptions. The lungworm flatworm *Paragonimus* is estimated to infect more than 22 million people annually, and *Trichinella* is a roundworm that deposits itself into muscle (Doanh et al., 2016).

### Protozoans

*Cyclospora* and *Cryptosporidium* are two species of protozoans of particular interest. Infection by these parasites tends to be through the fecal-oral route, and people become infected when they eat food or drink water contaminated by infected human or animal waste. Infection can also occur when people touch an object that has come in contact with infected fecal matter—so wash your hands! Fresh fruits and vegetables can become contaminated if they are irrigated with water that contains parasites. *Giardia* (Figure 20) and *Toxoplasma* are two other protozoan parasites that cause illness, but infection occurs largely through ingestion of water and not food. Wild and domestic animals in BC do shed feces containing parasites such as *Cryptosporidium*, *Giardia* and likely *Toxoplasma* into BC waters, and are well described (Bowie et al., 1997; Isaac-Renton, Cordeiro, Sarafis, & Shahriari, 1993; Ong , Moorehead, Ross, & Isaac-Renton, 1996). *Cyclospora*, however, has caused illnesses only in BC residents who ate imported foods contaminated with this protozoa, such as basil or cilantro (Hoang et al., 2005).

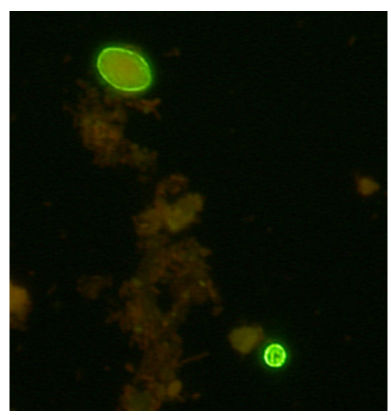


**Figure 20** — *Giardia* (upper left) and *Cryptosporidium* (lower right)

### Helminths (parasitic worms)

Helminths tend to be intracellular parasites, meaning that they fulfill their needs for food, protection, and transmission by living inside the host organism during part of their life cycles. Roundworms, flatworms, and tapeworms are important to food safety and can be found in fish, crustaceans, and meats. We are lucky in North America that controls in domestic pork production have virtually eliminated concerns for two types of worms, *Taenia* spp. and *Trichinella* spp. However, *Trichinella* spp. remain a problem in wild meats, such as wild bear meat (Figure 21). In 2005, these worms caused illness in twenty-six people who consumed undercooked meat (McIntyre et al., 2007). *Trichinella* has been found a variety of animal species that eat meat, including crows, owls, seals, whales, racoons, coyotes and dogs, and sometimes in domestic and wild pigs, goats and reindeer.


**Figure 21** — *Trichinella* worms retrieved from bear meat

We will discuss specific parasites in greater detail when we turn our attention to hazardous food groups and conditions wherein they become infected.

## Fungi

The fungi are a large and diverse group of microbes, which includes many species of moulds, yeasts, mushrooms, and truffles. The study of fungi is called mycology. Fungi are very important in decomposition, and so are often found in soils. Some fungi produce toxins that can cause disease when they get into our food supply, such as ergot in grains and bread, or patulin in decomposing apples. (Adeyeye, 2016)

### Fungi and the classification of organisms

Scientists classify the living things in our world to understand them better. However, how we classify living things is constantly changing at present as we learn more about their relationships based on their genetic material, DNA (Figure 22). Every year scientists sequence the DNA of more organisms, and from this we are learning that all living things are more interrelated than we previously imagined. The current classification system is referred to as the three-domain system. Based on similarities and differences in their DNA sequences, all organisms are grouped into one of three domains, which are:

- Eubacteria—true bacteria
- Archaea—ancient bacteria that live in extreme environments
- Eukaryota—fungi, plants, animals, protozoa, and parasites

The Tree of Life explains this modern classification system in more detail. The root of the tree describes Eubacteria, Archaea and Eukaryota.

**The Tree of Life**
http://tolweb.org/tree/phylogeny.html

**Figure 22** — Illustration of DNA

Fungi is a grouping in an older classification system that is made up of five domains or kingdoms. This system was based on the similarities and differences of the appearance of the organisms (i.e., their morphology) and organized using the concept of trees, with a central root and branches to describe the relationships among the domains. Bacteria (also called Monera) were placed at the bottom of the tree, followed by protozoa (Protists), and then three branches at the top for plants, animals, and fungi (Figure 23).

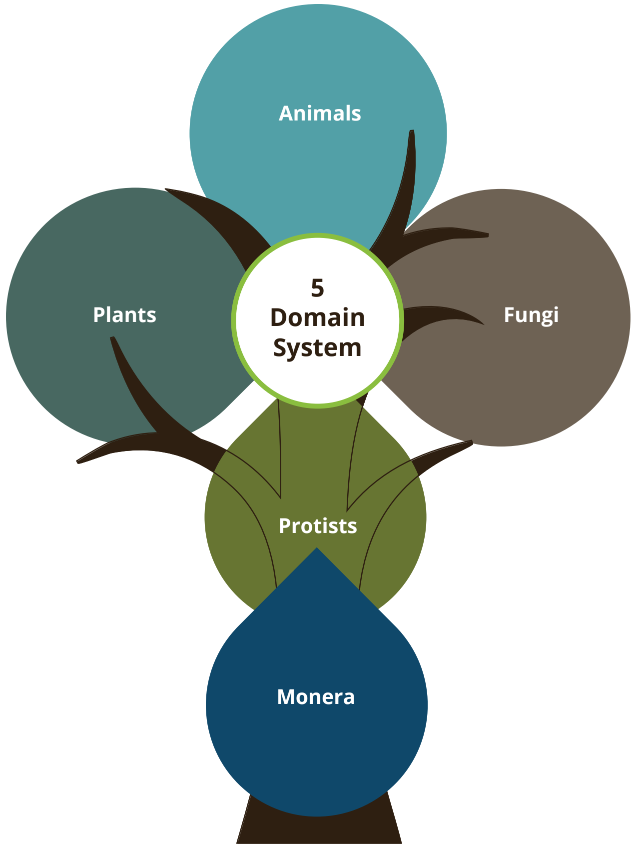



**Figure 23** — The five-domain system

## Groups of fungi

Although the five-domain system has now been superseded by the three-domain system, it is still conceptually useful. Within the fungi kingdom common names of fungi include yeasts, moulds and mushrooms.

Some fungi reproduce sexually by creating spores and fruiting bodies, and some reproduce asexually by a process called "budding off" to replicate themselves. Some fungi can reproduce using both methods. For example, black bread mould, Rhizopus (an important fermentative organism) reproduces sexually, while the fungi *Aspergillus* and *Penicillium* replicate asexually. Smuts, rusts, and galls are all terms associated with fungi.

To the food microbiologist, fungi can be beneficial when they assist fermentation, and can be a problem when they cause spoilage or add toxins to the food. For example, *Aspergillus* has many species. Some *Aspergillus* species cause spoilage, such as bread mould, but the species A. orzae is widely used as a fermenter to produce miso and soya sauce. Under a microscope, *Aspergillus* spp. appears to have many shapes (Figure 24). These organisms use long segmented threads called hyphae to grow through foods. *Aspergillus* spp. (asexual) fungi have reproductive structures labelled in Figure 24 as "vesicles" and "phialides."

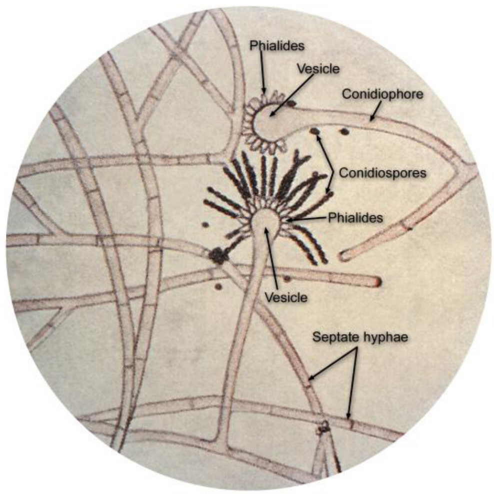



**Figure 24** — Common mould *Aspergillus*

Other fungi which reproduce sexually form fruiting bodies during reproduction. We eat the sex organs of fungi when we eat mushrooms! Mushrooms are the fruiting body, but most of the fungus actually resides underground in the soil as hyphae.

## Algae

Many types of algae are more commonly referred to as seaweeds (Figure 25). Although people think about seaweeds in the ocean as plants, they are more properly classified as algae. Edible seaweeds have been used for hundreds of years, and not just to wrap up sushi (Figure 26)!

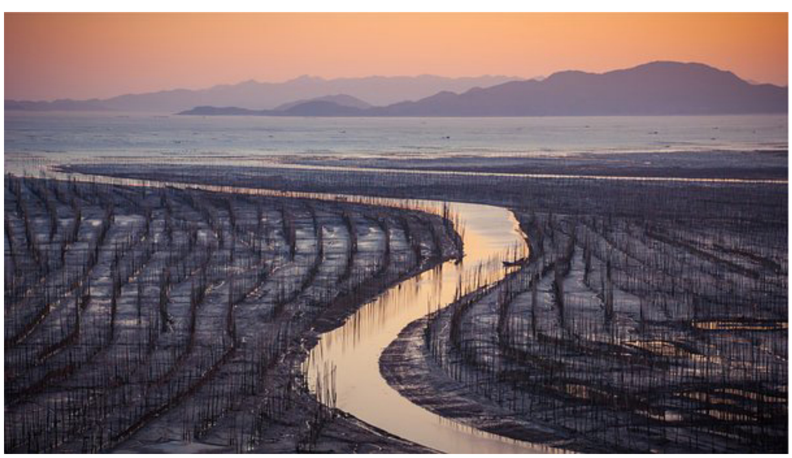


**Figure 25** — Seaweed farm

**Figure 26** — Red algae Pyropia, used to wrap sushi

Algae extracts are widely used in the food-processing world as a food additive, primarily as thickening and gelling agents (Figure 27). For example, carrageenan is used in many food products: desserts, condensed milks, patés and meats, beer, and other types of products such as shampoo and toothpaste (Cohen & Ito, 2002).

**Figure 27** — Algae is often used as a thickening agent in dairy products

From the microbiologist's perspective, algae is used every day! Did you know it is the base ingredient for agar plates used in the laboratory?

However, certain species can be devastating, particularly algae that form harmful algal blooms that poison marine and fresh waters. Overgrowth of algae can remove oxygen from the water, killing fish and other animals. Some types of toxin-producing algae can get into foods, particularly shellfish, and cause severe illnesses in human and animal if ingested. Saxitoxin, from algal species known as *Alexandrium* (Figure 28a) are very common in marine waters of the Pacific ocean. In fact, the first record of historical paralytic shellfish poisoning dates back to 1793, when one member of Captain Vancouver's crew died and four others became ill after eating mussels in Poison Cove, British Columbia (Quayle, 1966). *Dinophysis* (Figure 28b) in cooked mussels caused sixty-two illnesses in BC in 2011 (Taylor et al., 2013).

One emerging concern for the Pacific Northwest coast is from a diatom algae called *Pseudo-nitzia* (Figure 28 c)), which produces a very harmful neurologic toxin called domoic acid. The illness linked to this algae is called amnesic shellfish poisoning, because it can cause memory loss (Kathi A. Lefebvre et al., 2017; Perl, Bédard, Kosatsky, & Hockin, 1990).

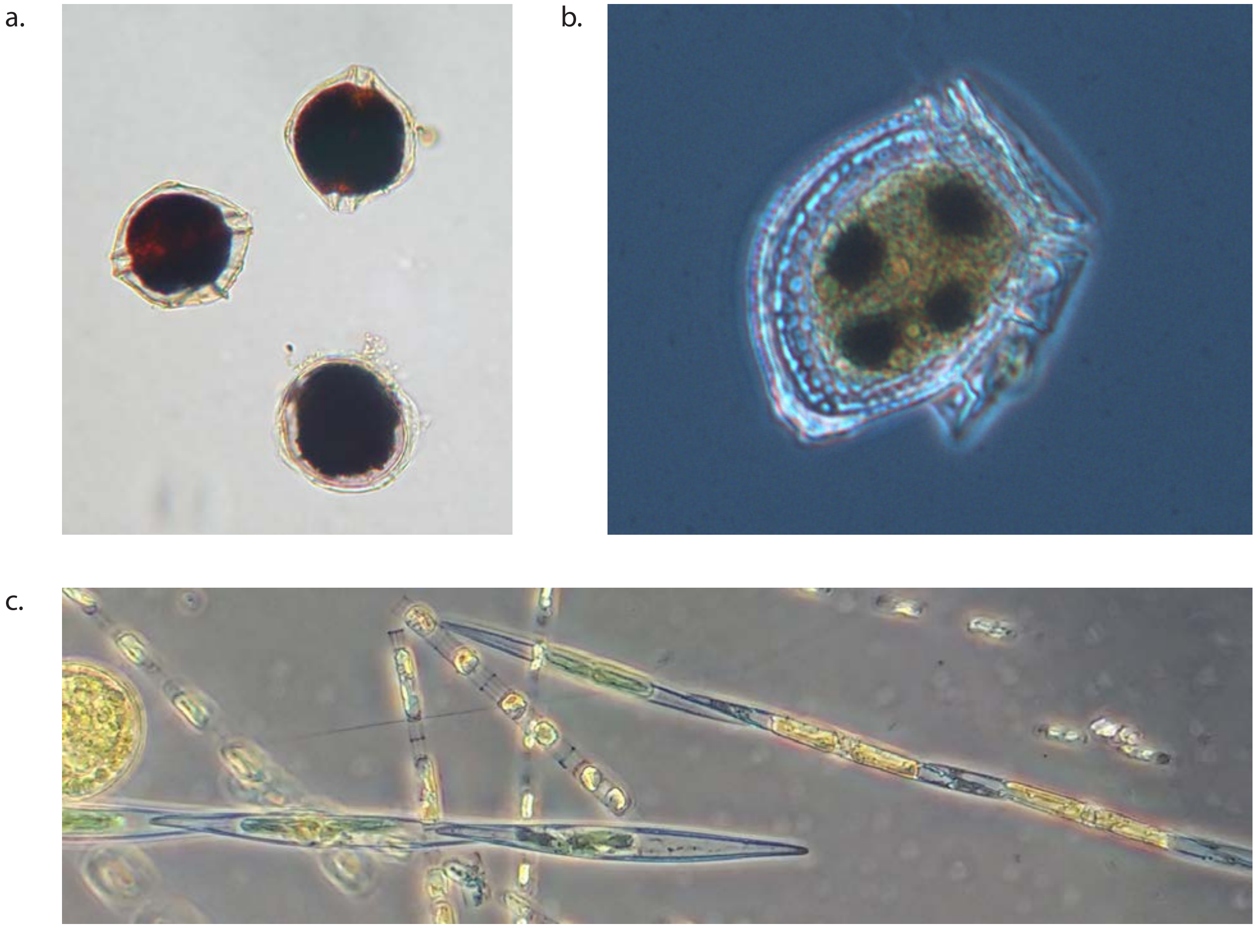



**Figure 28**—Harmful algae in British Columbia: a) *Alexandrium catenella*, b) *Dinophysis acuminata*, and c) *Pseudonitzschia pungens* and *australis*. Pictures courtesy of Nicky Haigh, Microthalassia Consultants Inc.

Algal toxins have also been linked to mass mortalities of whales and other sea animals (K. A. Lefebvre et al., 2016).

In the video in the following link, a sea lion on the beaches of Washington State can be seen having seizures from exposure to this algae.

**Climate Change: Sea Lion Seizures, Toxic Algae, and the Nightmare Scenario for Oceans**
https://www.youtube.com/watch?v=ote4a7hhW6A

Blooms of some algae release cyanotoxins, such as microcystins, which are potent liver toxins (Figure 29; National Collaborating Centre for Environmental Health, 2017). The responsible organisms are cyanobacteria, which is commonly referred to as blue-green algae. However, cyanobacteria are not in fact algae—in the three-domain system, cyanobacteria are in the Eubacteria domain, whereas algae are in the Eukaryota domain. Cyanotoxins can be a significant issue in freshwater, drinking water, irrigated water, and on food crops (National Collaborating Centre for Environmental Health, 2017).

**Figure 29** — Persisting cyanobacteria bloom in Lake Erie, Ontario, September 2017

*Spirulina* (Figure 30) is a beneficial cyanobacteria (although it is often referred to as an algae). Sprirulina is a source of protein, B-vitamins, and anti-oxidants, and so is used in smoothies.

**Figure 30** — Spirulina, a helpful cyanobacteria (blue-green algae)

# Lesson A Quiz

1. Which description applies to all microbes?

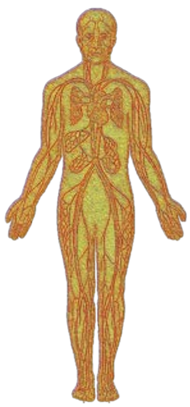
a.


**They need oxygen to survive.**

b.

**They cause infection.**

c.



**They reproduce in the human blood stream.**

d.

**They are too small to see without a microscope.**

2. Which statement describes microbial growth in the human body?
   a. Microbial growth is uniform in all individuals.
   b. Each person carries a unique combination of living bacteria, viruses and fungi.
   c. All people share the same healthy bacteria but are made ill by viruses or fungi.
3. Which type of bacteria is able to form its own protective coat?
   a. Vegetative bacteria
   b. Spore-forming bacteria
   c. Archeabacteria
4. Which phrase best describes how food microbes affect food production?
   a. Must be killed in food before it is consumed
   b. Can be beneficial, neutral, or harmful to humans
   c. Must be prevented from entering the food supply
   d. Always pose a threat to producers, processors and consumers

5. Which food product requires microbial fermentation in its preparation?

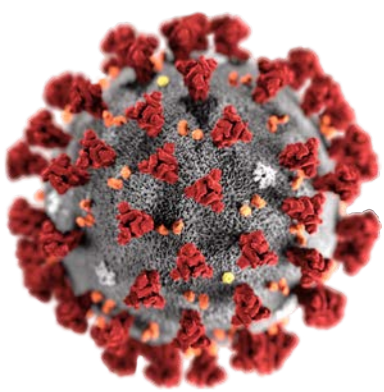
a. Pasta sauce

b. Yeast bread

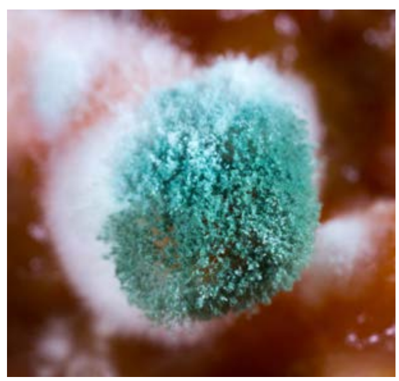
c. Vegetable soup

6. Which adjective describes microbes that are capable of causing illness?
   a. Aerobic
   b. Symbiotic
   c. Vegetative
   d. Pathogenic

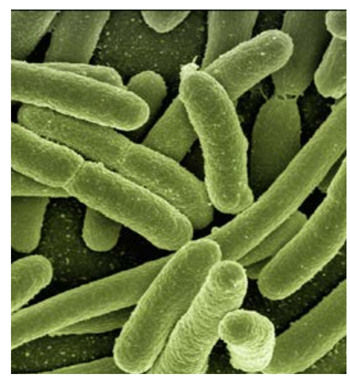
7. Which type of microbe has the following characteristics?
   - Non-living single-cell microbe
   - Produces no spores, eggs or cysts
   - Reproduction is neither sexual nor asexual



a. Virus



b. Mould



c. Bacteria

8. Which microbial characteristics are important for making yogurt? Select all that apply.
   a. habitats of microbes
   b. functions of microbes
   c. survival of microbes

9. Which microbial characteristics are important for packaging products in vacuum sealed containers? Select all that apply.
   a. habitats of microbes
   b. functions of microbes
   c. survival of microbes

10. Which microbial characteristics are important for checking the internal temperature of re-heated foods? Select all that apply.
    a. Habitats of microbes
    b. Functions of microbes
    c. Survival of microbes

11. What determines that a microbe is either aerobic or anaerobic?
    a. Whether it is parasitic or not
    b. Whether it is living or non-living
    c. Whether it requires oxygen or not
    d. Whether it is harmful or beneficial

12. Which statement best describes the relationship between food microbes and food production?
    a. Microbes come in a variety of forms that affect food quality
    b. Some microbes are visible in food, others require testing to identify
    c. Some microbes transform foods in a beneficial way, others may cause food spoilage or illness

13. How many groups can microbes can be classified into?
    a. 4 groups: bacteria, virus, spores, prions
    b. 5 groups: bacteria, virus, yeast, algae, parasites
    c. 6 groups: bacteria, virus, parasites, prions, algae and fungi

14. Which factor is not used to identify a microbe?
    a. By shape and colour
    b. By size
    c. By food preferences (substrates)
    d. By pH (acidity)
    e. By ability to move (motility)
    f. By ability to form a spore or a toxin

Check your answers with the answer key provided at the end of this textbook.

# Lesson B: Microbial Growth

## Introduction

In this lesson, we will explore what defines a food as being potentially hazardous by examining microbial growth and the factors that limit and promote microbial growth, as well as basic methods for how microbial growth is measured.

### Learning outcomes

Upon completion of this lesson learners will:

- Define a potentially hazardous food (PHF)
- Explain the key factors that affect microbial growth in foods
- Describe bacterial growth, doubling, and the phases of microbial growth
- Describe microbial growth limits for specific pathogens (max./min. pH, $a_w$, temperature, salt)
- Explain log reduction of bacteria during the thermalization processes
- Describe techniques microbiologists use to measure numbers of bacteria in/on foods
- Contrast microbial indicators and pathogens
- Interpret a laboratory report with results for indicators and pathogens

### Terminology

Key terms given in bold

aerotolerant
antibiotics
**antimicrobial**
binary fission
**colony forming units (CFUs)**
death phase
**extrinsic factors**
facultative
halotolerant
halophile
haloduric
hyperthemophile
**indicators** and **indicator tests**
**intrinsic factors**
lag phase
log phase
mesophile
microaerophilic
obligate
osmotolerant
**pH**
psychrophiles
**potentially hazardous food (PHF)**
**processing factors**
**sensitivity** and **specificity**
stationary phase
**substrate**
**thermal death point (TPD)**
thermophile
thermoduric
**thermalization**
toxins
viable but non-culturable
**water activity ($a_w$)**
xerotolerant

## Microbial growth—an overview

The work of cells is not that different from the activities and interests of humans. From the tiniest organism to the great blue whale, we all grow, eat, dispose of waste, protect our turf, and if possible, colonize new territories.

To understand bacterial growth, first consider human population growth. When we talk about population growth, we aren't talking about getting taller or bigger, but about getting more numerous. Bacterial growth is population growth. And like humans, in order to reproduce, bacteria require energy from food.

In microbiology, a bacterial food source is referred to as the **substrate**. A substrate may be in the form of meat, vegetables, debris on a countertop, soil on your hands, or crumbs that you spilled on your keyboard. Bacteria will reproduce wherever they can find substrates and convert them into usable resources—as long as the surrounding environmental conditions are suitable. By understanding the conditions under which bacteria reproduce, we can begin to understand how we can either harness that growth to our advantage (as in the production of cheese and wine) or inhibit the growth to prevent food spoilage and foodborne illnesses.

**Potentially hazardous food (PHF)** is the term we use to describe a food substrate in which microbes are able to survive, replicate, and colonize. The BC Food Premises Regulation (Government of British Columbia, 1999) defines a potentially hazardous food as a "food in any form or state that is capable of supporting the growth of disease-causing microorganisms or the production of toxins." Bacteria can easily grow in some types of food—for example, raw fish and dairy products—because there are plenty of nutrients and moisture in those foods.

**Figure 1** — Bacteria growing on a nutrient agar plate

In Figure 1, bacteria are seen growing on a nutrient agar plate. Dry foods such as crackers, however, are not hospitable to microbes, and so microbes do not survive or grow on that type of substrate.

What we will learn in this lesson is what factors affect microbial growth in foods and how these factors affect microbial growth, and about our tools for counting growth. We will also discuss measuring microbe destruction during cooking processes.

## Factors affecting microbial growth

Like other life forms, microbes require energy to grow and reproduce. They have evolved over millennia, and now inhabit diverse and sometimes extreme environments.

In the last section, we introduced two terms that describe microbes that live in extreme environments: *psychrophile* (liking cold environments) and *thermophile* (liking warm environments). Another term describing microbes that live in extreme environments is *halophile*, which refers to microbes that like high salt environments, such as the marine dwelling *Vibrio* spp. Should salt concentrations be too high though, salt-liking *Vibrio* bacteria can no longer survive. However, there are spoilage organisms that can survive very high salt conditions, such as those in salted meats. These organisms are called haloduric. Here the suffix "duric" (from the Latin word *durare*, which means "to last or endure") is used to differentiate bacteria that can survive in very extreme conditions from those that like less extreme conditions (recall that the Latin word *phila* means "to like"). Bacteria that are described as thermoduric are able to endure very high temperatures, including pasteurization temperatures.

When used in high enough quantities, salt is a preservative. Why? Salting cod is a very old practice that is part of our Canadian history (Figure 2). How can a perishable food such as cod be transformed into a shelf- and room-stable commodity when it is packed in salt (Figure 3)?

**Figure 2** — Cod preparation in Newfoundland, 1857–1859

**Figure 3** — Salted cod

We will look at four types of factors that affect how microbes grow in food (Figure 4):

1. What's in the food (**intrinsic factors**)?
2. What's outside of the food (**extrinsic factors**)?
3. What's being done to the food (**processing factors**)?
4. What other microbes are doing to the food (**microbe factors**)?

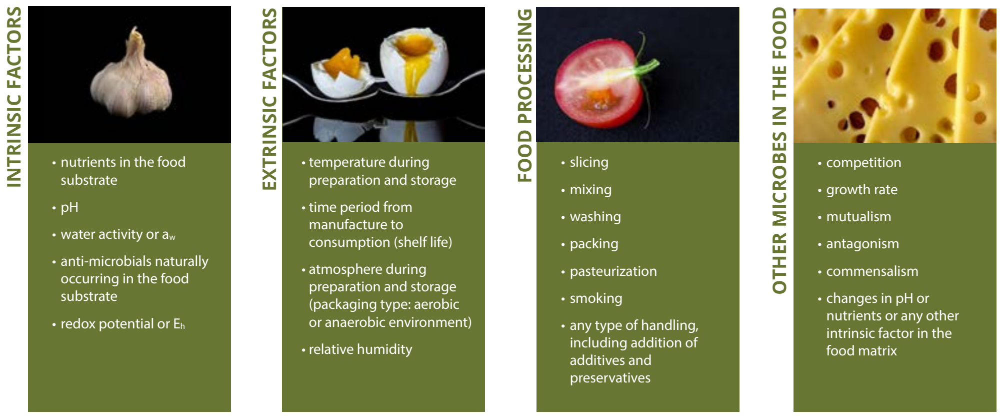



**Figure 4** — Summary of the four factors affecting microbial growth in foods

## Intrinsic factors

Factors intrinsic refer to aspects of the composition of the food itself. They include:

- Nutrients are available to microbes, including sugars, carbohydrates, proteins, and others
- Acidity/alkalinity or pH of the food
- The amount of water available in the food for microbes (water activity or $a_w$)
- Whether the food contains natural **antimicrobial** chemicals or features that limit growth
- How much natural oxygen is in the food (redox potential)

## Extrinsic factors

Factors extrinsic to the food are essentially the environmental surroundings of the food. They include:

- The temperature of the food during preparation and storage. Most microbes prefer warm conditions. If temperature is not managed and controlled, microbes can grow.
- The time available for microbes present in food to grow, from the time of manufacture to the time of consumption. This includes shelf life considerations for the food as well.
- The atmosphere for the food during preparation and storage. This is a very important concept—food packaging that changes the normal atmospheric conditions of oxygen, nitrogen, and carbon dioxide is called "reduced oxygen packaging" (covered in detail in Lesson F). As we learned in Lesson A, some bacteria survive best in normal aerobic conditions, while others, such as *C. botulinum*, prefer an anaerobic environment.
- Relative humidity. This is the level of moisture around the food—is it dry or wet?

### Processing factors

Food processing is often overlooked when foods are evaluated, but it is essential to understanding risk. Activities during processing can increase risk. For example, the act of slicing a tomato increases the risk of *Salmonella* growth. When a fruit is cut, such as a tomato or a melon, the outer skin and protective covering of the fruit is damaged. If the interior flesh of the fruit can support bacterial growth, when its outer skin (tomato) or rind (melon) is damaged, there is a risk that bacteria can enter the fruit and grow. This may occur if the skin is soiled (containing dirt and bacteria), or if the knife or utensil used to cut into the fruit is soiled. Processing can also decrease risk, such as when raw milk is pasteurized, which kills microbes during the heating step.

### Microbe factors

Foods—especially fresh foods—rarely have only one microbe present. A good example of this is fresh cabbage, which has likely hundreds of microbe varieties attached to the outer surface of the leaves, stalk, and roots. When fresh cabbage is submersed in a salty fluid and fermented, fast-growing populations of lactic acid bacteria change the environment to a more acidic, friendly one in which they can grow rapidly.

Next, we'll look in more detail at each of these four important factors that affect microbial growth.

## Intrinsic factors: Nutrients, pH, $a_w$, antimicrobials, and redox potential

Intrinsic factors refer to things that are naturally occurring in the food. These include: the available nutrients in the food; the natural acidity in the food; naturally occurring chemical compounds that may give the food natural resistance to other microbes, such as spoilage organisms; water availability for reactions; and the energy potential of the food.

### Available nutrients

The most important condition for microbial growth is a supply of suitable nutrients that can be used as metabolic fuel for growth and reproduction. All living cells need sources of carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorous, and other elements. If the supply of one of more nutrients is lacking in the composition of the food, microbes will have no fuel to maintain growth and reproduction. Bacteria can be specialized in terms of the types of nutrients they can consume. For example, different types of bacteria will consume different types of sugars, such as sucrose, lactose, glucose, and sorbitol.

### Acidity/alkalinity or pH of foods

The acidity or alkalinity of a food is given by its pH. **pH** is a measurement the number of hydrogen ions (H+) in water-soluble substances (pH stands for "potential of hydrogen"). Greater numbers of hydrogen ions makes foods more acidic. pH values are by numbers on a scale from 1 to 14. Substances pH 7, the middle value, are neither acidic or alkaline (basic), and are said to have a neutral pH. Substances with pH values below 7 are acidic. Acidity increases as the number decreases, with pH 1 being the most acidic. Substances with pH values above 7 are alkaline. Alkalinity increases as the number increases, with pH 14 being the most alkaline.

Some foods are acidic. For example, lemons contain natural citric acid and have a pH 3 to 4. Some foods are only slightly acidic or neutral, such as cabbage, which has a pH of 6.5 to 7.0. The pH of a food can be measured with paper strips called pH strips or indicator paper, which containing chemicals that change colour based on the amount of hydrogen ions in the food. Figure 5 shows the relative acidity/alkalinity of some common foods and substances, using the appropriate colours of indicator paper.

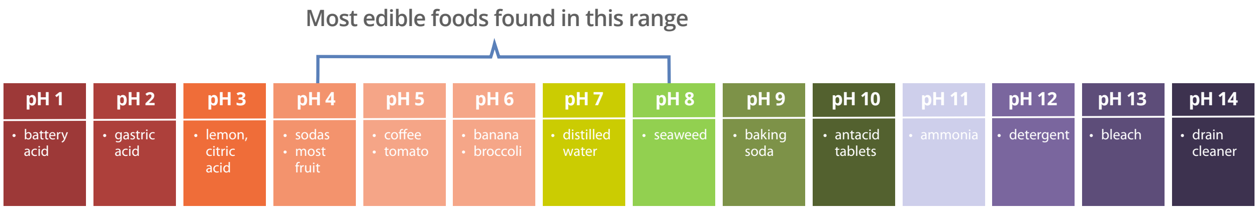



**Figure 5** — pH range of foods and other substances

Food microbiologists and food-safety specialists consider most foods with pH above 4.6 to be potentially hazardous. Most foodborne pathogens thrive in slightly acidic conditions, from pH above 4.6 to a slightly alkaline pH of 7.5. Most foods we eat also have a pH in this range. Many spoilage organisms, such as yeasts and moulds, tolerate pH values lower than 4.6 and are often the first microbes to start spoiling foods such as strawberries and tomatoes. Because lemons contain citric acid, they take a longer time to spoil; but will eventually also grow mould and deteriorate. Foodborne pathogens can survive, even if they don't grow, at higher and lower pH values as well.

A list of different foods and their pH of foods can be found at the following link.

**Approximate pH of Foods and Food Products**
http://ucfoodsafety.ucdavis.edu/files/266402.pdf

## Water activity ($a_w$ or $A_w$)

Because nutrients for microbes are predominantly in a soluble form, the availability of water is another factor that affects microbial growth. Enzymes (those specialized proteins that serve as tools to cells) use water to break down carbohydrates and other sources of food through a process called hydrolysis. Water is an essential ingredient for these activities. The availability of water in a food is referred to as **water activity ($a_w$)**. You might also see water activity given as $A_w$. While it is generally true that moist foods have greater water activity than dry foods, there are some exceptions.

Some foods are preserved by lowering their water activity. The use of salt to preserve foods does two things: it draws water away from cells, making it unavailable to microbes, and it binds excess water molecules, lowering the available water in the food substrate. These actions lower $a_w$ and limit the growth of all microbes, for example, pathogenic and spoilage-types of yeasts, moulds and bacteria. Sugar solutions can also limit the availability of water. Meat jerky is an example of a food that is preserved by lowering its water activity. The water activity of this food product usually ranges from 0.7 to 0.8, well below the level most bacteria can survive, but not below the level spoilage moulds can grow. Meat jerky is preserved through a combination of drying and addition of chemical preservatives (e.g., nitrite) to give this product a long shelf life. Drying is one of the oldest methods of food preservation. Lacking available water, meat jerky is an inhospitable substrate for bacteria. Bacteria generally require more water (i.e. have higher $a_w$ requirements) than some fungi, such as the spoilage moulds that grow on the outside of dried meats.

The following terms are used to describe microbes according to the water activity environments they can grow in:

- Halotolerant microbes grow in high concentrations of salt
- Osmotolerant microbes grow in high concentrations of organics (e.g., sugars)
- Xerotolerant microbes grow on dry foods

Figure 6 shows the water activity ($a_w$) levels of various foods and microbes that can grow at those levels.

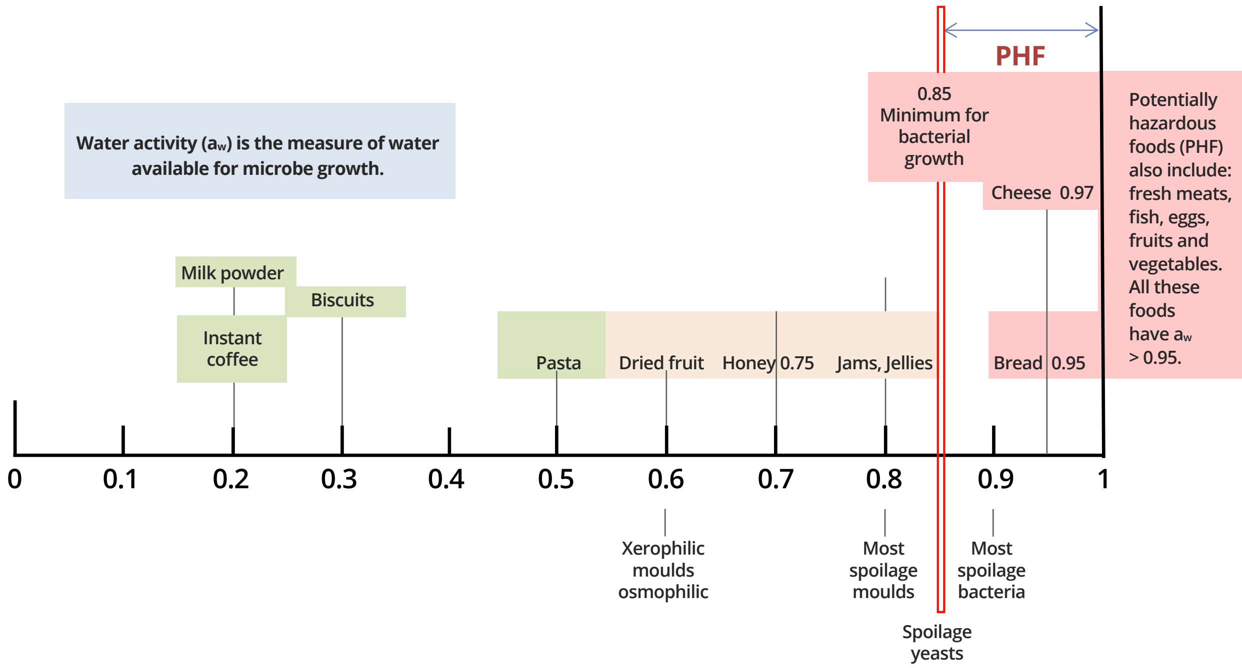



**Figure 6** — Water activity ($a_w$) values of various foods, and associated microbes

Water activity is not the same as percent moisture of a food. Although water activity can be expressed mathematically, this will not be covered in this course. Food with the same water activity can vary wildly in percent moisture. For example, each of the following foods may have a water activity of approximately 0.7, but very different percent moistures (Adams & Moss, 2000):

- Oil-rich nuts have a percent moisture of 4 to 9 %
- Protein-rich legumes have a percent moisture of 9 to 13 %
- Sucrose-rich dried fruits have a percent moisture of 18 to 25 %

One definition of a potentially hazardous food is that its water activity is above 0.85. Because their water activity is 0.7, the three foods above would not be considered potentially hazardous—even though their percent moisture varies widely. The cut-off for most microbial action (including fungi) is a water activity of around 0.6.

Based on the parameters of pH and water activity ($a_w$), the defining characteristics for a potentially hazardous food is a pH above 4.6 and a water activity above 0.85 (Figure 7).

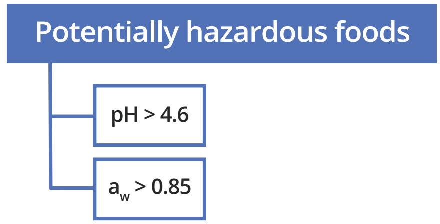



**Figure 7** — pH and water activity levels that make foods potentially hazardous

## Antimicrobials

Many foods have naturally occurring compounds that suppress the growth of microbes, called **antimicrobials**. Vegetables, fruits, and spices contain chemicals that prevent and suppress fungal and bacterial growth as a protective mechanism to plant injury. Plant injury can occur when insects or weather cause bruising or cutting of plant stems, roots, or leaves. Naturally occurring antimicrobials help to prevent infection by spoilage and pathogenic microbes. Many plants we use as spices contain antimicrobials, such as cloves(eugenol), oregano (carvacrol and thymol), thyme (thymol), garlic (allicin), and ginger (olioresin), along with many others (Liu et al., 2017). These compounds are used in the modern food industry during food production and food packaging to limit growth of undesirable microbes (Liu et al., 2017). Another example of naturally occurring antimicrobials may be found in eggs. Egg whites (albumen) contain several protein compounds, including the antimicrobial enzyme lysozyme (Clay, Lock, Dolman, Radford, & Board, 2003). This enzyme makes up 3.5 % of the albumen contents and protects the egg whites from bacterial growth by damaging the outer cell walls of bacteria (Clay et al., 2003). Other compounds in eggs raise the pH of the egg white to a more inhospitable alkaline pH that makes bacterial growth more difficult (Clay et al., 2003).

Although foods may contain intrinsic antimicrobial properties, these are not enough to overcome all pathogenic bacteria, especially when foods are not properly handled and temperature-controlled. For example, in BC, lack of proper temperature control (temperature abuse) has caused both botulism and salmonellosis (from growth of *Clostridium botulinum* and *Salmonella* respectively). Garlic-in-oil stored at room temperature caused thirty-six people to become severely ill from botulism (St Louis et al., 1988). Cases of salmonellosis were linked to temperature abuse and improper cooking of eggs in bakery products (McIntyre, Jorgenson, & Ritson, 2017; Strauss et al., 2000).

## Redox potential

**Redox potential ($E_h$)** refers to the likelihood that chemical components of a food (for e.g., vitamins and proteins that are part of the food) will accept or lose (transfer) electrons, and is determined based on a chemical assessment of a food. Electron transfers take place during oxidation-reduction reactions (or redox reactions). Transfer of electrons drives many chemical activities in microbes, such as the actions of enzymes and various living functions known as metabolic activities. The redox potential of a food can to some degree inform what microbes can survive in a food and what microbial activity has already occurred in a food. To explain this term in more detail is beyond what we wish to teach in this course.

The key concepts associated with this intrinsic factor are:

- Microbial growth in a food will reduce its redox potential
- Increasing air (oxygen) will increase the redox potential
- Increasing acidity will increase the redox potential

Of importance to the food industry is the use of redox dyes that measure microbial growth and activity in foods. For example, methylene blue is used in the brewing industry to tell whether yeast cells are alive and active (their cytoplasm does not stain blue) or inactive and dead (they do stain blue), as shown in Figure 8.

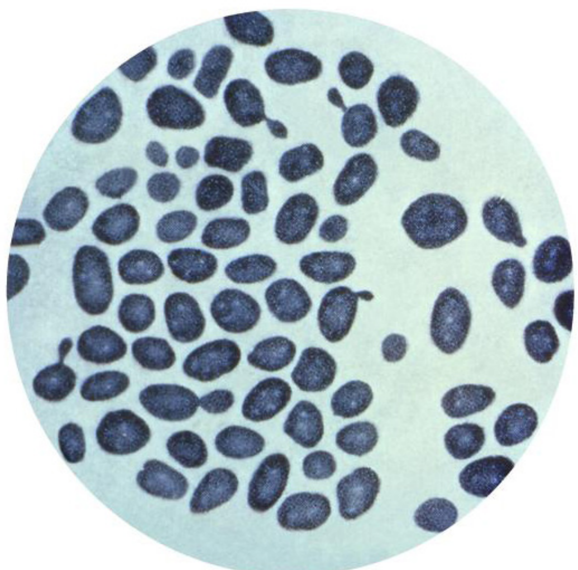


**Figure 8 —** Yeast (*Saccharomyces* sp.) stained with methylene blue dye

## Extrinsic factors: Temperature, time, atmosphere and relative humidity

Extrinsic factors refer to conditions present outside of the food, similar to the weather when we wake up: is it wet and muggy or dry and hot? For example, the potatoes you dug out of your garden will keep better if they are kept dry and in a cool basement, rather than stored wet in a hot room.

### Temperature

Temperature affects the rate of microbial growth. In this section, we will concentrate on the effect of temperature on bacterial growth. All bacteria have an optimum, maximum, and minimum temperature for growth. Temperature variations of only a few degrees may favour the growth of a completely different species of bacteria, so not only can a change in temperature alter the rate of growth of bacterial colonies, it can impact which species thrive. The **danger zone** is a term used to describe the temperatures between 4°C and 60°C (Figure 9). This is the optimal temperature range in which most food pathogens and spoilage bacteria grow.

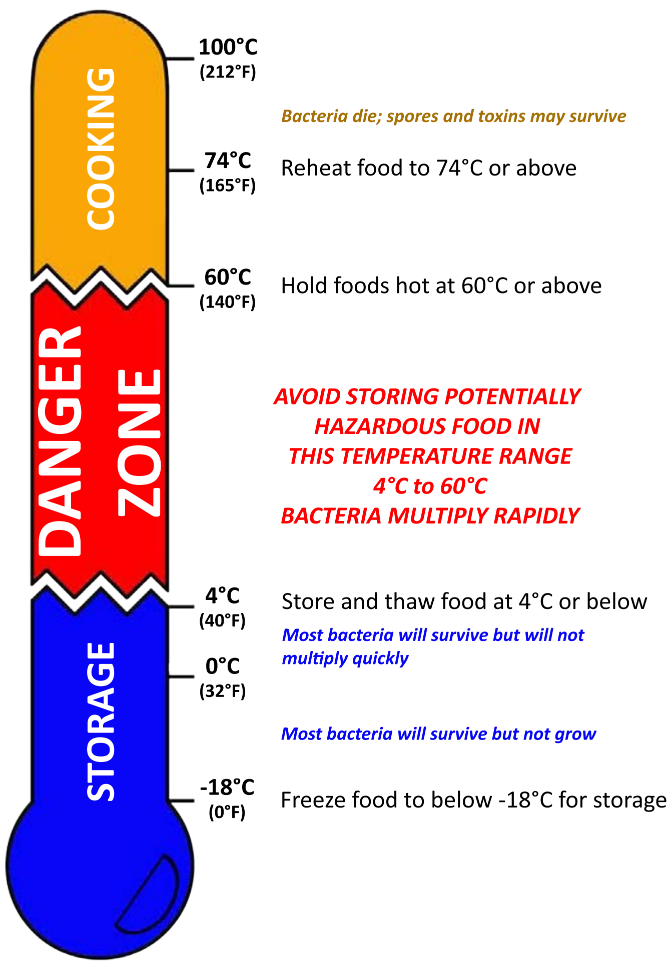



**Figure 9** — Growth of bacteria in the danger zone

Bacteria and other microbes can be classified into four groups, depending on the temperature zone in which they grow.

- Psychrophiles grow in cold temperatures between –20°C and 20°C, preferring temperatures in the range of 0°C to 10°C. These microbes will grow in refrigerators—and even on frozen foods!
- Mesophiles grow in moderate temperature between 10°C and 50°C, preferring temperatures between 20°C and 40°C. Most human pathogens are mesophiles and prefer body temperatures, which range from 35°C to 37°C.
- Thermophiles grow in hot temperatures between 20°C up to 120°C! Thermophiles prefer temperatures between 40°C and 80°C.
- Hyperthermophiles (a subset of the thermophiles group) have an optimum temperature greater than 75°C. This class of microorganisms tolerates the highest temperatures of any known organisms (some living at temperatures greater than 100°C and up to 120°C).

While freezing will not kill most bacteria, there is a defined **thermal death point (TDP)** above which bacteria can no longer survive. Food scientists use this value to calculate how much heat is required to cook a food to a safe end-point. For example, *E. coli* is a mesophile. Found in the human gut, these bacteria prefer a body temperature of 37 °C. While *E. coli* will not grow or multiply at refrigeration temperatures of 4°C or lower, they will not die. Instead, these bacteria will lie dormant until conditions become more favourable for growth. *E. coli* survives well on meat surfaces. Once meat is taken out of the freezer or refrigerator, the *E. coli* bacteria have an opportunity to begin growing. Cooking the meat to the thermal death point of the *E. coli* bacteria will ensure these bacteria are destroyed. We will learn more about microbial reduction later in this lesson when we talk about **thermalization** of microbes. Thermalization describes the heating process and population reduction (or logarithmic reduction) of microbes, and in layman terms, may be thought of as the cook step.

## Time

When considering time as a factor in food microbiology, consider the importance of time with respect to food hazards, food processes, and food shelf life.

1. For food hazards, time is most often linked to how long it takes bacteria to grow and double in numbers. Time is also needed for chemical hazards to develop, such as putrescine formation during decomposition (spoilage).
2. Food processes are often described in terms of temperature and time. Recipes and food-safety plans need to specify the temperature and the time—how long—the food is held at that temperature.
3. Food shelf life is described as a best-before date. This is the time given by a manufacturer for when the food will retain its best quality attributes (taste, texture, and smell).

Food hazards and food processes may be considered in seconds, minutes, hours and months, while shelf life is generally considered in hours, months, and in the case of canned foods, years. Time must be considered within the context of all of above examples, and many others.

## Atmospheric conditions

While many animals, plants, and bacteria need oxygen, it can be toxic and fatal to some species. Life on Earth had to adapt to the levels of oxygen in the air. The air we breathe is composed of 21 % oxygen, 78 % nitrogen, and 0.3 % carbon dioxide and other gases.

Different bacteria have a different tolerance for the presence or absence of oxygen, and are adapted to thrive in many different oxygen conditions. The following video explains the oxygen preferences (requirements) of various bacteria.

**Bacteria Oxygen Preferences**
https://www.youtube.com/watch?v=EjiAYCDMl7s

### Did you catch the error in this video?

In this video the professor provides examples of bacteria for each of the oxygen preference tubes:

- *Mycobacterium tuberculosis* (obligate aerobe)
- *E. coli* (facultative anaerobe)
- *Clostridium botulinum* (obligate anaerobe)
- *Lactobacillus* (aerotolerant bacteria)
- *Campylobacter jejuni* (microaerophilic bacteria)

The professor states that *C. botulinum* bacteria will grow inside the human gut after the canned food is ingested. This is incorrect. *C. botulinum* bacterial spores present in food, when they are not destroyed through a proper canning process, will produce toxin in the food.

Figure 10 illustrates the atmospheric conditions required by the following five types of bacteria:

Tube 1: Obligate aerobes require oxygen to live; they are growing at the top of the tube of nutrient broth.

Tube 2: Facultative anaerobes are bacteria that grow with or without oxygen but grow best in oxygen, with most growth near the top of the tube. These bacteria are growing throughout the tube of nutrient broth.

Tube 3: Obligate anaerobes do not grow in the presence of oxygen, shown growing at the bottom of the tube.

Tube 4: Aerotolerant bacteria are not affected by oxygen levels and can grow equally well in aerobic or anaerobic conditions (and anything in between).

Tube 5: Microaerophilic bacteria grow best in low concentrations of oxygen, just below the surface of the nutrient broth in the tube.

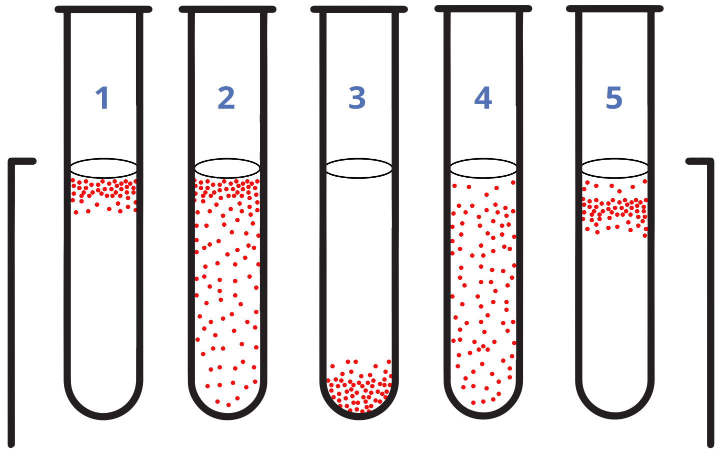



**Figure 10**—Growth of different types of bacteria in tubes of liquid nutrient broth solution

In Lesson F, we will look at different types of packaging that use reduced oxygen atmospheric conditions to limit the growth of spoilage bacteria. For example, dried jerky mentioned earlier is vacuum packaged to prevent the growth of aerobic spoilage moulds.

An excellent summary that combines microbial limits for pH and $a_w$ (intrinsic factors) with temperature and oxygen requirements (extrinsic factors) can be found in this Food and Drug Administration document.

**Appendix 3: Bacterial Growth and Inactivation**
https://www.fda.gov/downloads/Food/GuidanceRegulation/FSMA/UCM517405.pdf

## Relative humidity

Relative humidity is the amount of moisture in air or packaging. It is affected by temperature and pressure. For the food microbiologist, relative humidity is important as it can affect the water activity, $a_w$, of foods. A high relative humidity will allow water to be absorbed by food, while a low relative humidity may cause the food to lose water and shrivel (desiccate). High humidity can also cause food spoilage, as the excess moisture on the surface of foods allows growth of moulds, other fungi and microbes. Low humidity can change the effectiveness of cooking processes—without enough moisture, higher temperatures and longer times may be required to adequately cook foods (dry heat versus wet heat).

Like redox potential and water activity, relative humidity can be expressed mathematically. These expressions will not be explained in this course.

## Food processing

Food processing is often overlooked as a factor when evaluating a food for safety, but it is extremely important. As an example, let's look at a single ingredient, beef. Ground beef and mechanically tenderized beef have an inherently higher risk of *E. coli* contamination than whole cuts of beef. Why? This is due to differences in the handling of this ingredient; that is, in food processing. The actions of cutting and grinding for ground beef or hamburger, or needle-tenderizing for whole cuts of meat may introduce contamination. These activities also increase the availability of oxygen for microbes (the redox potential), which can increase the ability of microbes to grow.

Types of food processing activities that can affect microbial growth include:

- Slicing
- Mixing
- Washing
- Packing
- Pasteurization
- Smoking
- Irradiation
- Any type of handling, including the addition of additives and preservatives

Food additives and preservatives are often used to preserve flavour or limit undesirable microbial growth. Salt is an example of an additive that both affects flavour and limits bacteria growth by reducing the availability of water and lowering the water activity. Another example is sulphites, which are added to wine as a preservative. Many sulphites arise naturally in wine and other foods during the fermentation process—the addition of sulphur in the form of sulphites further preserves the wine and stops undesirable growth. Preservatives can be added in limited amounts as aids to keep food from spoiling, but adding too much of some preservatives can be toxic. The Health Canada website lists fifteen categories of permitted food additives, including preservatives, food enzymes, sweeteners, and many others. Go to this Health Canada webpage for more information on allowable preservatives and the amounts permitted to be added to foods.

**Lists of Permitted Food Additives**
https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted.html

## Other microbes in food

Bacteria, yeasts, moulds, and all other microbes require energy to grow and survive. They get energy from nutrients and water found in foods and other substrates. Microbes do not live alone as single species—they live in a mixed community. For example, a tree has microbes living in its roots, leaves and other structures, and a human body has many different microbes living on its skin and lung tissue and in the digestive tract.

### Relationships among microbes

Microbes can compete for the same resources. If one microbe species is better than another at utilizing nutrients to grow and so grows faster, we describe this as a competition—the one microbe species wins over the other. The successful microbe species outgrows or outcompetes the other species for the food and energy. Competition for essential nutrients impacts the rate at which different microbial species grow and reproduce.

A rapid increase in one species may well be at the detriment of another species. Changes in the substrate or food environment can also change the growth rate of microbes. This might occur when one microbe consumes all the food, so there is nothing left for a competing microbe to eat, which dies off. Or, this might occur when one microbe secretes a chemical into the food, which then changes the environmental conditions, making it more acidic and beneficial for the growth of other microbe species as occurs in fermentation.

Figure 11 explains some terms used to describe the relationships among microbe communities, and gives some examples.

| Terms | Description | Examples |
|---|---|---|
| **Competition and antagonism** | One microbe community harms another | The growth of lactic acid bacteria (LAB), *Leuconostoc,* produces acids that inhibit the growth of *E. coli.*<br>*Penicillium,* the black bread mould, secretes chemicals that kill bacteria. |
| **Mutualism** | Both microbe communities benefit each other. | The growth of one strain of LAB, *Leuconostoc*, promotes the growth of another type of LAB, *Lactobacillus.* |
| **Commensalism** | One microbe community benefits from growth but doesn't affect the other community. | During fermentation of apples into vinegar, yeast will break down sugars into alcohol. *Acetobacter* bacteria benefit from this and break down the alcohol into acetic acid. This end-product is not harmful to yeasts. |

**Figure 11** — Relationships among microbe communities

Let's use fermentation of grape juice by yeast as an example of competition and antagonism. Yeasts grow rapidly in a liquid environment. Although yeasts can be held inactive in a dry and cool environment for many months, once water and sugar are added, the yeast cells will start actively growing. Without yeast present, freshly squeezed grape juice left out on a counter would likely spoil through the growth of other types of fungal moulds. When yeast is added to grape juice, however, the yeast will actively grow and rapidly digest the sugars in the juice to produce alcohol, changing the grape juice into wine. The production of the alcohol will inhibit many other types of bacterial and fungal microbes. The rapid growth of the yeast is *competition*, and the production of alcohol is *antagonism*. Not all bacteria are inhibited by alcohol, however. *Acetobacter* require alcohol as a food and metabolize this substrate into acetic acid. Acetic acid is not harmful to yeast, and so this is an example of commensalism.

It's important to remember that during the processes of breaking down food sources into usable nutrients and emitting waste, bacteria and microbes alter the substrate. This can be used to human advantage in the case of fermentation, but can also cause humans problems, as in the cases of food spoilage and infection. For the food microbiologist, what happens to the food substrate over the shelf life of the food is an important consideration—does the acidity of the food change over the food's lifetime?

## Factors affecting microbial growth in foods: Consider the tomato

For each tomato product that follows, review the factors that are important when considering the risk of microbial growth. The list of factors is as follows:

Intrinsic factors:

- Nutrients
- pH
- $a_w$
- Antimicrobials
- Redox potential

Extrinsic factors:

- Temperature
- Time
- Atmospheric conditions
- Relative humidity

### Whole tomato

| Intrinsic factors |
|---|
| **Nutrients:** Whole tomatoes have carbohydrates as sugars, water, and very little protein or fats. As there are no proteins (amine groups or nitrogen), growth of some types of microbes and pathogens will be limited. However, available water in the tomato will allow mould and spoilage microbe growth over time. |
|  **Figure 12** — Whole uncut tomato |
| **pH:** Tomatoes are acidic, ranging from a pH of 4 up to a pH of 5 (generally pH 4.3 to 4.9). Spoilage microbes and pathogens can grow in this pH range. |
| **Water activity ($a_w$):** Whole tomatoes are full of water, estimated at 95% water content. Very little of the water has bound sugars that would lower the overall water activity, and tomatoes have $a_w$ of 0.99. |
| **Antimicrobials:** Like many fresh fruits and vegetables, tomatoes do exhibit antimicrobial activity. Bioactive compounds, such as sugar glycosides, lycopene, provide anti-oxidant and anti-microbial properties. This means that tomatoes can inhibit the growth of microbes such as moulds, fungi, bacteria and virus to a degree. |
| **Redox potential:** The redox potential of fresh whole tomato is quite high, but as the fruit ages (respires) the redox potential will drop. |

| Extrinsic factors |
| --- |
| **Temperature:** Whole tomatoes can be held at room temperature, refrigerated, or frozen. The outer skin of whole tomatoes, as with other fruits and vegetables, serve as a barrier to microbes. |
| **Time:** Whole tomatoes will ripen after picking in one to two weeks or longer. In the refrigerator they can be stored after ripening for another one to two weeks, or longer, depending on the packaging. |
| **Atmospheric conditions:** Whole tomatoes continue to respire (give off ethylene gas) while they ripen. In the refrigerator, putting them into a plastic bag with air removed, i.e. into anaerobic condition, will delay spoilage from aerobic microbes, extending useable lifespan. In controlled atmosphere conditions 3% $O_2$, 5% $CO_2$, 92% $N_2$), green tomatoes can be stored for 6 to 10 weeks. |
| **Relative humidity:** Optimal relative humidity during storage for whole tomatoes is 85% to 95%. Lower humidity may result in wilting, while higher humidity will speed up aerobic mould growth. |
| **Food processing:** Workers need clean hands and a gentle touch during picking. Rough handling results in bruising and damage that can speed up mould damage. Whole field and greenhouse tomatoes are washed to remove dust and debris by using gentle brush rollers and chlorine at consumer-safe levels (below 200 ppm, e.g. 125 ppm). Air-blast dryers are used to dry before packing. Learn more at:<br><br>**Postharvest Cooling and Handling of Field- and Greenhouse-Grown Tomatoes**<br>https://resources.sdspecialtyproducers.org/wp-content/uploads/2023/10/Postharvest-Cooling-and-Handling-of-Field-and-Greenhouse-Grown-Tomatoes-_-NC-State-Extension-Publications.pdf |
| **Other microbial interactions:** Many types of moulds and fungus are associated with tomato spoilage: Botrytis, Rhizopus and Alternaria rots are common. Bacterial soft rot is caused by several genera (Pectobacterium, Pseudomonas, Xanthoomonas, Bacillus) of bacteria that break down tissue causing softening of tomatoes, particular on damaged surfaces with moisture/wet areas. |

### Sliced tomato

| Intrinsic factors |
| --- |
| **Nutrients:** Sliced tomatoes have the same properties as whole tomatoes: carbohydrates as sugars, water, and very little protein or fats. As there are no proteins (amine groups or nitrogen) growth of some types of microbes and pathogens will be limited. However, available water in the tomato will allow mould and spoilage microbe growth over time.<br><br>**Figure 13** — Sliced raw tomato<br><br>**pH:** Sliced tomatoes are acidic, ranging from a pH of 4 up to a pH of 5 (generally pH 4.3 to 4.9). Spoilage microbes and pathogens can grow in this pH range.<br><br>**Water activity ($a_w$):** Tomatoes are full of water, estimated at 95% water content, very little of the water has bound sugars that would lower the overall water activity, and tomatoes have $a_w$ of 0.99. Dehydration of tomatoes by further processing to transform into 'sun-dried tomatoes' for example, will lower water activity levels to 0.85 or less and prevent bacterial growth.<br><br>**Antimicrobials:** Like many fresh fruits and vegetables, tomatoes do exhibit antimicrobial activity. Bioactive compounds, such as sugar glycosides, lycopene, provide anti-oxidant and anti-microbial properties. This means that tomatoes can inhibit the growth of microbes such as moulds, fungi, bacteria and virus to a degree.<br><br>**Redox potential:** The redox potential of sliced tomatoes are high, additional surfaces exposed through slicing will slightly increase the redox potential (electron transfers to aerobic bacteria, for example, become higher). |

| Extrinsic factors |
|---|
| **Temperature:** Sliced tomatoes are now categorized as a potentially hazardous food and should be refrigerated to 4°C within two hours of slicing, or frozen for later use. |
| **Time:** Sliced tomatoes are now categorized as a potentially hazardous food and should be refrigerated to 4°C within two hours of slicing. |
| **Atmospheric conditions:** Sliced tomatoes should be protected (covered) to reduce oxygen available to aerobic spoilage microbes. |
| **Relative humidity:** Sliced tomatoes should be covered and stored under the same relative humidity as whole tomatoes, 85% or higher, to avoid becoming wilted. |
| **Food processing:** By slicing a tomato, the skin of the tomato is punctured and surface exposure is increased. These changes increase risk that pathogenic microbes may grow. The concern is that surface bacteria, particularly Salmonella, may be transferred from the drier skin of the fruit to the moist interior. Salmonella can grow in slightly acidic conditions (minimum pH of 4.2). While whole tomatoes can be stored at ambient temperatures, once a tomato sliced, it should be treated as a PHF and temperature controlled for safety (refrigerated). |
| **Other microbial interactions:** Spoilage fungi, moulds and bacteria described for whole tomatoes remain a concern. Pathogenic bacteria can also grow. Leuconostoc and Lactobacillus bacteria can soften and cause spoilage, too. However, if salt is added to sliced and diced tomatoes, these lactic acid bacteria are able to tolerate salt, and are normally found on the surface of the tomato. They will begin producing acids and reduce the pH, turning the tomatoes into fermented salsa. |

### Tomato on pizza

| Intrinsic factors |
| --- |
| **Nutrients:** Tomato sauce on pizza usually comes from canned tomato paste or crushed tomatoes. Many products have added sugar (glucose-fructose) and salt to improve flavour, and less water. No proteins are added and calories (energy) in the sauce or paste still come from carbohydrates in the form of sugar and fibre. Other nutrients will come from the other ingredients on the pizza, and fats and proteins will be available to microbes from oils, meats (pepperoni), and cheeses (pizza mozzarella and others).<br><br>**Figure 14** — Cooked tomato on pizza<br><br>**pH:** The pH of canned tomato sauce is generally the same as for whole tomatoes, ranging between a pH of 4 (or slightly lower if ascorbic acids or other preservatives are added) to a pH of 4.7 or slightly higher depending on the types of tomatoes used. The pH of pizza, once made, will be higher from other ingredients, from a pH of 5 to 6.<br><br>**Water activity ($a_w$):** The water activity of tomato pastes will be much lower than whole or sliced tomatoes. Instead of 0.99 the $a_w$ will be 0.93 to 0.97. This is high enough to permit growth of many spoilage and pathogenic microbes. Cooked pizza will have a water activity similar to breads at 0.93, toppings will vary and may increase overall $a_w$ to 0.95.<br><br>**Antimicrobials:** Bioactive compounds described in whole and sliced tomatoes will still be present after canning, and these antimicrobial compounds will limit growth of spoilers. Another term used for these compounds is "phytochemicals"; note that phyto means derived from a plant. Research has demonstrated that tomatoes are a source of antioxidants with cancer fighting potential.<br><br>**Redox potential:** The redox potential of canned pastes and sauces will be lower than for fresh tomatoes. Aerobic spoilage organisms can still grow on the surface once the can is opened given enough time. |

| Extrinsic factors |
|---|
| **Temperature:** Pizza is a nutrient-rich environment, and this food is considered potentially hazardous. After cooking, if pizza is left too long out of temperature control, spores can grow out and release toxins that may result in foodborne illness. Refrigerate leftover pizza to 4°C or lower within two hours. |
| **Time:** Pizza is a nutrient rich environment, and this food is considered potentially hazardous. After cooking, if pizza is left too long out of temperature control, spores can grow out and release toxins that may result in foodborne illness. Refrigerate leftover pizza to 4°C or lower within two hours. |
| **Atmospheric conditions:** Pizza dough is generally proofed in a humid environment under normal (aerobic) atmosphere conditions. Packaging for fresh and frozen uncooked pizza is usually an overwrap of plastic, but not intentionally vacuum packaged, as this food is meant to be cooked and served right away. Cooked and delivered pizza is transferred from the oven to a breathable cardboard box. |
| **Relative humidity:** Pizza ovens will have lower relative humidity than the preparation areas. Relative humidity is not important when cooking pizza at high temperatures. Relative humidity for other ingredients, for example salami, on the pizza can be important. During salami fermentation, the relative humidity must be higher than 75% for E. coli pathogens to be reduced. Drier conditions will allow a hard crust to form on the outer surface of the salami and during aging moisture loss from the inside is reduced, allowing harmful E. coli to grow. |
| **Food processing:** Canned tomato paste and sauce in a can or foil retort pouch will undergo a classic canning retort operation. This means heat, time and pressure in a commercial pressure cooker will provide for a 12D pathogen log reduction. Put another way, 99.9999999999% (there are 12 9's in that value) of all microbes including spores will be inactivated. Pizza is usually cooked at a very high heat (230°C +) for a short time. Food handlers will have a lot of hand food contact with the pizza before it is cooked. Spices or ingredients that may contain microbes, and microbes from food handlers will be inactivated during the cook step (or thermalization process). |
| **Other microbial interactions:** Yeast in the pizza dough will out-compete other microbes while the dough is proofing. In the photo fresh basil is added to the cooked pizza before serving. This ingredient can be a source of microbes and potentially cross-contaminate the pizza. Although spores are killed in the tomato paste or sauce, they may not be killed in the spices or other ingredients. Pizza is a nutrient-rich environment. Spores can still grow out and form toxins in the pizza if it is left too long out of temperature control. |

### Tomato soup

| Intrinsic factors |
| --- |
| **Nutrients:** Many products have added sugar (glucose-fructose) and salt to improve flavour, and less water. No proteins are added and calories (energy) in the sauce or paste still come from carbohydrates in the form of sugar and fibre. Soups may contain thickening agents, such as wheat dextrin and maltodextrin and added preservatives such as ascorbic and citric acid. <br><br><br>**Figure 15** — Canned tomato soup<br><br>**pH:** The pH of canned tomato soup is generally the same as for whole tomatoes, added ingredients (alkaline starches and acidic preservatives) will result in a pH of 4.5 to 4.7.<br><br>**Water activity ($a_w$):** Most tomato soups are condensed, water is removed, and the consumer is directed to add it back in. Some of the water is bound up by sugars and starches, moisture content is still high, and the $a_w$ is estimated to be approximately 0.97 to 0.98. This is high enough for most microbial growth.<br><br>**Antimicrobials:** Bioactive compounds described in whole and sliced tomatoes will still be present after canning, and these antimicrobial compounds will limit growth of spoilers. Another term used for these compounds is "phytochemicals"; note that phyto means derived from a plant. Research has demonstrated that tomatoes are a source of antioxidants with cancer fighting potential.<br><br>**Redox potential:** The redox potential of canned tomato soup will be lower than for fresh tomatoes. Aerobic spoilage organisms can still grow on the surface once the can is opened given enough time. |

| Extrinsic factors |
| --- |
| **Temperature:** Typically canned soups are held at room temperature and are stable. Once opened, canned soups are vulnerable to microbes in the environment. If they enter a partially consumed can, many microbes would be able to grow at ambient temperatures, but not refrigerated temperatures.<br><br>**Time:** Typically canned soups are marked with a best before date and are stable for several years after manufacture. Food banks will use canned foods for up to two years after the best before or use by date. Canned foods are shelf-stable for a very long time.<br><br>**Atmospheric conditions:** The canned soup is in an anaerobic oxygen-free environment. During the canning process (also called retort), altitude of where the canning occurs is important. More pressure is required at higher altitudes, because atmospheric pressure is lower at higher altitudes, temperatures will also be lower.<br><br>**Relative humidity:** The relative humidity in the environment is not important because the tomato soup is in a hermetically sealed container (can or pouch).<br><br>**Food processing:** Canned tomato soup in a can or foil retort pouch will undergo a classic canning retort operation. This means heat, time and pressure in a commercial pressure cooker will provide for a 12D pathogen log reduction. Put another way, 99.9999999999% (there are 12 9s in that value) of all microbes including spores will be inactivated. When the can is opened, any bacteria or other microbes introduced during handling or from added water or milk, will be removed by heating the soup through to a minimum of 74°C.<br><br>**Other microbial interactions:** There will be no microbial interactions in properly canned soup. However, if the canning process was not done properly, for example, if the seams on a can were not aligned, then it is possible that spoilage organisms can enter. This can occur during cooling of retorted soup cans. Organisms in the cooling water or microexchange of atmospheric air will allow intrusion of microbes. This can result in microbial growth, leading to gas formation in the can, or off-flavors and sourness, usually from lactic acid bacterial growth. Anaerobic conditions are a risk for C. botulism, but lack of protein sources and acidic conditions of tomatoes would likely inhibit growth of this harmful bacteria in canned  tomato soup. |

## Growth characteristics of bacteria

How fast a strain of bacteria can grow in the food environment will also factor into food risk. Some types of bacteria grow quickly, while others are grow slowly.

### Bacterial growth

Bacteria typically reproduce by a process known as binary fission: splitting in two. This is a type of asexual reproduction and produces two bacterial cells that are genetically identical. During binary fission, the cell's DNA molecule replicates and attaches to the cell membrane. The cell membrane then begins to grow between the two DNA molecules, and then a cell wall forms to give complete separation, resulting in two bacteria cells. The two cells become four and the four cells become eight and so on, the number of cells continuing to grow exponentially until the environment can no longer sustain them. Because there is a limited supply of food and habitable space, bacterial growth does not go on forever. Once all the food is gone, required resources are depleted, and the environment is full of waste products, life is no longer sustainable.

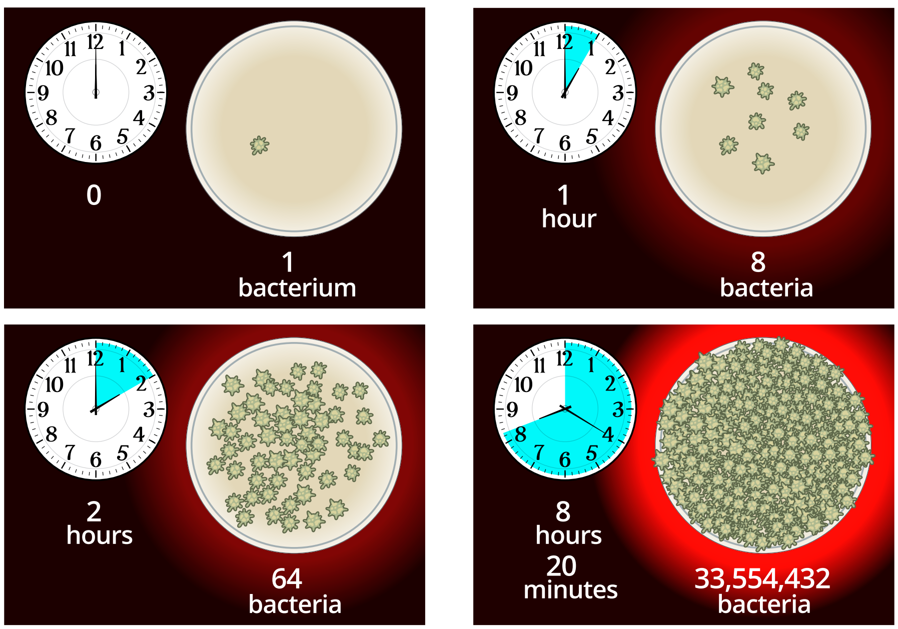



**Figure 16** — Bacterial growth in a nutrient agar plate over an 8+ hour period.

In Figure 16, note the number of bacteria growing at two hours and at eight hours. At two hours, we estimate sixty-four bacteria **colony forming units (CFU)**, with more than 33 million bacteria CFU at eight hours. Foods kept for more than two hours at temperatures above 10°C (i.e., in the temperature danger zone) have the potential to quickly become overgrown with bacteria. This is also why inspectors will discard potentially hazardous foods that have been in the danger zone for two or more hours.

Foods that are temperature abused in this manner will likely have many different types of bacteria and other microbes present, growing very rapidly. For *E. coli*, reproduction times can be as short as 20 minutes under ideal conditions and excess substrate. That means one single *E. coli* bacterium could duplicate into 472,236,648,300,000,000,000 identical bacteria cells in only twenty-four hours. If this number of bacteria were placed side-by-side, they would form a continuous line stretching from Earth to the Sun and back almost 8 000 times! The amount of *E. coli* O157:H7 needed to cause an illness, particularly in susceptible people, is as low as ten bacteria. *Salmonella* infection is believed to occur at 100 to 1000 bacteria. Lesson D, about foodborne illnesses, will describe infectivity in more detail. Keeping all bacterial numbers low in food by controlling temperature and other factors will keep the risk of infections low as well.

## Phases of bacterial growth

There are four distinct phases to growth of bacteria: lag phase, log phase, stationary phase, and death phase (Figure 17).

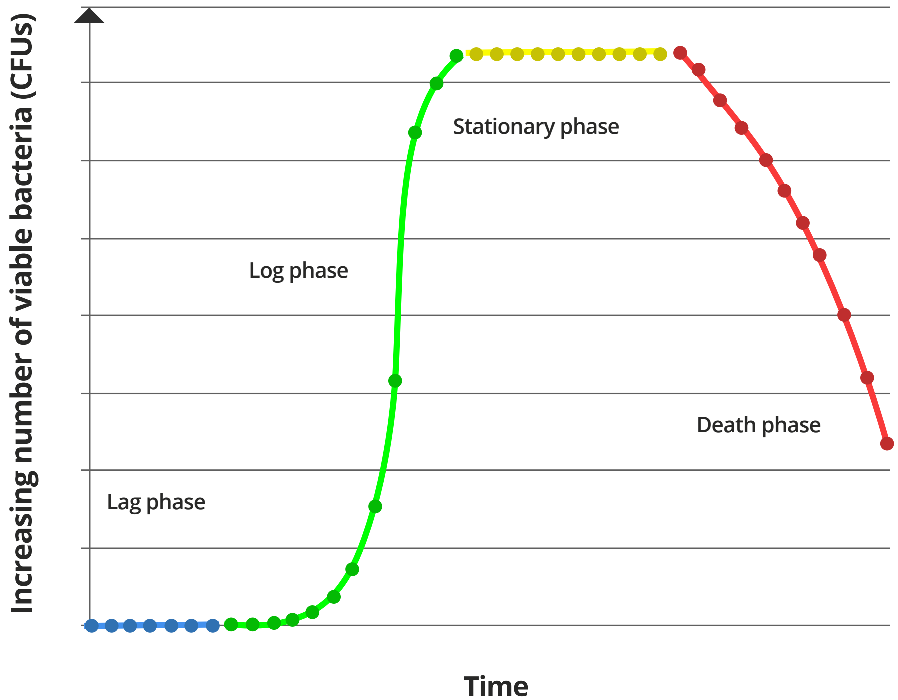



**Figure 17** — Growth phases of bacteria

The following video explains each of the four phases, as well as the process of binary fission.

**Bacterial Growth Curve and Binary Fission**
https://www.youtube.com/watch?v=ckAHRAC48nY ).

### Lag phase

The first phase of bacterial growth is called the lag phase. This phase occurs when bacteria first enter a nutrient-rich environment. During this phase, the growth rate is quite slow, as bacteria begin adapting to their new environment.

While in the lag phase, bacteria produce the enzymes they will need to break down the substrate or food source. Provided the environment is nutrient-rich and conditions are favourable, the lag phase tends to be quite short. However, the length of the lag phase is the most variable of the four phases and the one most susceptible to intervention. For example, the lag phase can be prolonged if the temperature isn't ideal for bacterial growth. Other environmental factors that can impact the length of the lag phase include pH, water activity, and competition with other microbial species for nutrients.

### Log phase

As bacteria begin to multiply more quickly, they enter the growth phase known as the log phase, in which the bacteria experience very fast growth characterized by doubling of the population after each generation. Doubling means, for example, that eight bacteria divide into sixteen bacteria, sixteen bacteria into thirty-two bacteria, and so on. Logarithmic values (log values) are used to count the rapidly increasing numbers of bacteria in the log phase. The mathematical expression for this is $\log_{10}$. Each time a $\log_{10}$ value increases by 1, the number of bacteria increases by a multiple of 10, as explained in Figure 18A. So, a $\log_{10}$ value of 1 means 10 bacteria, a $\log_{10}$ value of 2 means $10 \times 10 = 100$ bacteria, a $\log_{10}$ value of 3 means $10 \times 10 \times 10 = 1000$, and so on. The log phase may also be called the exponential growth phase.

| $\mathbf{Log_{10}}$ | **Bacterial counts (CFU)** |
|---|---|
| 0 | 1 |
| 1 | 10 |
| 2 | 100 |
| 3 | 1 000 |
| 4 | 10 000 |
| 5 | 100 000 |
| 6 | 1 000 000 |
| 7 | 10 000 000 |

**Figure 18 —** $\text{Log}_{10}$ values and corresponding bacterial counts

During the log phase, bacterial numbers increase dramatically. If it takes twenty minutes for the population to double, after twenty-four hours there will be hundreds of billions of bacteria. The length and rapidity of bacterial growth during the log phase depends on a variety of factors, including nutrient availability, the build-up of bacterial waste products, and temperature. In optimal conditions, most bacteria can double in only ten to thirty minutes. This time period for binary fission to complete is called the generation phase.

While bacteria may continue to grow under refrigeration, the rate of growth will generally be slower than at temperatures in the danger zone (between 4°C and 60°C). The log phase comes to an end when resources are exhausted.

### Stationary phase

As growth slows, the bacteria enter the stationary phase. During this period, the bacteria are still alive, but the environment will not support more growth and the rate of bacterial growth is the same as the rate of bacterial death. A steady state has been reached between the availability of nutrients and the increase in bacterial waste products. As food becomes scarce and waste products build up, the environment will become toxic for the remaining bacteria. It is during this phase that bacteria may produce chemicals such as antibiotics or toxins to inhibit other types of bacteria from growing.

Some types of bacteria may begin what is known as the endospore phase, during which they change their shape to form **spores** for longer term survival. This form of the bacteria is tougher and more resistant to heat and ultraviolet radiation. Figure 19 and Figure 20 show *Clostridium* bacteria spores. The swollen looking or club-shaped bacteria in Figure 19 (a very-high magnification 3D image) are *Clostridium* cells in the spore form and the long straight rods are cells in the normal (vegetative) form. In Figure 20 (a lower magnification 2D image of stained cells), the spores are those cells with unstained swollen ends and mid-regions.

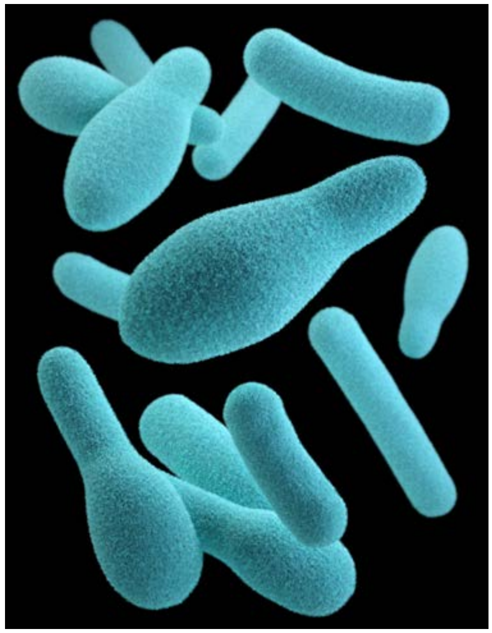


**Figure 19 —** Scanning electron micrograph showing *Clostridium* spores and normal cells

**Figure 20 —** Light micrograph of stained *Clostridium* bacteria spores and normal cells

### Death phase

Once nutrients are exhausted, bacteria will enter the death phase and begin to die off in large numbers. Some bacteria enter into a viable but non-culturable form. This is important to the food microbiologist, because it means that bacteria may not grow in the laboratory, but are still alive. It is unlikely that 100 % of the bacteria die during the death phase. The bacteria that do survive, however, are adaptable and able to begin growing again once more favourable conditions occur.

As mentioned, one way that bacteria can survive for long periods is to form a spore. Bacteria spores will remain viable but dormant until conditions improve, and so serve as a way for bacteria to ensure the survival of their species. For the food industry, spore-forming bacteria are of concern because bacterial spores can withstand common preservation and sanitation methods such as heating, freezing, chemicals, and other processes that are used to ensure our food is safe to eat. For example, consider cooked rice. Would you agree that once rice has been boiled, there should be no bacteria present? If you agreed with this statement, you would be overlooking that there might be spores of bacteria present in rice that are highly resistant to boiling. Rice, dried beans, and other grains are grown in or near soils and prone to contamination with spore-forming bacteria such as *Bacillus* and *Clostridium* spp. Boiling these foods will destroy the vegetative or normal forms of these bacteria, but not the spore forms. That is why it is important to keep cooked rice and other foods out of the temperature danger zone (4°C to 60°C).

## Cooking (thermalization) and log reduction of bacteria

Have you heard the expression "your burger's done at 71"? Recommendations are to cook ground beef (hamburger) to an internal temperature of 71°C (160°F). To adequately cook a turkey and most other food proteins, an internal temperature of at least 74°C (165°F) is recommended. Some recommendations are for even higher temperatures for turkey, especially when it is stuffed, such as temperatures of 82°C (180°F) or even higher.

In this section, we are going to address two basic but very important questions:

1. Why are there different recommended temperatures for different foods, such as beef and turkey?
2. How do food microbiologists determine the temperature needed to adequately cook a food?

Earlier, we learned that bacteria multiply by binary fission and that their numbers essentially double after every division. Food microbiologists use the term "thermalization" to describe the application of heat to a food and population reduction (or logarithmic reduction) of bacteria to mathematically estimate how many bacteria die off during thermalization processes. Thermalization is more commonly called the cook step. To calculate the rate of thermalization or the rate of bacterial die-off (the thermal death curve), we must first understand logarithmic (log) reduction. Recall from our discussion of the log growth phase that each time a $\log_{10}$ value increases by 1, the number of bacteria increases by a multiple of 10 over each unit of time (e.g., each hour). Log values can also be used to measure the decrease in the number of bacteria during thermalization. During log reduction, the number of bacteria decrease by a factor of 10 each time. That is, during log reduction, the number of bacteria decline or are divided by 10 over each unit of time.

Another way of using logs is to apply this to a percentage decrease in the log values. The percentage decrease is calculated using the following simple formula:

$$\text{percentage decrease} = (\text{decrease} / \text{original number}) \times 100$$

So, a single log reduction ($log_{10}$ value decreases by 1) means the bacterial numbers are reduced by 90 %, and a two log reduction ($log_{10}$ value decreases by 2) means the bacterial count is reduced by 99 %, and so on.

| $Log_{10}$ reduction | Percent decreases in bacterial counts (CFU) |
|---|---|
| 0 | |
| 1 | 90% |
| 2 | 99% |
| 3 | 99.9% |
| 4 | 99.99% |
| 5 | 99.999% |
| 6 | 99.9999% |
| 7 | 99.99999% |
| 8 | 99.999999% |
| 9 | 99.9999999% |
| 10 | 99.99999999% |
| 11 | 99.999999999% |
| 12 | 99.9999999999% |

**Figure 21** — Log10 reductions

This basic mathematical information is used to determine the amount of time food needs to be cooked. The D-value, or decimal reduction dose, is the amount of time needed at a specific temperature to obtain a single log reduction (or a 90 % reduction) in the population of a specific species and strain of bacteria. It's important to remember that the D-value represents:

- A specific length of time
- At a specific temperature
- For a specific bacteria

How do food scientists and microbiologists determine D-values? Using very sensitive temperature probes, they inoculate samples of turkey or beef with a population of bacteria and then cook the food at specific temperatures. The log reduction value is measured at each specific temperature, and then mathematical formulas are used to model how long it would take to achieve a specific log reduction.

The reasons why the recommended cooking temperatures for beef and turkey are different in part concerns a combination of the composition of the protein in these foods and the type of bacteria used in the calculation. *E. coli* is a bacteria of concern in beef, and was the bacteria used in laboratory experiments to test how long it took for log reductions of *E. coli* bacteria. *Salmonella* is the bacteria used for log reductions in turkey.  Both these bacteria have different D-values, and different minimum and maximum temperature requirements. The amount of fat in a food can also change the D-value, which is why food regulatory agencies provide a range of D-values for thermalization of different foods.

Information on where these values can be found at the following links.

**Hazard Analysis and Risk-Based Preventative Controls for Human Food: Draft Guidance for Industry**
https://www.fda.gov/downloads/Food/GuidanceRegulation/FSMA/UCM517405.pdf

**Annex D: Cooking Time/Temperature Tables (Canadian Food Inspection Agency)**
http://www.inspection.gc.ca/food/meat-and-poultry-products/manual-of-procedures/chapter-4/annex-d/eng/1370527526866/1370527574493

Most recommended cook times represent a 6.0 or 6.5 log reduction (for meat products) or 7 log reduction (for poultry). However, the canning process requires a much more stringent 12 log reduction or "12D bot cook" step, which allows canned foods to be shelf-stable for years. This process includes enough time, temperature, and pressure to destroy any bacterial spores present in foods, specifically those of *C. botulinum*—the temperature and time required is 121°C for 2.5 minutes. As shown in the table above, a 12D *C. bot* cook process is a 99.9999999999% reduction in bacteria.

The following can be used for further reading and review:

**12 D Concept and Bot Cook**
https://www.youtube.com/watch?v=TqG8xf550b8

**Optional:** For the mathematically inclined, follow these links:

**Determining D-value from Survivor Data**
https://www.youtube.com/watch?v=PM2S2dH8z_o

**Thermal Death Time and F value**
https://www.youtube.com/watch?v=r7GfEFnDMq4

## Techniques microbiologists use to measure numbers of bacteria

Because bacteria are too small to see, various techniques have been developed to enable us to measure bacterial growth. In this section, we will introduce how to grow (or culture) bacteria and how to count them. More detail on these techniques is provided in the Lesson B Supplemental Materials.

### Streaking techniques

"Streaking" is a term referring to how bacteria are applied to nutrient agar plates. There are many different reasons for streaking; for example, bacteria may be streaked out for isolation (i.e., to provide a single bacterial colony), for counting, or for testing for resistance to antibiotics or disinfection agents.

Basically, streaking involves putting a bacteria sample onto a previously sterilized loop (e.g., a flame-heated piece of wire), which is then run along the surface of a semi-solid nutrient agar plate. Wherever a bacteria cell are deposited, it will grow on the nutrient agar plate.

### Counting techniques

Several different laboratory methods are used to determine how many bacteria are in a food sample. Two of the most common are as follows:

1. Visualize the number of bacteria as CFU on semi-solid agar plates to give an actual number
2. Examine for growth in nutrient broth tubes to give an estimated number

Figure 22 and Figure 23 show how bacteria appear on semi-solid agar and in nutrient broth. These two techniques are further explained in the supplementary material for Lesson B.

**Figure 22** — Counts on semi-solid nutrient agar following a serial dilution (left: 1X dilution; right: 10X dilution)

**Figure 23** — Growth of *E. coli* in letheen broth: positive reaction (left tube), negative reaction (right tube)

More information can be found in Appendix A: Techniques for Counting and Characterizing Bacteria

## Important concepts for microbial growth

Measuring the growth of bacteria involves more than counting. There are different types of tests to detect bacteria, including different performance ratings and abilities. This is similar to how gasoline comes in different formulations—more expensive gasoline may improve engine performance, but all types of gas still allow an engine to run. Two important ratings for a microbial test is its **sensitivity** and its **specificity**. The sensitivity of a test describes how many bacteria it can detect, and the specificity of a test describes how often it correctly identifies the target bacteria. These two ratings determine how credible a test result is, based on the number of false-negative results (sensitivity) and false-positive results (specificity) it gives.

For more details regarding sensitivity and specificity, read the article and watch the video at the following links.

**Sensitivity and Specificity**
https://en.wikipedia.org/wiki/Sensitivity_and_specificity

**Sensitivity and Specificity—Explained in Three Minutes**
https://www.youtube.com/watch?v=FnJ3L-63Cf8

## Detection limit of a test and sensitivity

In the supplemental material to this lesson, we learned how bacteria are counted in solid and liquid media and used probability statistics to estimate growth in the most probable number (MPN) method. However, it is important to realize that not finding any bacteria in a sample doesn't mean that there were in fact no bacteria present in the food. The result that is observed is only for the small portion of the food that was tested. The only way to be 100 % certain that a food did not have any bacteria in it would be to test all of the food, and that would not leave any food left to eat. It would be also be too expensive and wasteful of everyone's time and resources.

Food microbiologists prefer to report results as "detected" or "not detected," rather than saying a sample was positive or negative for bacteria. Whenever a result is reported as 'not detected," this is accompanied by a detection limit for the test. The detection limit for a given test tells you the threshold of detection (i.e., the lowest number of bacteria that can be detected) for that test. The higher the detection limit, the poorer the sensitivity of the test.

For example, suppose you submit your cheese to a private lab to test for *Listeria*. Test A has a detection limit of 3 CFU per gram, and Test B has a detection limit of 300 CFU per gram. If you receive a result of "not detected" for Test A, then *Listeria* might be present in your cheese, but it will be below a level of 3 CFU per gram. So, perhaps 1 CFU per gram was present, but it was not detected. However, if you receive a result of "not detected" for Test B, then *Listeria* might be present at 290 CFU per gram, but this test is not sensitive enough to detect it. So, Test B would likely not meet your regulatory requirements for sensitivity.

## Viable non-culturable

The term "viable non-culturable" is applied to bacteria that are alive, but cannot be cultured (grown in nutrient media in a laboratory environment). Some species of bacteria do not grow well under laboratory culture conditions, but once ingested in food or inhaled, are able to grow in their host. This is another reason why it is difficult to have 100 % certainty that a result of "non-detectable" result means that no bacteria are present.

## Indicators for food quality and food safety

Testing for pathogens (food safety), spoilage organisms (food quality), and toxins of concern (food safety and food quality) in food can be expensive. Therefore, **indicator tests** are often used for guidance on whether there is a risk that foods have these microbes or toxins present or have the potential to support the growth of these microbes. **Indicators** used for testing are often microbes themselves. For example, it is much faster and less costly to check whether a processed food has any fecal coliforms (*E. coli*) than to check specifically for only vertoxigenic *E. coli* O157:H7. The presence of fecal coliforms in a sample indicates the potential presence of sewage or that unsanitary handling occurred.

There are several items to consider when choosing an indicator to measure the quality and safety of a specific food. Ideally, indicators can be quantified and easily detected. The indicator should be present in greater numbers and persist longer than the microbe of interest, and its presence should only be evident if there is an issue with the process or if spoilage has occurred.

Indicators may be used to assess the following:

- The hygiene of an ingredient
- The standards of process control
- Whether human or fecal contamination has occurred
- The effectiveness of post-processing contamination
- Whether a product or process meets a regulatory requirement
- Whether potential pathogens survived after thermal treatment
- The time before spoilage, or the shelf life of a product

For example, if you are adding almonds to ice-cream, as the manufacturer of the ice-cream you want to ensure the nuts are free of *Salmonella,* since this bacteria has been linked to salmonellosis in people consuming nut ice-creams. An indicator test for *Enterobacteriaceae* (Gram-negative bacilli) would provide information about the hygiene of the nuts. Process controls can also be assessed for hygiene standards using indicators. A process control is any practice or procedure that controls the operation during the food manufacture, such as when to clean equipment. Indicator bacteria tests may be used to find the optimal time to clean equipment in a processing plant that operates twenty-four hours a day. A coliform test for ground meat may be useful to assess the potential presence of *E. coli* and when a cleaning process needs to be initiated. The quality assurance team first decides how much bacteria represents a hygiene risk; for example, *E.coli* values exceeding 100 MPN/g. If the coliform threshold (i.e. any value >100 MPN/g) is reached after eight hours of production, then the grinding line needs to be disassembled and cleaned before going back into operation again.

Common microbial indicators include:

- Aerobic plate count (APC, also referred to as standard, total, or heterotrophic plate count, SPC, TPC, or HPC)
- Coliforms
- *Enterobacteriaceae*
- *E. coli*
- Yeasts
- Moulds

Not all indicators are microbial. Indicators may also test for chemical parameters, based on enzymes or energy chemicals. A phosphatase test is used to determine if complete pasteurization of raw milk has occurred. Phosphatase is an enzyme found in live cells. It is denatured (rendered inactive) when pasteurization temperatures are achieved. Testing for the enzyme is a fast and inexpensive way to verify that proper heating of raw milk (pasteurization) has occurred. Normally, pasteurization is verified by keeping a paper record of the temperatures in the vat pasteurizer (the equipment used to heat the milk). But what if the paper ran out? It would be costly to discard the day's production, and doing this simple enzymatic test would act as verification that pasteurization was achieved.

The effectiveness of cleaning can be assessed by a protein or sugar indicator test, or by measuring the amount of adenosine triphosphate (ATP) present. ATP is an energy-producing chemical present in many different types of microbes, and in the cells of all living things. These types of tests are rapid and often preferable to microbial indicator tests. But, ATP tests can only tell you if this energy chemical is present, not if the bacteria are alive or dead. For example, if you use a sanitizer on your food preparation area but the surface still has the bacteria on it, you might wonder if the bacteria is alive or dead. It is possible that the ATP test will show that bacteria is present, but the bacteria may, in fact, be dead. The only way to know would be to do a surface sponge swab-test for bacteria growth, which involves culture. It also doesn't tell you whether the positive signal is from a plant cell, a bacterial cell, or your own skin—it only shows that the ATP chemical is there. Each indicator test has its pros and cons, and you need to determine which test is right for what you need.

Quick and inexpensive protein and sugar residue tests are also available for the food industry. The tests work by detecting amino acids, peptides for protein residues or glucose and lactose for sugar residues. These types of tests are also available for specific allergens.

Example videos from two companies are shown on the following sites.

**3M™ Clean-Trace™ Surface Protein Plus Demo**
In this video a meat slicer is tested for protein residue.
A purple colour indicates protein contamination.
https://www.youtube.com/watch?v=qxOP6YTi6Rc

**Hygiena SpotCheck Plus Food Safety Residue Test (Sugars)**
In this product the slogan is "if it's green it **isn't** clean" , meaning that sugars are present.
https://www.youtube.com/watch?v=SM5CBEpXWA4&feature=c4-overview-vl&list=PL4ogy1NbREQN5p5RUaShg0p1XlC3eDmvF

## Activity: Testing for proper cleaning and sanitation

1. In a processing plant making candy, if you wanted to verify the cutting and packaging table was cleaned properly, what indicator tests would work? Select all that apply.
   a. ATP test
   b. Protein test
   c. Sugar test
   d. Total plate count test

2. In a processing plant making deli meat, if you wanted to verify the cutting and packaging table was cleaned properly before the next lot was processed on the same day, what indicator tests would work? Select all that apply.
   a. ATP test
   b. Protein test
   c. Sugar test
   d. Total plate count test

## Activity: Understanding laboratory reports

### Part A

Imagine this scenario: You would like to establish the safety and quality (shelf life) for a food you are planning to market in your local retail store, called "Clara's Chocolate Raspberry Bliss." The food product is a chocolate sauce that you've hot-filled into glass jars. You ask these two questions to the lab providing the testing service:

1. What tests do I need to determine if my chocolate sauce needs refrigeration?
2. What tests do I need to determine shelf life and quality?

The answer to the first question, does this food need refrigeration, is based on whether or not the food is a potentially hazardous food (PHF). These tests are also required by your health inspector before they will approve your food application. Look back at the lesson to determine the parameters that define a PHF.

1. What two tests from the following list do you need to ask the laboratory to run in order to determine if a food is considered a PHF?
    a. Total aerobic plate count
    b. Percent glucose
    c. Water activity, $a_w$
    d. *E. coli*
    e. Acidity of foods, pH
    f. Yeasts and moulds
    g. Total coliforms
    h. *Staphylococcus aureus*
    i. *Salmonella* spp.
    j. *Listeria* spp.
2. From the same list, what INDICATOR tests could you ask for to establish the shelf life?
3. From the same list, what PATHOGEN tests might you ask for to establish safety?

## Part B

After chatting with the laboratory service provider, you decide to get the following tests, pH, $a_w$, *E. coli* and *Staphylococcus aureus*. You submitted the products last week to a local laboratory and have just received an e-mail with the results in a report called "Certificate of Analysis."

Reading and interpreting reports of results you receive from a laboratory can sometimes be challenging. Different providers will have slightly different ways of displaying information in the report, but the elements of each should be similar. Result reports should provide you with the following pieces of information:

- The name of the laboratory that performed the analyses, including its address and contact information (phone number, fax number)
- A number for the report, which may be called the COA (certificate of analysis) number, the P.O. number or job number, and that will be quoted on the invoice
- The name of the submitter or company who/that requested the test (i.e., you, the person, or company they will invoice for the test)
- The date of the report and the date the sample(s) were submitted
- A description of each sample submitted, along with a sample number if one was provided
- A unique identifier and number for each sample submitted
- A description of the tests requested
- The result

- The units for the result
- The reference method for each test requested
- The limit of detection for the test
- The date the test was performed
- A signing authority of the microbiologist to verify the results

Questions:

1. Is your chocolate sauce PHF?
    a. Partially, because *E. coli* and *Staphylococcus aureus* grew in one batch
    b. No, because the *E. coli* and *Staphylococcus aureus* results were both not detected at < 10 CFU/g
    c. No, because the combination of pH and water activity will prevent microbial growth in this food
    d. Yes, because the combination of pH and water activity will not prevent microbial growth in this food
2. Does it need refrigeration?
    a. Yes, because the acidity is not low enough
    b. This food only needs to be refrigerated after opening
    c. No, because the food has a high sugar content and will crystallize
    d. No, because *Salmonella* cannot grow at water activity below 0.97
3. Were any *E. coli* found in the first batch that has the Best by / Date code of 2019JA15?
    a. Yes, 37 *E. coli* were found
    b. Yes, up to 9 *E. coli* are present
    c. Yes, up to 10 *E. coli* are present
    d. No, the < 10 means there were not any *E. coli* detected
4. What is different between the two batches, and what results should you be concerned about?
    a. The dates of production are different and the results show the process must have been incorrect in one batch.
    b. The second batch has detectable levels of E. coli and Staphylococcus aureus, along with higher pH and water activity.
    c. The date of testing for both batches was the same when the production lot date was different so the results cannot be fairly comparable.
    d. The first batch is different from the second batch and shows that by keeping a lower pH and water activity the shelf life can be extended further.
5. What can you do to reformulate this product to improve it?
    a. Add citric acid or lemon juice to bring down the pH.
    b. Use a boiling water method or other heating process to eliminate any bacteria.
    c. Repackage the sauce into vacuum packaging so spoilage bacteria cannot grow.
    d. Find a supplier of chocolate that tests their chocolate for the absence of pathogens (*Salmonella* and *E. coli*) and has a certificate of analysis (COA).

Check your answers with the answer key provided at the end of this textbook.

COMPANY A -
Awesome Testing
Awesome Testing Laboratories, ULC
Unit 7-200 Street West, Greentown, BC V1C 2L0
Phone: 250.851.7732 Fax: 250.821.7735

**CERTIFICATE OF ANALYSIS**

| | |
|---|---|
| **COA No:** | XRV-451 |
| **Supersedes:** | |
| **COA Date** | 10/29/19 |
| **Page 1 of 1** | |

**ORIGINAL TO:**
Ms. Maria Lewis
Title: Owner
Lewma Ltd.
673 Smith Street
Greentown, BC V1C 8X2

| | |
|---|---|
| **Received From:** | Jack Husband |
| **Received Date:** | 10/25/19 |

**Location of Test: (except where noted)**
Greentown, BC

## Analytical Results

**Desc. 1:** Clara's Chocolate Raspberry Bliss
**Desc 2:** **Best by/Date code:** 2020JA15

**Laboratory ID:** 377777721
**Condition Rec'd:** NORMAL
**Temp Rec'd (°C):** 5.8

| Analyte | Result | Units | Method Reference | Method | Test Date | Loc. |
|---|---|---|---|---|---|---|
| E. coli - Petrifilm | <10 | CFU/g | MFHPB-34 | 78X | 1/28/18 | |
| Staphylcoccus aureus | <10 | CFU/g | MFHPB 21 | 54C | 1/28/18 | |
| pH | 4.7 | N/A | MFHPB-03 | 10A | 1/28/18 | |
| Water activity | 0.93 | N/A | MFLP-66 | 12A | 1/28/18 | |

**Laboratory ID:** 377777721
**Condition Rec'd:** NORMAL

**Desc. 1:** Clara's Chocolate Raspberry Bliss
**Desc 2:** Best by/Date code: 2020JA30

**Temp Rec'd (°C):** 5.8

| Analyte | Result | Units | Method Reference | Method | Test Date | Loc. |
|---|---|---|---|---|---|---|
| E. coli - Petrifilm | 37 | CFU/g | MFHPB-34 | 78X | 1/28/18 | |
| Staphylcoccus aureus | 100 | CFU/g | MFHPB 21 | 54C | 1/28/18 | |
| pH | 5.3 | N/A | MFHPB-03 | 10A | 1/28/18 | |
| Water activity | 0.96 | N/A | MFLP-66 | 12A | 1/28/18 | |

Dag Lakewalker — **Laboratory Director**

**LOD**
MFHPB-34 <10 CFU/g; MFHPB21 <10 CFU/g; MFHPB-03 ± 0.05 pH units; MFLP-66 ± 0.003

You can find further information at the following links to testing methods in Canadian and American food governance:

**The Compendium of Analytical Methods** (Canadian source)
https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods.html

**Bacteriological Analytical Manual (BAM)** (American source)
https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

# Lesson B Quiz

1. Select the appropriate term associated with microbial growth based on the following conditions:
   - Microbes are present
   - Substrate is accessible
   - Nutrients are available
   - Level of humidity is high

   a. Indicator tests
   b. Potentially hazardous foods
   c. Processing factors

2. Select the appropriate term associated with microbial growth based on the following conditions:
   - Microbes are present
   - Level of humidity is high
   - pH is greater than 4.6
   - Temperatures are uncontrolled

   a. Indicator tests
   b. Potentially hazardous foods
   c. Processing factors

3. Select the appropriate term associated with microbial growth based on the following conditions:
   - Microbes are present
   - Substrate is accessible
   - ACC is <103

   a. Indicator tests
   b. Potentially hazardous foods
   c. Processing factors

4. Which factor that affects microbial growth is associated with the pH of a food?
   a. Intrinsic factors
   b. Extrinsic factors
   c. Food processing
   d. Other microbes in the food

5. Which factor that affects microbial growth is associated with relative humidity?
   a. Intrinsic factors
   b. Extrinsic factors
   c. Food processing
   d. Other microbes in the food

6. Which factor that affects microbial growth is associated with mixing?
   a. Intrinsic factors
   b. Extrinsic factors
   c. Food processing
   d. Other microbes in the food

7. Which factor that affects microbial growth is associated with mutualism?
    a. Intrinsic factors
    b. Extrinsic factors
    c. Food processing
    d. Other microbes in the food

8. Why is the level of acidity in food products an important concern for food producers and handlers?
    a. The high acidity level of fruits and vegetables accelerates food spoilage.
    b. The low acidity level of heavily salted foods stimulates microbial growth.
    c. The acidity levels that allow pathogen growth are found in most foods we eat.
    d. The acidity levels used to prevent bacterial growth in frozen foods must be carefully monitored.

9. What is the meaning of the term "redox"?
    a. An intrinsic factor of a foods ability to accept or transfer electrons
    b. A time period in which bacteria in a population of bacteria will double
    c. The surface or material on which bacteria will grow and get oxygen

10. What extrinsic factor for microbial growth does the term "danger zone" refer to?
    a. Temperature
    b. Atmosphere
    c. Relative humidity

11. Which extrinsic factor affecting microbial growth is described in the following statement?
    *Dried meat products are vacuum packaged.*
    a. Temperature
    b. Time
    c. Atmosphere
    d. Relative humidity

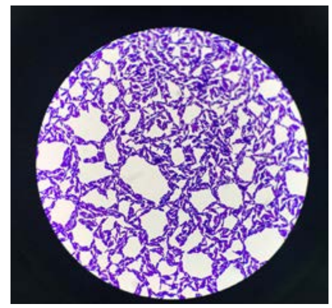
12. Which extrinsic factor affecting microbial growth is described in the following statement?
    *Yeast packages are labelled with use by dates*.
    a. Temperature
    b. Time
    c. Atmosphere
    d. Relative humidity

13. Which extrinsic factor affecting microbial growth is described in the following statement?
    *Milk must be pasteurized before distribution in BC*.
    a. Temperature
    b. Time
    c. Atmosphere
    d. Relative humidity

14. Which extrinsic factor affecting microbial growth is described in the following statement?
    *Condensation in the walk-in cooler caused mould issues.*
    a. Temperature
    b. Time
    c. Atmosphere
    d. Relative humidity

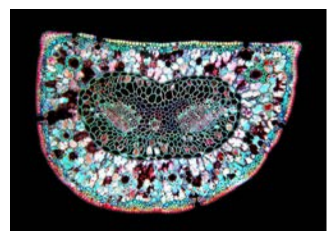
15. Which phase of bacterial growth is the period of rapid increase?
    a. The lag phase
    b. The log phase
    c. The stationary phase
    d. The lug phase

16. Which description applies to the class of bacteria known as psychrophiles?
    a. Will grow in refrigerators and on frozen foods
    b. Most human pathogens are of this class
    c. Tolerate the highest temperatures of any known organism



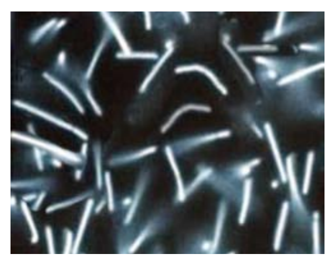
Psychrophiles

17. Which description applies to the class of bacteria known as mesophiles?
    a. Will grow in refrigerators and on frozen foods
    b. Most human pathogens are of this class
    c. Tolerate the highest temperatures of any known organism



Mesophiles

18. Which description applies to the class of bacteria known as hyperthermophiles?
    a. Will grow in refrigerators and on frozen foods
    b. Most human pathogens are of this class
    c. Tolerate the highest temperatures of any known organism



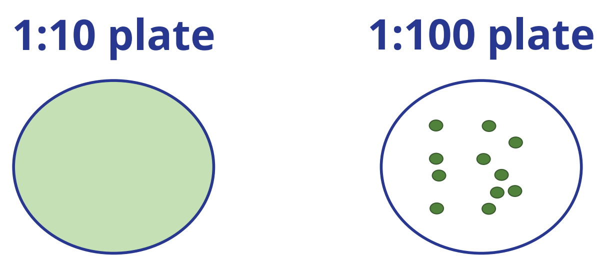
Hyperthermophiles

19. In terms of the relationship between pathogens and microbial indicators, which statement best describes indicators?
    a. Indicators show that pathogens may be present in a sample.
    b. Indicators prove that pathogens have entered the death phase.
    c. Indicators will combine with other bacteria to create pathogens.

20. Why is it important for a meat processor to know about bacterial log growth and bacterial log reduction?
    a. So all meat products can be cooked to eliminate all viable microbes
    b. So all meat process controls can be developed to ensure opportunities for pathogen growth are limited and microbial counts are reduced by temperature and time
    c. So all lots of meat products getting tested before and after processing have acceptable *E. coli* levels

21. You are a quality control technician for a food company. Your daily procedure consists of checking the bacterial growth in your juice sample. You're not allowed to have more than 1000 CFU (colony forming units) of bacteria in the juice sample. You always plate out a 1:10 and 1:100 dilution of the juice.

    The first plate has too many bacteria to count on it, and on the $2^{nd}$ plate you find 12 CFU.




    Did your juice pass the quality control check or not?

    a. Yes
    b. No

22. Your company makes packaged juice for the refrigerated retail market. There are several fresh fruit flavoring agents that have been causing spoilage issues earlier than the best before date on your label claim. Normally you thermalize (heat treat) the juice to get a 3 log reduction but you implement a new heating process to get a 5 log reduction.

    You take a sample of the juice before the thermal process and after the thermal process. Your QC technician report says the raw juice sample had 12,439,000 aerobic plate counts per mL (or $12.4 \times 10^6$ CFU/mL). What result is needed to achieve a 5 log reduction in your sample?

    a. Any number at or below 12,400
    b. Any number at or below 1240
    c. Any number at or below 2,487,800
    d. Any number at or below 124

Check your answers with the answer key provided at the end of this textbook.

# Lesson C: Fermentation and Spoilage

## Introduction

In this lesson, we will explore two processes that are by-productions of microbial growth, fermentation, and spoilage. While fermentation is desirable for many food businesses, spoilage is not. We will take a look at the effects of microbial growth on foods and how it impacts the food industry.

Now watch this video introduction. Note that foodborne illnesses will be covered in the Lesson D.

**Microbes in our Food**
https://www.youtube.com/watch?v=ur2tItApqBU

### Learning outcomes

Upon completion of this lesson, learners will:

- Describe types of food and beverage fermentations, including lactic acid bacteria (LAB) fermentations, yeast fermentations, mould fermentations, and mixed fermentations
- Define probiotic foods and describe their potential role in human and animal health
- Describe types of spoilage and how spoilage occurs in foods
- Identify microbes (bacteria, yeast, or moulds) that spoil foods and contributing factors that enhance spoilage
- Describe how spoilage microbes may lead to the development of toxins in foods

### Terminology

Key terms given in bold

bacterial succession
**backslopping**
**bacteriocin**
bacteriophage
**catabolism**
**fermentation**
glycolysis
generally recognized as safe (GRAS)
gut microbiome
homofermentive
heterofermentive
histamine poisoning
**lactic acid bacteria (LAB)**
lactic acid fermentation
metabolism
**mycotoxin**
oxidation
prebiotic
primary fermentation
**probiotic**
pyruvate
**rancidity**
**SCOBY**
secondary fermentation
spoilage
**starter culture**
**symbiosis**
**wild fermentation**

## Fermentation—an overview

**Fermentation** is a natural process of microbial metabolism in which microbes produce energy by converting sugars into simpler compounds (acids, gases, and/or alcohol), most often under anaerobic conditions. Fermentation microbes may be bacteria, yeast, or moulds. Figure 1 shows blue cheese, a type of mould-ripened cheese. The blue colour is the mould *Penicillium*, the microbe that gives the cheese is characteristic colour, taste, and odour.

**Figure 1** — Blue cheese

Fermentation begins with a cellular process called glycolysis, during which glucose is converted into a compound called pyruvate. The next stage of fermentation depends on the species of microbe. Some microbes convert pyruvate into lactate (e.g., lactic acid fermentation, the first step in making blue cheese). Other microbes convert pyruvate into alcohol and carbon dioxide (e.g., yeast fermentation).

Catabolism is metabolism that breaks down molecules to release energy. Through the catabolic process of fermentation, microbes break down sugars to release the energy they need as they break down sugars to form organic acids, peroxides, and **bacteriocins**, which have an inhibitory effect on the growth and survival of other microbial species. Fermentation is one of the earliest biotechnologies used for food preservation. Recent archaeological evidence suggests grapes were preserved as wine in Neolithic times, dating back to 6,000 BC (McGovern et al., 2017). The process of fermentation often creates an environment in which dangerous food pathogens cannot grow. Fermentation microbes that rapidly bring down pH – making the environment more acidic – prevent the growth of many harmful pathogens. The reduction in pH that occurs during fermentation preserves food and increases shelf life of foods such as pickles, sausage, and sauerkraut.

Fermentation is essential to our health and ability to digest foods. Bacteria in the large intestine that form our gut microbiome synthesize vitamin K and B-group vitamins, in addition to breaking down foods into smaller compounds (Rowland et al., 2018). This metabolic activity of bacteria in our guts can be considered a form of fermentation that takes place within our bodies. Without these bacteria, we would not be able to digest the foods we eat. Our relationship with these permanent gut bacteria is an example of mutualism, we derive benefit from the bacteria and they also have a residence. Beneficial bacteria that we ingest in our foods are called **probiotics** (the opposite of antibiotics). Our relationship with probiotic bacteria may be described as commensalism. Probiotic bacteria get nutrients from the food but not directly from us as they may or may not become gut residents. Evidence is growing that probiotics do confer human and animal health benefits, but whether they permanently change our microbiome after ingesting these bacteria will be debated later in this lesson.

As well as making foods we consume, industrial fermentation is used in the production of medicines. For example, the antibiotic penicillin is produced during a fermentation process involving various types of fungi, and the important glucose regulator, insulin, is produced for the pharmaceutical industry using bacteria (Advemeg Inc., 2018; General MicroScience, 2018).

In this lesson, we will review three broad groups of food fermentations: lactic acid fermentations, yeast fermentations, and mixed types of fermentations such as kombucha tea, which is made with a **SCOBY**. SCOBY stands for Symbiotic Culture of Bacteria and Yeast. **Symbiosis** is a close relationship between two different organisms. When symbiotic organisms work together for mutual benefit, the relationship is mutualistic. In the case of fermentations, many microbes are symbiotic.

## Lactic acid fermentations

**Lactic acid bacteria (LAB)** are the primary microbes used for the process of lactic acid fermentation, which is used in the production of a variety of products, including yogurt, cheeses, pickles, sauerkraut, kimchi, salami, kvass (a beverage made from rye bread) and many other foods.

Members of the LAB group are acidophilic, facultative anaerobes, and are largely Gram positive. Many different genus and species make up this group of bacteria; you might recognize the names of some LAB members from food advertisements, such as *Lactobacillus acidophilus* or *Lactobacillus delbrueckii* subsp. *bulgaricus* from yogurt ads, or *Lactobacillus saki* used to ferment rice into saki. Although these three examples are all members of the same family: *Lactobacillaceae*, many other families are also included in the LAB group, such as Leuconostoc spp. from the family *Leuconostocaceae* and *Streptococcus* spp. from the family *Streptococcaceae*.

> **Did you know?**
> Lactic acid fermentation occurs in human muscles during periods of intense exercise.
>
> Lactic acid is produced when there isn't enough oxygen available to meet energy needs through aerobic metabolism. Muscles then begin to produce energy through lactic acid fermentation, an anaerobic process.

You don't need to know all the family, genus, or species names listed above, but it is important to realize that the LAB group contains more than one type of bacteria. Why is this important? Carrying out a LAB fermentation successfully is much like a building a home successfully. To build a home, different contractors are employed, who all have skills to do different tasks, such as laying foundation, putting up drywall, or doing electrical or plumbing work. Each of these tasks must be done in sequential order: electrical work can't be done before walls are built, and the walls can't be built before the concrete foundation. Similarly, with LAB fermentations, there is a sequence of bacteria used during the fermentation. This sequence is referred to as bacterial succession.

Within the LAB group, bacteria can be either homofermentive or heterofermentive. The homofermentive LAB produce only lactic acid through the digestion of glucose, and include *Lactococcus*, *Lactobacillus*, *Pediococcus*, and others. The heterofermentive LAB produce lactic acid AND alcohols, carbon dioxide, esters, and alcohol. Heterofermentive LAB include *Leuconostoc*, *Weisella*, and others. These bacteria will often appear first during fermentations and quickly bring down the pH of a food matrix, suppressing the growth of some microbes that might be pathogens. Heterofermentive LAB are not as acid-tolerant as the homofermentive LAB, so as the fermentation progresses and the pH drops, bacteria in the homofermentive group will take over.

LAB produce several organic acids and compounds that inhibit microbial growth, including acetic and lactic acid, bacteriocins, hydrogen peroxide, ethanol, and diacetyl. Lactic and acetic acid production lowers pH to less than 4.0. Bacteriocins are small proteins that have bacteriocidal properties. One bacteriocin, nisin, has been commercialized as a food preservative that is particularly effective against Gram-positive spoilage- and spore-forming microbes, and is often used in the dairy and meat products (Gharsallaoui, Oulahal, Joly, & Degraeve, 2016). LAB also lower the redox potential in substrates such as yogurt, inhibiting growth and creating specific aroma profiles in fermented foods like yogurt (Martin et al., 2013). Figure 2 describes these factors and how they inhibit microbial growth.

| Factor | Action |
|---|---|
| Acetic acid and/or lactic acid | Lowers pH of the ferment<br>Inhibits growth of Gram-negative bacteria and some types of fungi |
| Bacteriocins | Inhibits growth of Gram-positive bacteria (e.g., *Listeria*) |
| Diacetyl | Antimicrobial to all types of bacteria and fungi |
| Ethanol | Destroys proteins and the outer layer of bacteria |
| Hydrogen peroxide | Antimicrobial chemical that LAB are less sensitive to than are non-LAB bacteria |
| Nutrient depletion | LAB microbes grow fast and out-compete other microbes for nutrients |
| Redox potential | Reduces available oxygen and affects aroma |

**Figure 2** — LAB-produced factors that inhibit microbial growth during fermentation

Lactic acid fermentations can proceed naturally in many food products, also known as "wild" fermentations. Sauerkraut and kimchi are very good examples of natural fermentations. The food substrate, cabbage, is chopped up and then sprinkled with salt. The salt inhibits growth of non-LAB species, promoting LAB growth. In sauerkraut manufacture, the percentage of salt added (2 to 2.5%) is a critical factor: too much or too little will change the bacterial succession in the sauerkraut (Fan, Hansen, Sharpe, Chen, & Zhang, 2015). Salt is sprinkled onto the shredded cabbage and then mixed/macerated in. During this process, liquid is generated. In a successful fermentation, the cabbage will be completely submersed below the liquid. Any cabbage left above the surface, and therefore not under anaerobic conditions, is vulnerable to spoilage from aerobic moulds and yeasts (Fan et al., 2015). Temperature is another control point in sauerkraut manufacture – if the temperature is too high, it will not favour LAB growth, and if the temperature is too low, it will slow LAB growth. Optimal temperatures for LAB ferments are generally between 15°C and 25°C.

## Starter culture

Manufacturers of commercially produced sauerkrauts may choose to purchase LAB, or they may use liquid from the previous sauerkraut batch to start the next batch, a process known as **backslopping**. These are both a form of **starter culture**, which is the live microbial culture needed to begin the fermentation process. For most large-scale manufacturers, purchasing a reliable, validated, and certified starter culture is the most reliable way to ensure the highest product quality and consistency. There are pros and cons to using wild, commercial, or backslopped starter cultures. **Wild fermentations** have no purchase costs, but they rely on having the microbes you want available to begin the fermentation being present. If they are not present at all or only in very small numbers, undesirable spoilage or growth of pathogenic microbes may occur at the beginning of the fermentation that can cause off odour, off flavour, and potential for illness. Commercial cultures are reliable and pure (i.e., no other microbes or contaminants are present), but may add costs or be unavailable from the supplier. Backslopped cultures can be wonderful starters because they are adapted to the food substrate, but they are also prone to cross-contamination and may become infected (bacteria can get infected with viruses called bacteriophages). If this occurs in a wild starter culture or during backslopping, it can cause problems in the ferment.

### Generally recognized as safe (GRAS)

This term is used by the U.S. Food and Drug Administration (FDA) to describe food additives that have been recognized among experts as safe under conditions of its intended use. It allows the substance to be used in foods without additional regulatory approval.

Visit the following link for more information. Note that not all FDA-approved GRAS substances are approved for use by Health Canada.

**Generally Recognized as Safe (GRAS)**
https://www.fda.gov/food/ingredientspackaginglabeling/gras/

## Sanitary conditions

LAB are generally recognized as safe (GRAS), and LAB fermentations are usually considered low risk as well. But, problems can occur when the incoming ingredients or the environment where the fermentation takes place is unsanitary. Several outbreaks of foodborne illnesses caused by kimchi occurred in Korea, through a combination of two factors: poor ingredients that likely were contaminated by pathogenic fecal coliform bacteria, and a fermentation that was rushed and so not given enough time to fully acidify (Shin et al., 2016).

## Yeast fermentations

Yeasts are famously used for rising breads, and are also used by food manufacturers to produce beer, cider, wine, and other beverages. Figure 3 shows the bubbling action of bread yeast, with rising bread dough shown in Figure 4. Sourdough breads are made with a symbiotic combination of yeast and *Lactobacillus*, giving these breads a slightly sour taste.

**Figure 3** — Bread-yeast culture

**Figure 4** — Yeast causing bread dough to rise

When used to make fermented beverages, yeasts change sugars into ethanol and carbon dioxide. For example, yeast is used to ferment sugars in grain to make beer and in grape juice to make wine. In this metabolic process, both ethanol and carbon dioxide ($CO_2$) are produced. Many cultures make fermented beverages with yeasts. For these fermentations to proceed, sugars must first be extracted from the substrate.

If the substrate is barley or wheat (for beer), then regular brewing yeast, *Saccharomyces cerevisiae*, will not be able to break down the starch (a carbohydrate) in the grains. Therefore the first step is to germinate and sprout the seeds (grains) into a mash to make a wort. More than one type of yeast will be present during a typical fermentation, sometimes referred to as bottom fermenters for lagers and top fermenters for ales.

Watch the video in the following link to learn more about the history of beer and the importance of microbes in the fermentation process.

**History of Beer—Seven Wonders of the Microbe World (1/7)**
https://www.youtube.com/watch?v=_ob1c-n_oNY

Other beverages are made by first using the enzyme amylase to break down the carbohydrates in the substrate. Once the sugars are available, yeasts and some other bacterial groups complete the fermentation. For example, to make the traditional South America alcoholic beverage called *chicha*, amylase is added to soaked corn before fermentation begins. Beware – the traditional source of amylase for this fermented beverage is saliva—tribe members spit into the soaking corn to break down the starch. Chicha produced in this traditional manner is a known risk factor for acquiring hepatitis.

## Mixed fermentations

Kombucha (shown in Figure 2) is a beverage is made of brewed tea, sugar, and live microbial culture. The live microbial culture or SCOBY (Symbiotic Culture Of Bacteria and Yeast) is acquired from a small portion of the previous culture, from the kombucha liquid, the floating mat of culture, or a mixture of both. Kombucha is reported to have health benefits, although none of these claims have undergone rigorous clinical trial testing. It is thought that the acidity and variety of live microorganisms in the culture confer beneficial effects to digestive health, as well as other health benefits. However, illnesses have also been linked to drinking some kombucha tea recipes. For example, acidosis, a condition where blood acidity becomes too high, was linked to illness (and a death) caused by consuming too much kombucha which presumably was over-fermented (BC Centre for Disease Control, 2020). Incidences of bottles of kombucha exploding from gas build-up have also occurred, caused by over-fermentation. Residual sugars left in the tea may continue to ferment after bottling, resulting in excess gas and alcohol formation. Unhygienic kombucha production has led to a variety of illnesses, including a wound infection caused by handling contaminated SCOBY (BC Centre for Disease Control, 2020).

SCOBY contains a mixture of yeast and various bacteria, including LAB and acetic acid bacteria. The LAB rapidly drops the pH of the kombucha substrate, making it more acidic. Ethanol and carbon dioxide is produced as the yeasts ferment sugars. Then, the ethanol that was produced is further broken down by acetic acid bacteria into vinegar, driving the pH even lower. Kombucha should not be permitted to reach a pH lower than 2.5. To prevent excessive acidity and gas formation, commercially bottled kombucha should be pasteurized to stop the fermentation. Alternatively, fermentation may be slowed through refrigeration or addition of preservatives, such as benzoate or sorbate, to the tea (BC Centre for Disease Control, 2020).

Figure 5 outlines the three types of fermentation occurring simultaneously in this symbiotic culture.

| | Alcoholic fermentation | Lactic acid fermentation | Acetic acid fermentation |
|---|---|---|---|
| Microbe | Yeast | LAB | *Acetobacter* |
| Substrate | Sugar | Sugar | Alcohol |
| End product | Alcohol + $CO_2$ | Lactic acid | Acetic acid |

**Figure 5** — Symbiotic fermentations that occur during kombucha production

It usually takes up to one week to complete the fermentation cycle in kombucha production. During this period, the vessel in which the tea is made should be loosely covered to allow for aerobic conditions. When the fermentation is complete, the kombucha is usually placed into a sealed carboy or container and refrigerated.

Some kombucha is flavoured after the fermentation is complete. When flavouring occurs, there are at least two potential problems that can occur, identified in the table for the following activity. You should be able to identify what type of fermentation contributed to each problem.

### Activity: Fermentation problems with kombucha tea

Describe the type of fermentation occurring that led to the following problems with kombucha tea.

| Problem | **Fermentation type**<br>Choose from (i) alcohol, (ii) lactic acid, or (iii) acetic acid |
|---|---|
| Dizziness and headache was reported in a young child after drinking kombucha, and led to an investigation. The parents felt fine. The owner said the kombucha was first fermented, then a sugar-based flavouring was added. The kombucha was kept at room temperature and sold during an event. | |
| Emergency room visit occurred where the patient was diagnosed with acidosis. Patient had drank kombucha that had been fermenting for two weeks. | |

Check your answers with the answer key provided at the end of this textbook.

There are many mixed fermentations involving yeast and LAB. Food products from fermentations by these combinations of microbes include kefir, salami, and lambic beer. Fungi other than yeasts are also regularly used to many types of fermented foods, including blue cheese (see Figure 1). Before the mould is added to blue cheese, a first stage of a primary fermentation with LAB occurs, followed by a secondary fermentation wherein the mould *Penicillium* is added to create the blue veins in the cheese. Other types of moulds (*Rhizopus*) work in combination with LAB to make tempe, a traditional dish of Indonesia. Soy sauce and miso, food staples in China and Japan, are made in combination with yeasts, LAB, and mould (*Aspergillus*).

## Probiotics

Fermented foods can be a dietary source of the live organisms known as probiotics. According to the World Health Organization, a probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host"(Morelli & Capurso, 2012). Probiotics can benefit the health of us (human hosts) but also the health of animals.

Prebiotics are the nutrients necessary for probiotic microbial growth, such as carbohydrates, vitamins, and proteins (the substrates). In this view of probiotics and health, it is not only what you eat that promotes health, but what your gut bacteria eat, which keeps your immune system and gut healthy (Cremon, Barbaro, Ventura, & Barbara, 2018). Prebiotics are often non-digestible to the human or animal host, but are good sources of food (substrates) to the microorganism. One example would be the sugar lactose, which is indigestible to a large segment of the human population. LAB contain lactase enzymes that help to break lactose down into the digestible sugar glucose. As described earlier in the LAB section, strains such as *Lactobacillus acidophilus* and *Lb. delbrueckii* subsp. bulgaricus are commonly used to manufacture yogurt.

Many fermented foods purchased at retail outlets generally have $10^4$ to $10^7$ LAB per gram (ten thousand to ten million), although cultured dairy products have much higher levels, up to $10^9$ LAB per gram (one billion; (Rezac, Kok, Heermann, & Hutkins, 2018). Fermented foods that contain prebiotics include vegetables, cheese and dairy products, soy and miso fermented products, and fermented cereals that contain glucans, oligosaccharides and polyphenolic compounds (Marco et al, 2021). Probiotic cultured dairy products include yogurts with live *Lactobacillus* and *Bifidobacterium* bacteria. These bacteria are considered exclusively beneficial, with no harmful effects.

## Probiotics in human and animal health

How do probiotic foods provide health benefits? Recall from Lesson A that a microbiome is the community of microbes in a particular environment. Our microbiome – the community of microbes in and on our bodies – can have profound health impacts. The science of microbiome interactions and their impacts on our functions, ranging from gut health to lung health, is a fairly recent science. Animal husbandry studies of microbiomes and optimization of probiotics is also a new field. Examples of research in this area range from the use of prebiotics and probiotics in reducing cattle methane emissions to improving early weaning of young pigs and cattle.

To see the microbial variety (also termed microbiota) of several plants and animals, review the open-access diagram from (Ikeda-Ohtsubo et al., 2018) available at the following link.

**Microbiomes in Agriculture**
https://www.frontiersin.org/files/Articles/406036/fnut-05-00090-HTML/image_m/fnut-05-00090-g001.jpg

The microbiome can be defined as the library of microbial genomes (genetic material) that are contained within and on a human or animal host. Your microbiome library is unique to your past and present microbial exposure, derived from the foods you eat and interactions with your environment. The books in your microbiome library will change over time with the most significant changes occurring during the first few years of your life (Arrieta, Stiemsma, Amenyogbe, Brown, & Finlay, 2014a). Microbiota (microbe varieties) that establish the microbiome also differ depending on where they are found, skin microbiota are different from gut microbiota. The idea that microbes affect human health is now fairly well established and developed in the scientific community. Microbes that inhabit our bodies are active partners in gut health, neurological health, and also play roles in bowel disease, obesity, and asthma (Arrieta, Stiemsma, Amenyogbe, Brown, & Finlay, 2014b; Rowland et al., 2018; Tremlett, Bauer, Appel-Cresswell, Finlay, & Waubant, 2017). These links are known as host-microbial interactions. Gut microbiota that are permanent residents provide one of the books in your microbiome library. This type of relationship is mutualistic: the gut microbiota benefit from having a safe place to live and we as hosts derive benefit from these bacteria through digestion of foods and other benefits. While these linkages are well established, the exact mechanisms by which probiotics and prebiotics interact with the human microbiome and confer health benefits or create health problems is less understood (Cremon et al., 2018). Probiotic foods, for example, do not become permanent members of human gut, i.e. microbiota in probiotic foods do not become books in the microbiome library. The microbes in probiotic foods have transient effects on the digestive system which may last only a few hours to days and we lack evidence that probiotic foods confer permanent changes to the human microbiome. This uncertainty is illustrated in Figure 6. The interaction between beneficial microbiota and the human microbiome is well established, indicated by "✓". The interaction between prebiotic factors in prebiotic foods and the functioning of probiotics in foods is also well established. However, the science for how prebiotics and probiotics affect overall microbiota health and the human microbiome is hazy, indicated by "?" Will consuming probiotic foods make one healthy? Maybe.

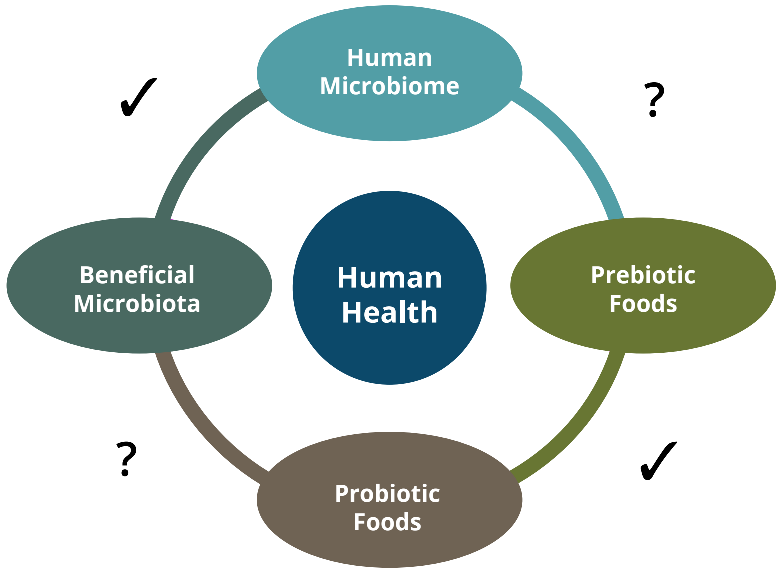



**Figure 6**—Interaction between human health, microbiota, and foods

Animal health is dependent on feed formulations to maintain health and promote growth. For example, once piglets are weaned from their mother (the sow) and no longer being fed milk, they are prone to diarrhea. A major cause of death in piglets is infection by *Salmonella* Typhimurium and shigatoxigenic strains of *E. coli* (Brugman et al., 2018). The use of antibiotics in feeds to counteract these diseases impacts the microbiota of the animals. Also, antibiotic use is discouraged (or restricted, in some jurisdictions) to limit impacts of antimicrobial resistance in the food chain. Restoring the gut health of piglets through the use of prebiotics and probiotics would be ideal. However, similar to the evidence gaps in humans, the research in this area has given inconsistent results. While the majority of studies show some reduction in diarrhea levels, none demonstrate elimination of pathogens or improved production (Barba-Vidal, Martín-Orúe, & Castillejos, 2018).

In Canada, actions to address the issue of antimicrobial resistance in animals include improvements in oversight and use, reporting of drug use to improve surveillance, and improving regulations for veterinary health products. Read more about these actions at this Government of Canada site.

**Antibiotic Resistance and Animals**
https://www.canada.ca/en/public-health/services/antibiotic-antimicrobial-resistance/animals.html

More encouraging is the use of probiotics in recently hatched chicks, where probiotic use was linked to improved gut microbiota and weight gains (Baldwin, Hughes, Hao Van, Moore, & Stanley, 2018). They found that fewer pathogens became established when probiotics were given to chicks. This study supports the use of probiotics, showing more benefits when they were used for chicks that had just hatched. If the central blue circle in Figure 7 above was relabelled "Chicken health," this article supports an association between probiotic foods and beneficial microbiota that establish the permanent microbiome.

**Figure 7** — Chicks hatching from the egg

## Spoilage—an overview

Food spoilage is the undesirable deterioration of food quality that may result in changes in odour, taste, and appearance of food. Another definition of *food spoilage* is "to destroy the value or quality of a food." *Spoilage* refers to the process of decay itself. Spoilage can occur through both microbial and non-microbial routes. Spoilage does not necessarily mean that the food is dangerous to eat. It just means that the food has been transformed in ways that make it less appealing for human consumption. For example, if you want to drink fresh milk, LAB in milk are undesirable and will cause spoilage. However, if you want to make yogurt, LAB in milk is desirable and does not cause spoilage.

The rate and type of spoilage can be affected by low or high temperatures, the amount of moisture in food or the humidity where foods are stored, the amount of light, time and presence or absence of oxygen (FAO /WHO, 2001). You should recognize these factors as intrinsic and extrinsic conditions important for microbial growth. They also affect non-microbial spoilage factors. We will describe the three most important routes of spoilage, shown as follows in the order they occur:

1. Autolysis or enzymatic spoilage
2. Microbial spoilage
3. Chemical spoilage (fat oxidation)

Autolysis refers to natural stages of decomposition that occur in living organisms shortly after death. This can also be called self-digestion, and refers to the actions of the organisms' own enzymes that result in cellular destruction.

*Microbial spoilage* refers to the external actions of bacteria and fungi on foods. This is the same process as microbial fermentation, except that the actions of the bacteria and fungi result in undesirable traits in the food during spoilage. Like fermentation, spoilage is a consequence of metabolic processes. Whenever microbes feed on a substrate (i.e., food), they change it. Microbes also use enzymes to break down carbohydrates, fats, proteins, and other nutrients in the substrate to form molecules they can transport inside them to use as energy and building blocks.

Chemical spoilage can be the last stage of spoilage for some kinds of foods stored for long periods, such as frozen foods. If they are not properly packaged or stored for too long, chemical changes can occur to make the foods unpalatable.

Other agents of spoilage in foods include various pests, such as insects, mice, rats, and other animals. Besides eating foods, they might also introduce undesirable microbes that will cause foods to spoil, or even contaminate foods with pathogens. Improper storage conditions can speed up spoilage, such as when foods are stored at too high temperatures or with too much moisture. These factors will be covered briefly under Topic 6: Microbial spoilage.

## Enzymatic spoilage

An enzyme is a chemical substance (a protein) that performs a specific action. For example, people who own contact lenses may use a cleaner containing hydrogen peroxide to digest away unwanted protein deposits on the lens that disrupts vision. Putting contact lenses with active hydrogen peroxide directly into your eyes could cause injury. To inactivate hydrogen peroxide a protein enzyme called catalase is added in tablet form to the saline storage solution. Catalase is a common protective enzyme found in most living organisms (animals, plants and microbes) because it converts harmful hydrogen peroxide into water and carbon dioxide.

What happens to a living organism when it dies? In the absence of respiration there is no energy provided to cells to maintain normal cellular activity. When living organisms die, many enzymes such as catalase are released from cells within the tissues of the organism. Catalase is normally encased in cellular structures that make fats. Following death excess hydrogen peroxide builds up and is no longer controlled by catalase causing damage. Lysosomes are a type of cell structure containing autolytic enzyme groups that affect lipids (fats), sugars and proteins. Following death the lysosomes structures break open releasing their enzymatic contents. When the organism is alive, its enzymes are regulated by processes in the living cells. When the organism dies, its enzymes are still active, but are no longer regulated or functioning. These enzymes begin the process of self-digestion or decomposition, and result in tissue softening and other activities (Powers, 2005).

In fresh fish, for example, enzymatic spoilage begins as soon as the fish dies. Removing the guts of the fish and refrigerating it rapidly will help to lessen the effects of enzymatic spoilage. The enzymes involved in spoilage include trypsins, collagenases, and glycolytic enzymes (Huss, 1995). Figure 8 shows gaping in a filet of chum fish, which is the result of connective tissue destruction by collagenase enzymes. Gaping is a quality indicator for fish, and is linked to temperature abuse (BC Salmon Marketing Council, 1995; Huss, 1995).

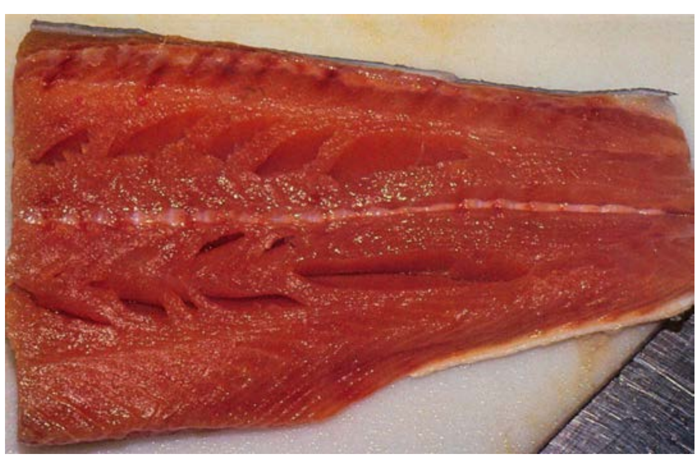


**Figure 8** — Enzymatic spoilage from temperature abuse causing gaping in a chum filet

Enzymatic spoilage also causes weakening of muscles and pokes holes into other tissues, such as intestinal tissues. Any breaches in the intestine will result in the release of live microbes from the gut that will perform the next stage of decomposition.

## Microbial spoilage

Microbial spoilage can be caused by bacteria and fungi (yeast and moulds). Through fermentation or spoilage, bacteria, yeast, and moulds may change the colour, smell, and/or taste of our food. In some cases, bacteria will produce odours to ward off competitors, including humans.

Factors that affect microbial food spoilage include:

- Intrinsic factors
- Extrinsic factors
- Species of microbes present

### Activity: Extrinsic and intrinsic characteristics in microbial growth

Name at least three extrinsic and two intrinsic characteristics that will be important to microbial growth and decomposition of the food source. (Hint: refer back to Lesson B.)

Check your answers with the answer key provided at the end of this textbook.

Moisture content and pH are both intrinsic characteristics of foods. Microbial spoilage occurs in all types of foods: fish, meats, vegetables, fruits, nut, and grains. Highly perishable foods include fresh meat, milk, vegetables, and some fruits. These foods have higher moisture contents. Within this group, foods with low acidity, such as milk, vegetables, and meats, will spoil more quickly than foods with higher acidity, such as fruits.

Among extrinsic factors, perhaps the most important is temperature. It is common knowledge that foods stored without refrigeration will spoil more quickly than refrigerated foods, simply because microbes grow faster at warmer temperatures. Another important extrinsic factor for food spoilage is the environment where the food came from. For example, consider an apple. Was the apple picked from a tree or collected off the ground? If it was on the ground, fungi, moulds, and other microbes in the leaf litter and soil could get on the apple. Any damage to the exterior of the apple would increase the rate of food spoilage, since microbes could enter through any cuts and nicks on its surface. If there were any fruit flies and other external pests that carry microbes, these pests could introduce microbes to the exterior of fruit, allowing them to penetrate and spoil it. Another extrinsic factor is how the food is handled and processed. Which would spoil faster, a whole cut of round beef or the same cut ground into hamburger? If you said the hamburger, you would be right. In ground meat, there is more exposed surface area for spoilage microbes to access the food source.

When you buy strawberries, you may not notice mould until the next day. Where did it come from? Moulds present in the soil and in the air were on the leaves and fruit of the berry even though they were not yet visible (Figure 9). Watch the 10 second time-lapse video at the following link to see the progression of fungal spoilage on a strawberry.

**Strawberry Moulding**
https://www.youtube.com/watch?v=fRRmCPTKL9E

**Figure 9** — Mould on a strawberry

The species of microbes present on produce or meat depend in part on the microbes in the environment the food has been in contact with and on the food handling. However, microbes associated with food spoilage are typically specific to the substrate. Meats are often spoiled by *Pseudomonas*. Vegetables and fruits and more prone to fungal microbial spoilage from *Aspergillus* (black mould), *Penicillium* (blue mould), and *Rhizopus* (softening). Yeasts can grow in higher sugar levels, alcohol, and salty foods, and can produce gas (causing swelling in food containers), sour tastes (e.g., the wrong yeast in the wine), and turbidity.

Typical food spoilage bacteria include *Aeromonas*, *Acinetobacter*, *Erwinia*, *Moraxella*, *Shewenella putrefaciens*, *Pseudomonas*, *Bacillus, and Clostridium*. Typical food spoilage moulds include *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhizopus*. Typical food spoilage yeasts include: *Candida*, *Zygosaccharomyces*, and *Saccharomyces*.

## Signs of microbial spoilage

Microbial spoilage can change the smell, texture, colour, and taste of food, making it less palatable or appealing. Some examples of changes in food due to microbial spoilage include:

- Smell
    - ◊ Rotten egg smell from sulfur-producing microbes
    - ◊ Other off-odours from nitrogenous compounds, such as ammonia and amines
- Texture
    - ◊ Sliminess from overgrowth of microbes
    - ◊ Gas formation and bubbling
    - ◊ Sponginess in meats

- Colour
  - ◊ Changes from moulds, such as green and blue moulds (Figure 10)
  - ◊ Greening from sulfide-producing bacteria
  - ◊ Rainbow sheen from fluorescent pseudomonads
- Taste
  - ◊ Souring from acid production
  - ◊ Other off-tastes

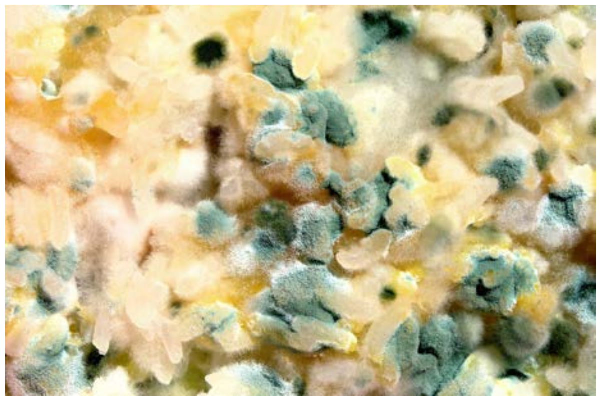


**Figure 10** — Colour change in mouldy food

## Preventing microbial spoilage

Microbes will be more likely to grow in some environmental conditions. For example, most moulds prefer environments that are drier, and will thrive in a wide pH range, from highly acid environments (pH 3) to slightly alkaline (pH 8). Moulds (as well as yeasts) can grow on highly acidic foods such as fruit, tomatoes, jams, jellies, and pickles. Moulds require oxygen to grow, so if you leave an opened jar of pickles in the refrigerator too long, psychrophilic mould strains may start to grow on top of the pickling solution, in the oxygen-containing air space between the food and jar lid. Once any jar is opened, it is open to circulating mould spores in the environment. As moulds grow, they will produce enzymes that break down the substrate (food), resulting in food softening and spoilage.

Microbial spoilage can be limited by controlling the environment through one or more of the following food preservation strategies:

- Refrigerating
- Adding acid
- Adding salt
- Using packaging to limit aerobic microbial growth (i.e., reduced oxygen packaging)
- Cooking
- Canning
- Drying
- Irradiating
- Limiting pest contact with foods in storage or on display

Watch the video at the following link to hear about various food preservation strategies to limit harmful microbial growth.

**Food Preservation—Seven Wonders of the Microbe World (3/7)**
https://www.youtube.com/watch?v=LPWvzW82kWU&index=3&list=PL4473936D327B7C69

## Microbial spoilage and foodborne illness

Most microbial spoilage is detectable by smell, taste, or visual appearance. Some microbes can be beneficial, can cause spoilage, or cause illness, depending on the circumstances. Of importance, microbial spoilage can change foods in a way that is not detectable and creates a new hazard. **Mycotoxins** are chemicals produced by moulds that can cause foodborne illness if the contaminated foods are consumed. Moulds that produce mycotoxins are very common and cause significant losses of agricultural crops worldwide (Bennett & Klich, 2003). These moulds are usually associated with cereals, legumes, and flours. In some countries, as much as 50 % of grains are contaminated by mycotoxins (Adeyeye, 2016). For example, aflatoxins, produced by the fungus *Aspergillus flavus,* are one of the most potent liver carcinogens known. Aflatoxins cause significant health problems in developing countries, where it commonly contaminates grains and animal feeds. *Aspergillus* is often associated with drought-stress in plants just prior to harvesting. Poor drying techniques and storage conditions that do not control moisture create conditions for fungal growth and mycotoxin formation in grains (Adeyeye, 2016). Another example of a mycotoxin is ergot. Ergot is a potent alkaloid mycotoxin associated with breads and grains, which is produced by a mould called *Claviceps*. This mycotoxin was behind infamous mass poisonings during the Middle Ages, wherein it caused convulsions and gangrene.

Scombroid or histamine poisoning is a foodborne illness caused by eating tuna that has undergone microbial spoilage (Figure 11). This type of food poisoning is entirely preventable by controlling refrigeration. Histamine is a chemical that produces facial flushing and swelling, often associated with allergic responses. Histamine is not a naturally occurring chemical in tuna. However, the muscle flesh of tuna can contain high amounts of an amino acid called histidine, which can be converted into histamine by bacteria that are present on the surface of tuna (e.g., *Morgonella*). These bacteria will grow during temperature abuse and bacterial decomposition of the tuna, and the conversion to histamine will only take place in the presence of large numbers of these bacteria, over one to ten million bacteria per gram of tuna. The histamine formed is heat stable and so resistant to canning temperatures (Emborg & Dalgaard, 2008).

**Figure 11** — Canned tuna can contain histamine due to microbial spoilage prior to canning.

## Chemical spoilage

**Rancidity** (or the process of rancidification) is a form of chemical spoilage that occurs from oxidation of fats and lipids. The reaction of the food with oxygen in the air causes decomposition of fats and oils. The great tasting fats in foods are transformed into foods containing undesirable tastes, from chemicals such as aldehydes and ketones. This problem arises in foods when either the packaging is poor or the food has been in prolonged storage. Peanut butter and frozen seafoods (for example, salmon) are susceptible to this kind of spoilage.

Nuts and nut butters, such as peanut butter (Figure 12) are foods have a fairly high content of oil and fat. Prolonged storage of peanut butter at room temperature can result in rancidity. Although nuts and nut butters can be stored at room temperature, their shelf life is approximately one year, after which the oils and fats they contain will oxidize and go rancid. While this is a food quality issue and will not cause foodborne illness, the taste of rancid peanut butter is not acceptable to most consumers.

**Figure 12** — Peanut butter will go rancid after prolonged storage at room temperature.

Have you ever found a forgotten a bag of frozen prawns in the bottom of your freezer, and noticed that the tips of the prawns have turned white or yellow in colour? This change is known as freezer burn, and is caused by oxidation (Figure 13). Oxidation in prawns and other seafoods can change their colour and their taste. This type of chemical spoilage can occur after long-term frozen storage or when frozen storage takes place in poor packaging. Whitening of edges or yellow colour development in any frozen seafoods can indicate this type of chemical spoilage.

**Figure 13** — Oxidization (freezer burn) of prawns from prolonged storage

An excellent review and summary of the three types of spoilage affecting meats can be found in the following Canadian article (Dave & Ghaly, 2011). An extrinsic factor not covered that is explained in the beginning of this article is the effect of pre-slaughter conditions on spoilage. The colour and texture of meat is affected by the amount of stress experienced by animals during slaughter. Stress changes the pH of the meat (in the muscle) during lactic acid breakdown of sugars (to see this effect open the following link and look at Figure 2). Higher acid contents leads to poorer quality meat more prone to bacterial spoilage.

**Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review**
https://thescipub.com/abstract/10.3844/ajabssp.2011.486.510

# Lesson C Quiz

1. What is fermentation?
   a. A process by which mycotoxins are produced in moulds that cause foodborne illness
   b. A process by which food reacts to oxygen in the air to cause decomposition of fats and oils
   c. A process by which microbes produce energy by converting sugars into simpler compounds

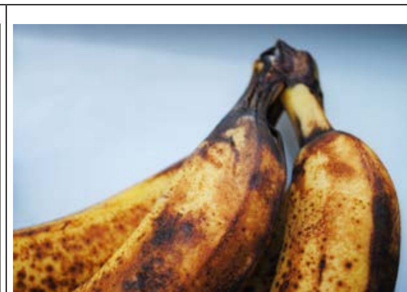
2. What is backslopping?
   a. Enzymes break down carbohydrates and alter the taste and texture of food.
   b. Spoilage occurs due to the introduction of external bacteria and fungi in food.
   c. A portion of a batch of food is used to create a starter for a new batch of food.
   d. A batch of food is contaminated by microbes in ingredients used in its preparation.

3. Which type of fermentation is present in these foods?

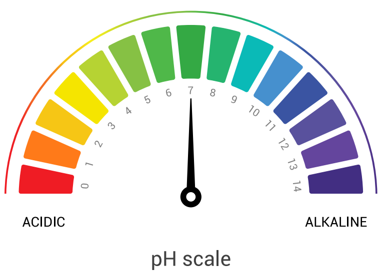
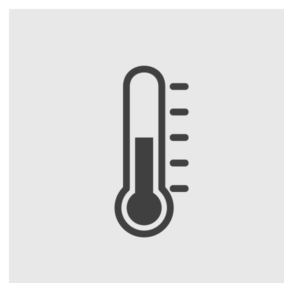
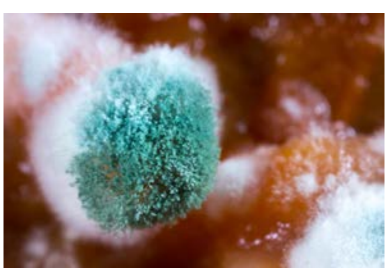
| | |
|---|---|
| <br>**Yogurt** | <br>**Beer** |
| a. Yeast fermentation<br>b. Mixed fermentations<br>c. Lactic acid fermentation | a. Yeast fermentation<br>b. Mixed fermentations<br>c. Lactic acid fermentation |
| <br>**Kombucha** | <br><br>**Sauerkraut / salami** |
| a. Yeast fermentation<br>b. Mixed fermentations<br>c. Lactic acid fermentation | a. Yeast fermentation<br>b. Mixed fermentations<br>c. Lactic acid fermentation |

4. What is believed to be the benefit of consuming probiotic foods?
    a. Probiotics neutralize undigestible prebiotics present in fermented foods.
    b. Probiotics provide nutrients to the bacterial microbes that are essential to gut health.
    c. Probiotics offer permanent improvements to gut health by eliminating other microbes.

5. Which category of spoilage is shown in each photo?

| | | |
|---|---|---|
|  **Slimy cucumbers** |  **Freezer burnt turkey** |  **Overripe bananas** |
| a. Chemical spoilage<br>b. Enzymatic spoilage<br>c. Microbial spoilage | a. Chemical spoilage<br>b. Enzymatic spoilage<br>c. Microbial spoilage | a. Chemical spoilage<br>b. Enzymatic spoilage<br>c. Microbial spoilage |

6. Which of these factors affecting food spoilage are intrinsic and which are extrinsic?

| | |
|---|---|
|   **Acidity** |  **Temperature** |
| a. Intrinsic<br>b. Extrinsic | a. Intrinsic<br>b. Extrinsic |
|  **Mould** |   **Moisture content** |
| a. Intrinsic<br>b. Extrinsic | a. Intrinsic<br>b. Extrinsic |

7. What is the best way to limit microbial spoilage from mould growth?
   a. Reduce oxygen
   b. Lower pH
   c. Cool temperatures
   d. Increase the humidity environment

8. Which of the following produces the toxic substance mycotoxin?
   a. Fungal organisms
   b. Lactic acid bacteria
   c. Probiotic microbes
   d. Primary fermentation

9. Why does enzymatic spoilage occur?
   a. After death, the cells of an organism can no longer control enzyme activity.
   b. After death, enzymes are introduced to an organism through contamination.
   c. After death, the proteins in an organism combine with hydrogen to create enzymes.

10. What is the main idea about fermentation and spoilage presented in this lesson, that food workers should understand?
    a. The sources of harmful microbes in food
    b. The biproducts created by microbial growth in foods
    c. That even helpful bacteria in food must be controlled
    d. That microbial growth in food is caused by careless actions

Check your answers with the answer key provided at the end of this textbook.

# Lesson D: Foodborne Illnesses

## Introduction

Four million Canadians, or approximately one in eight people, become ill each year from contaminated food. This results in an estimated 11,500 hospitalizations and 240 deaths every year. See the Public Health Agency of Canada infographic at the link that follows for a visual representations.

**Infographic: Food-related illnesses, hospitalizations and deaths in Canada**
https://www.canada.ca/content/dam/hc-sc/healthy-canadians/migration/publications/eating-nutrition/foodborne-illness-infographic-maladies-origine-alimentaire-infographie/alt/pub-eng.pdf

The most common term used to describe foodborne illness is **food poisoning**. "Food poisoning" is a little misleading, because the word "poison" on its own is usually accepted to mean a chemical toxin causing illness. Microbes can cause illness in more than one way: they can cause an infection in the body, or they can release a toxin (poison). Some microbes can do both: they infect cells in the body causing damage, and they also release toxins. There are many microbes that may cause illness. When a microbe causes illness, it is called a **pathogen**.

As much as we require microbes to maintain our health, harmful microbes (i.e., pathogens) can result in major public health issues and are one of the leading causes of illnesses and death around the world. *Norovirus* causes 1 000 000 illnesses and twenty-one deaths a year in Canada. *Listeria* infections cause fewer illnesses (178), but are responsible for thirty-five deaths annually. *Salmonella* causes one in four hospitalizations of all the foodborne illnesses.

### Learning outcomes

Upon completion of this lesson, learners will:

- List common pathogenic microbes responsible for foodborne illness and intoxication
- Differentiate between foodborne infection and foodborne intoxication
- Describe the disease, incubation period, and symptoms of illness caused by common pathogens
- Explain why some people are more susceptible to infection and disease than others and identify those groups at-risk
- Describe how foodborne illness can cause acute and chronic disease
- Describe allergens risk
- Match pathogens to foods most likely to be vehicles for these illnesses

### Required readings and resources

- Diseases & Conditions: A to Z list of diseases & conditions (BC CDC)
- Centers for Disease Control and Prevention: Food Safety
- Bad Bug Book (FDA)
- Diagnosis and Management of Foodborne Illnesses
- Investigation of a Foodborne Outbreak

## Terminology

Key terms given in bold

**acute disease**
**allergen**
allergenic
**attribution**
**case**
**chronic disease**
clinical specimen
**clinical symptoms of illness**
confirmed illness
congenital infections
**contagious**
**differential diagnosis**
**disease sequelae**
**disease transmission**
duration of illness
etiology
**etiologic agent**
**fecal-oral transmission**
food intolerance
**foodborne pathogen**
food poisoning
gastroenteritis
**gastrointestinal symptoms**
**host**
**immunocompromised**
incubation period
infectious
odds ratio
**outbreak**
**pathogen**
pathogenesis
persistent diarrhea
person-to-person transmission
reservoir
stages of disease
suspected illness
systemic illness
toxigenic
**vulnerable population**
**YOPI (young, old pregnant, immunocompromised)**

# Foodborne illness: Infection and intoxication

Foodborne infections and foodborne intoxications were briefly described in Lesson A: Introduction to Food Microbiology: both cause foodborne illness. Figure 1 shows some common pathogens that cause foodborne infections, and categorizes them as bacterial, parasitic, and viral pathogens. These pathogens cause illness by infecting the body of a **host**. Infectious pathogens damage tissues and cells during growth in the host and sometimes release toxins that cause illness. The term "host" refers to the animal in which a pathogen is found. Figure 1 shows some common examples of pathogens that cause foodborne intoxication when toxins are released into foods. Illness occurs when the contaminated food is ingested along with the toxin.

| Bacterial pathogens | Parasitic pathogens | Viral pathogens |
|---|---|---|
| *Bacillus cereus* | *Cryptosporidium* | Hepatitis A virus (HAV) |
| *Campylobacter jejuni* | *Cyclospora* | Norovirus |
| *Clostridium botulinum* | *Giardia* | |
| *Clostridium perfringens* | *Toxoplasma gondii* | |
| some *Escherichia coli* strains (e.g., *E.coli* O157:H7 and shigatoxigenic strains) | *Trichinella* | |
| *Listeria monocytogenes* | | |
| *Salmonella* spp. | | |
| *Shigella* spp. | | |
| *Vibrio* spp. | | |
| *Yersinia enterocolytica* | | |

**Figure 1** — Foodborne pathogens that cause illness by infection

| Pathogen | Toxin produced by pathogen | Disease caused by toxin | Example of commonly contaminated food |
|---|---|---|---|
| *Aspergillus* spp. (mould) | aflatoxin | cancers and other diseases of the liver | peanuts |
| *Amanita phalloides* (mushroom) | amanitin | death cap poisoning | death cap mushroom |
| *Clostridium botulinum* | *botulinum* | botulism | canned green beans |
| *Pseudo-nitschia* spp. (marine diatoms) | domoic acid | amnesic shellfish poisoning | razor clams |
| *Bacillus cereus* | enterotoxin | food poisoning | rice |
| *Staphylococcus aureus* | enterotoxin | food poisoning | potato salad |
| *Claviceps* mould | ergot | ergotism (St. Anthony's fire) | rye bread |
| *Morgonella* bacteria | histamine | scombroid poisoning | tuna |
| *Alexandrium* spp. (marine dinoflagellates) | saxitoxin | paralytic shellfish poisoning | mussels |

**Figure 2** — Foodborne pathogens that cause illness by intoxication

The location where a pathogen releases its toxin determines whether it is listed in Figure 1, Figure 2, or both. If a pathogen releases a toxin only in the body of its host during the course of illness, that pathogen is listed in Figure 1 only. If a pathogen only releases toxins in the food before it is ingested (i.e., the toxin was pre-formed in the food), the pathogen is listed only in Figure 2. Some pathogens, however, can infect and release toxins within a host's body and can also infect and release toxins into food. These pathogens are listed in both tables.

For example, *Clostridium botulinum* are pathogenic bacteria listed in both tables. If *botulinum* intoxication was to occur in an individual who ate contaminated canned green beans, it is most likely the individual ingested the toxin directly from the food. If *botulinum* intoxication was to occur in a young baby, it is more likely that the baby ingested *Clostridium botulinum* spores first. The *C. botulinum* bacteria then grow in the gut of the infant, causing an intestinal infection. The bacteria infecting the intestines then release the toxin inside the host's body, leading to illness. The source of the toxin in both cases is the bacteria *C. botulinum*. However, the manner in which the foodborne illness occurs is very different.

- **Foodborne infection** is caused by eating food contaminated with pathogens, including bacteria, parasites, and viruses. Once ingested, these pathogens infect the body—often in the intestinal tract—causing infection and disease.
- **Foodborne intoxication** is caused by ingesting food that contains pre-formed toxins produced by pathogens. Toxins may be produced by bacteria, mushrooms, moulds, or marine organisms. During intoxication, the pathogen may no longer be present in the food.

In summary, an infection requires the consumption of pathogens that multiply as they infect and colonize cells and tissues in the body. Intoxication requires ingesting a pre-formed bacterial, fungal, or microbial toxin.

Appendix C: Pathogens contains more information about common pathogens. Descriptions include species and photos, sources, outbreaks, symptoms, incubation, duration, and prevention measures.

## Canadian foodborne illness estimates

The **etiologic agent** of a disease or illness is the pathogen found to be the cause of a foodborne illness. The pathogens may be found in food eaten by someone with foodborne illness, or blood or feces collected from an ill person and tested in the laboratory.

Figure 3 shows the proportion of pathogens that caused foodborne illnesses in the Canadian population, based on surveillance between 2000 and 2010 (Thomas et al., 2013). As mentioned, norovirus causes sixty-five percent of all foodborne illnesses, or approximately 1 000 000 cases each year. Note that the word "cases" is bolded: when referring to foodborne illness and to the ill person, the word "case" indicates the ill person. *Clostridium perfringens* is next on the list, followed by *Campylobacter*, *Salmonella*, and *Bacillus cereus*. *Staphylococcus aureus* is not shown. Included as one of the other causes, it is estimated to account for 1.5% of illnesses. Some foodborne illnesses, like *Staphylococcus aureus*, are less frequently reported by the public. It is important to emphasise the data shown here is an estimate based on available data.

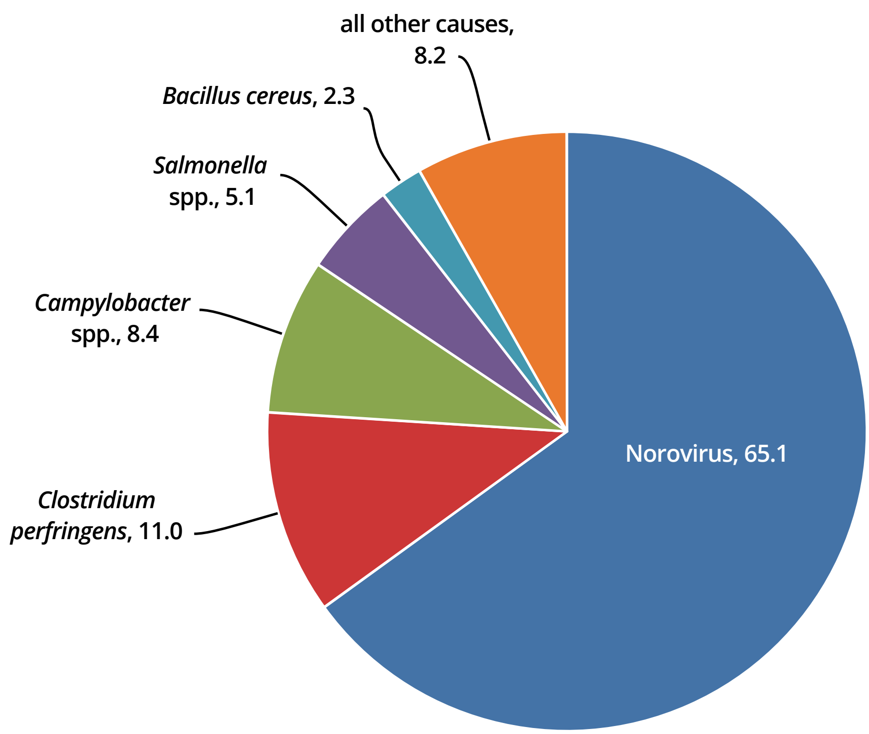



**Figure 3** — Causes of foodborne illness in the Canadian population

You may wonder how these pathogens are able to cause disease and what types of foods may be linked to them. We will explore this later in this module. Our next objective is to explain how foodborne illness is medically diagnosed.

More information can be found in Appendix B: Statistics on Foodborne Illness through links to disease statistics sites in B.C. and Canada.

## Foodborne illness

In general, for both foodborne intoxications and foodborne infections, two conditions must be met for foodborne illness to occur:

1. The pathogen or toxin must be present in the food in sufficient quantity to cause illness.
2. The food must be eaten.

For bacterial foodborne infections, conditions must also be suitable for microbial survival and/or growth. Remember all the intrinsic and extrinsic conditions that allow for microbial growth? These conditions include:

- A food substrate that provides nutrients to the pathogen;
- A pH above 4.6;
- A water activity ($a_w$) above 0.85;
- An aerobic or anaerobic environment, depending on the pathogen; and
- A high redox potential ($E_h$, redox provides energy in form of oxygen).

Depending on how a bacterial pathogen causes infection, the following two conditions are needed before or after the contaminated food is consumed:

- The temperature of the food or the host must allow for microbial growth, and
- There must be enough time for microbes to grow to disease-causing numbers and/or to produce secondary toxins.

## How do public health practitioners diagnose and investigate foodborne illness?

A simplified overview of an investigation of a foodborne illness is shown in Figure 4. We will use this as a guide to explain what takes place during investigations of foodborne illness.


**Figure 4** — Steps to investigate a foodborne illness

### Step 1: The interview

When we suspect someone has a foodborne illness, it is common for us to first ask them, "What did you eat?" But, this is not usually one of the first questions a public health practitioner (PHP) might ask.

Who are public health practitioners? These are people in occupations that investigate illnesses, including:

- Doctors
- Nurses
- Medical health officers
- Environmental health officers
- Public health inspectors
- Epidemiologists
- Medical technologists (laboratory specialists)
- Occupational health specialists
- Food safety specialists
- Quality assurance and control specialists, and
- …others!

Figure 5 shows questions that a PHP asks during the interview of an ill person suspected of having a foodborne illness. The first question usually asked is, "What symptoms do you have?" This is because, similar to all other types of illnesses, diagnosis of foodborne illness is based partially on the ill persons' symptoms. Symptoms of foodborne illness commonly include:

- Diarrhea
- Vomiting
- Abdominal cramps

These three symptoms are collectively referred to as **gastrointestinal symptoms** or **gastroenteritis** (from the Greek word for stomach, *gaster*). These terms are used because the areas where these symptoms occur are in the stomach and digestive tract.

When an ill person has diarrhea, it might be due to foodborne illness. However, diarrhea is also a symptom of many other conditions, such as irritable bowel syndrome or a reaction to a drug prescription. A PHP will ask many questions to determine whether foodborne illness is suspected. The interview is a crucial part of the initial investigation, as it provides information for the PHP to perform a **differential diagnosis**. A differential diagnosis is necessary when there may be different potential reasons for the same symptoms to occur. In the case of diarrhea, the PHP will need to know if the case (i.e. the ill person) has a history that suggests a reaction to prescription medicine, or if foodborne illness could be possible. Does the case have contact with other people that might also be ill, such as a spouse who works in a hospital?

Other questions asked during an interview for a differential diagnosis specific to foodborne illness are also shown in Figure 5. Based on the information gathered during the interview, foodborne illness may or may not be suspected.

| Questions an ill person is asked | Information that answers provide |
|---|---|
| What symptoms do you have? | clinical symptomology |
| What was the first symptom you had? | |
| When did your symptoms first start? | |
| Is anyone else you know also ill with the same symptoms? | population and exposure information |
| What foods did you eat? What were the last few meals you ate? | food history |
| Did you travel recently? Where? | travel history |
| Have you visited a farm or been camping? What events have you attended recently? | exposure information |
| Do you work in a hospital or daycare or have children attending a daycare? | |
| How long have you been ill? | duration of illness |
| Did you eat any raw or undercooked foods? | identification of potentially hazardous foods in the diet |
| How do you prepare your foods at home? | identification of improper food-preparation practices |
| How long after eating a suspect food did the symptoms begin? | incubation period |

**Figure 5** — Questions asked during case interviews to inform a differential diagnosis of suspected foodborne illness

**Question:** Do you think a foodborne illness would be suspected if the ill person was a child who attended a daycare?

**Answer:** Maybe. It depends on all the information collected during the interview. If other children at the daycare were also ill, it could be foodborne illness. Or, it could be some other **contagious** illness that is easily spread, such as a rotavirus infection. This is a non-foodborne virus common in children that is spread by **person-to-person transmission**. That means one child was likely ill first, and then spread the infection to another child. If all the children were healthy at the daycare and became ill at the same time, it could be foodborne illness. But if one child was ill first and the others became ill over the next few days, a foodborne illness is less likely. Other evidence can be collected to help rule foodborne illness in or out.

Figure 6 explains in more detail how and why the information collected for each of the questions in the interview is important to making a differential diagnosis of foodborne illness.

| Questions an ill person is asked | Details on information that answers provide |
| --- | --- |
| What symptoms do you have? | As well as gastroenteritis (vomiting, diarrhea and abdominal cramps), symptoms associated with foodborne illness include fever, nausea, fatigue, numbness, tingling, weakness, dizziness, unsteady gait (incoordination), difficulty breathing, paralysis, watery diarrhea, and bloody diarrhea. Each pathogen has specific symptoms associated with it. For example, bloody diarrhea is often linked to *E.coli* and *Salmonella* infections. |
| What was the first symptom you had? | Symptoms can be progressive. For example, nausea will often precede vomiting, followed by diarrhea. Toxin poisonings might start with tingling, then numbness, and then proceed to difficulty breathing. |
| When did your symptoms first start? | Noting the date and time that symptoms first started is important to establish the onset and incubation period. These factors are specific to various pathogens. |
| Is anyone else you know also ill with the same symptoms? | This will assist in identifying a common exposure. Did other people attending the same event also become ill? |
| What foods did you eat? What were the last few meals you ate? | Most (but not all) foodborne illnesses are linked to meals that were consumed within the last three days. PHPs will ask for a three-day food history, in which the ill person provides an account of all the foods and beverages he/she consumed over the last seventy-two hours. |
| Did you travel recently? Where? | A travel history is also related to exposure. Many foodborne illnesses and some types of infections can be acquired during trips to countries where a particular pathogen is common in food and drinking water. For example, hepatitis A virus is common in Mexico and can be acquired from drinking water or eating ice or any foods in contact with the water supply. |
| Have you visited a farm or been camping? What events have you attended recently? | Farms are known sources of exposure to enteric sources of pathogens from farm animals such as cattle, goats, and chickens. The feces of cattle and goats are a known source of *E.coli* O157:H7, whereas poultry such as chickens, turkey, and ducks carry *Salmonella*. When an illness is linked to a farm, foodborne illness may be ruled out.<br><br>Camping trips are often linked to exposure to contaminated drinking water sources. Depending on how foods were stored, temperature abuse of potentially hazardous foods can mean a suspicion of foodborne illness. |
| Do you work in a hospital or daycare or have children attending a daycare? | This exposure information will indicate if the symptoms and illness may be acquired from a high-risk setting such as a hospital or daycare. This may indicate the illness is not foodborne. |

| | |
|---|---|
| How long have you been ill? | The length of time or duration of a foodborne illness is specific to a particular pathogen. Many foodborne illnesses are of short duration, but some can persist longer. |
| Did you eat any raw or undercooked foods? | When the diet contains obvious potentially hazardous foods, such as raw proteins or undercooked foods, this points to a possible source of food-related illness. Undercooked beef burgers, undercooked eggs, raw shellfish, and raw bean sprouts are examples of higher-risk foods. |
| How do you prepare your foods at home? | Preparation and storage of foods, if done incorrectly, can lead to temperature abuse of potentially hazardous foods and microbial growth of pathogens in those foods. |
| How long after eating a suspect food did the symptoms begin? | The incubation period or incubation time is specific to each pathogen. A PHP will ask the case when their illness began after eating the suspect meal. When more than one person is ill from eating the meal, the PHP will establish the time period for each case. Some cases may get ill sooner, others later. This allows the PHP to determine the time range for the incubation period; i.e., the shortest to longest time period for illnesses in a group of cases. This provides important data to the type of pathogen that may have caused the illness. |

**Figure 6** — Further details on differential diagnosis information

### Step 2: Collect specimens

It can be difficult to diagnose foodborne illness in a single individual. To confirm the initial diagnosis, a PHP may request a clinical specimen, i.e., a blood or fecal specimen from the ill person.

Figure 7 shows the procedure for collecting a fecal specimen. A routine screen for gastrointestinal infections (gastrointestinal panel) from a fecal specimen for bacteria includes testing for:

- *Campylobacter*
- *E. coli*
- *Salmonella*
- *Shigella*

If foodborne illness is suspected, depending on the food history and other evidence, other tests can be requested. These can include testing for:

- *Staphylococcus aureus*
- *Bacillus cereus*
- *Vibrio*
- *Yersinia*
- Viral sources, such as norovirus and hepatitis
- Parasitic agents, such as *Cyclospora* and *Giardia*


**Figure 7** — Collection bottle and instructions for fecal (stool) samples for diagnosing foodborne illness

While results from blood or stool specimens from an ill person will provide objective evidence of an infection, it is important to realize that having a negative result does not necessarily mean there was no infection or illness. It could be that the tests done on the specimen did not include the microbial cause of the illness. The microbial cause of the illness is also termed the **etiologic agent** of the illness. For example, suppose the illness was caused by *Staphylococcus aureus* but the tests included only the four pathogens in a routine gastrointestinal panel (*Campylobacter*, *E. coli*, *Salmonella*, and *Shigella*). The test results would be negative, as the etiologic agent of the illness, *Staphylococcus aureus*, would not be detected. As the fictional detective Sherlock Holmes often said, "absence of evidence is not evidence of absence."

Another reason it is difficult to **confirm** an illness is that it is also extremely difficult to get ill persons to submit a clinical sample; it is uncomfortable to collect fecal specimens in a small bottle!

PHP will divide cases into two categories, depending on the clinical differential diagnosis and the laboratory clinical specimen results:

1. **Suspected illness:** based on the presence of symptoms, exposure, and history that fit a specific differential diagnosis, (e.g., ate undercooked hamburger at a barbeque where others were also ill and presented with bloody diarrhea)

2. **Confirmed illness:** based on the same criteria in the first category PLUS a positive laboratory test result in the clinical specimen (e.g., *E. coli* O157 was found in the fecal sample from the ill case)

During the interview, it is important to ask whether anyone else was ill. If two or more people shared a meal and all became ill, foodborne illness is more likely. This is because it is less likely that those people would all have the same medical issues individually, such as, for example, all having irritable bowel syndrome or similar reactions to a prescription drug.

The other type of specimen that may be collected is a food specimen. Food specimens may be examined for presence of a foodborne illness causing bacteria (e.g., *Listeria monocytogenes*) or their toxin (e.g., *Bacillus cereus* or *Staphylococcus aureus* enterotoxins) but these are not routine tests. Food specimens may be leftovers from the actual meal, foods from the same batch, or samples of the ingredients. Food specimens might be collected from the home of a person with a foodborne illness, a retail grocery, a restaurant, or a farm, depending on the specifics of the investigation. When a clinical test result is positive for a specific etiologic agent, it will inform the foods most likely to contain that pathogen. For example, if a clinical test is positive for *Salmonella,* poultry is a food that is common source of this pathogen. Other potential sources (also called "reservoirs") of *Salmonella* include raw sprouted seeds, nuts, and chocolate. Note that water is also considered to be an ingredient in foods, and it is known to be a reservoir of some microbial pathogens, including hepatitis and *Giardia*. The relationships among pathogens and foods will be explored later in this section. Food specimens are usually collected during the environmental investigation (see Step 3).

### Step 3. Further environmental investigations

When a suspected foodborne illness is reported to public health agencies, these agencies may conduct further environmental investigations. What occurs during further investigation of an outbreak will be based on the results of interviews and sample collections (i.e., steps 1 and 2). Note that a full environmental investigation is comprised of steps 1 and 2 along with any further activity.

Environmental investigation activities normally include visiting where the common exposure occurred and conducting an on-site investigation of the restaurant or business (for example) where food was prepared. PHPs will examine health of staff, handwashing practices and infection control, how food is handled (including temperatures and process control), cleaning and sanitizing in the premises, and will generally perform a risk based inspection. Examples of three outbreaks and the environmental investigations performed are described next.

## Examples of scenarios and investigations of foodborne illness outbreaks

An **outbreak** occurs when two or more individuals are linked by a common exposure to foodborne illness. Common scenarios of exposures in foodborne outbreaks include:

a. People in different households eat the same type of food (e.g., contaminated peanut butter is the common exposure)
b. Foodborne illness occurs in a large gathering of people attending an event (e.g., a wedding)
c. Different groups of people go to the same place to eat on different days and become ill (e.g., to a restaurant)

To describe what further steps may be taken, sample investigations of each of these scenarios follow, including the interviews and specimen collection.

**Figure 8** — Peanut butter

## Outbreak of foodborne illness caused by peanut butter

### Interviews

Individual illnesses linked to foods purchased in grocery stores can be difficult to identify. For cases of illness caused by peanut butter, the common exposure is normally uncovered during secondary interviews of cases. This often occurs after clinical samples that have been laboratory tested link cases of illness together with a specific strain or biotype of the etiologic agent. For example, *Salmonella* is a pathogen that has been linked to contaminated peanut butter. If PHPs discover ill people in different households have the same *Salmonella* biotype, they will conduct follow-up interviews to find what foods they might have in common. Sometimes, PHPs will ask for permission to examine the cases' grocery shopping history using their store loyalty-card information. This information can identify common foods bought by the different cases and the retail stores where they were purchased.

### Collecting and testing food

Leftover jars of peanut butter would be the best samples to test to confirm whether the source was peanut butter. However, it cannot be ruled out that an ill case accidentally contaminated a jar themselves after opening it because they were already ill from some other source. Therefore, to prove it was the peanut butter, unopened jars of the same batch of peanut butter purchased by all the cases should also be tested.

### Preparation of the food

It is unlikely that all of the containers of peanut butter would be contaminated with *Salmonella*. Some might contain only a few bacteria while others might contain thousands. Even within the same container, bacteria might not be evenly distributed. This can explain why some people become ill while others do not, even if they all eat from the same jar of peanut butter. Peanut butter is made by grinding roasted peanuts (Figure 8), and can include mixing the grind with natural and added oils (such as soybean or canola oil) and adding salt and sugar. *Salmonella* can contaminate raw peanuts or be present in the factory. Peanut butter can become contaminated if the raw peanuts are not roasted properly or after roasting, during grinding and before packaging.

### Environmental investigation

Unless a local factory made the peanut butter, there is no on-site investigation of the factory in this type of outbreak. PHPs will contact other jurisdictions to inquire whether they have information about the plant. This requires provincial- and federal-level PHPs' assistance and investigation to liaise with other jurisdictions. As described above, peanut butter can be contaminated by improper hygiene and sanitation in the plant or from the source ingredient (the peanuts). Further work would be needed to determine what occurred.

For more details about an actual peanut butter outbreak consult the following link:

**Multistate Outbreak of Salmonella Typhimurium Infections Linked to Peanut Butter, 2008–2009 (FINAL UPDATE)**
https://www.cdc.gov/salmonella/2009/peanut-butter-2008-2009.html

**Figure 9** — Chafing dishes at a buffet

## Outbreak of foodborne illness at a wedding

### Interviews

During events, only one specific food may be contaminated, but dozens of foods are served. This is particularly true for an outbreak at a buffet wedding service. During the outbreak investigation, PHPs will interview those who attended the event and ask what food items they consumed, whether they were ill, and if so, get detailed information about their symptoms. It can be challenging for PHPs to do hundreds of case interviews in a few days, because interviews are usually conducted by telephone. In recent years, there has been a move to collect this information via online questionnaires. However, maintaining the privacy of case information is also critically important, and so collecting information over cell phones and websites while maintaining client confidentiality is a concern for PHPs. In this example, 350 people attended the wedding, and fifteen became ill with diarrhea. Four people agreed to collect and submit a stool sample.

### Collecting and testing food

During event outbreaks, PHPs employ tools to determine the food most likely to have led to illness. People who attended the event but did not eat the contaminated food should not be ill. Based on interview information, PHPs make a mathematical calculation to figure out how many people were ill and were not ill for each specific food consumed or not consumed (e.g., for pasta salad, for green salad, for fish, for chicken). This calculation is called an odds ratio, which is based on a two by two table that simply divides up the population (the people at the wedding, in this example) into those who did or did not eat a particular food, and whether they were ill or not ill after eating that food. The higher the value of the odds ratio, the more likely it is that the food in question caused the illness. PHPs then collect those foods for testing. The odds ratio for the pasta salad and chicken in this outbreak was high.

### Preparation of the food

At large events, the same food (for example a pasta salad) may be prepared in multiple batches. With multiple batches of food, there may be differences for each batch in who did the preparation, in the ingredient sources, and in the handling of the food. During the environmental investigation of food preparation in our example , the PHPs inquired about the cooking and cooling of the pasta, the storage of the pasta , and the temperature control during its transport to the event. They asked similar questions for the preparation of the *sous vide* chicken. They looked at each ingredient in the salad and chicken. There were no raw ingredients used in the chicken, but the pasta salad included raw red onions, sugar snap peas, and cilantro.

**Environmental investigation**

Although there were leftovers of the chicken and the pasta salad from the wedding, the food-poisoning tests on these samples came back negative. The clinical stool samples were also negative. One person sent in a second stool sample for persistent watery diarrhea, and the result came back positive for a parasite, *Cyclospora*. The PHPs now had more information to help inform their investigation. Cilantro is a known reservoir for *Cyclospora*. Following a close examination of the pasta salad batches, they discovered two interesting facts. It was the bride's wish to have all locally sourced food at her wedding, so the first batches of the pasta salad contained locally sourced cilantro from a farm in Langley. However, the caterers ran out of the local cilantro, so they used imported cilantro to prepare the last batch of pasta salad. *Cyclospora* is not found in British Columbia, so it could only have come from imported produce. Only the people at the wedding who ate the salad at tables 30 to 35 became ill, and they ate the last batch of prepared pasta salad. The PHPs concluded the outbreak was caused by the imported cilantro. They contacted the federal Canadian Food Inspection Agency, who conducted a recall for this ingredient.

## Outbreak of foodborne illness at a restaurant

**Interviews**

When more than one group of people become ill after dining at a restaurant, usually at least one person will call and report this to the public health department. The PHP will contact the case, conduct an interview, and follow-up by contacting the other people in the group and the restaurant. Sometimes, the restaurant owner will also track and keep records of complaints. Other useful information to collect from the restaurant and cases are receipts or electronic records of what was served. The restaurant can inquire how many servings of that particular meal were sold during that period. In this example, four parties of diners complained of illness: one party from the first evening, and three the following day. The foods eaten included a wide variety of dinner and breakfast items.

**Collecting and testing food**

Unless the case has leftovers from the meal, there is often no food from the batch available. The PHP can visit the restaurant to determine if any ingredients or foods matching descriptions from the cases can be collected.

**Preparation of the food**

During preparation of foods in a restaurant on different days, foods may be prepared multiple times each day, sometimes by a variety of staff. If one kitchen staff worker was not trained properly on how to prepare a food, or if one kitchen staff worker had an infection that contaminated the food, only the food subject to the improper preparation or to contamination would be affected.

**Environmental investigation**

During an environmental investigation, PHPs will inspect the kitchens where food was prepared, interview the kitchen and catering staff, and look at the food safety plans and methods of preparation for foods. PHPs pay particular attention to sanitation and practices within the facilities to look for risk. Examples of practices and sanitation issues that have been linked to foodborne illness that PHPs assess include proper storage of food after delivery, whether foods have been correctly cooled after cooking, whether staff have been adequately trained to perform tasks, if there is an established sick policy in place and staff know about them, and the methods for and how often washrooms are cleaned and sanitized. In our example, the PHP learned that one staff member was ill that same evening and was sent home after vomiting in the washroom. This person did not serve any guests and had duties that included dishwashing, cutting up fruit for the morning shift the next day, cleaning public toilet areas, and mopping the floors. Cases that became ill from the evening group had visited the bathrooms before leaving the restaurant. Cases from the breakfast service the following morning reported eating the fruit on their plates. The PHP asked two cases to submit stool samples, and the test result came back positive for norovirus. The PHP concluded that the illness was due to the ill food-handler. The PHP recommended deep cleaning of washrooms and kitchen areas, and reviewed the handwashing and sickness policies with the manager. The PHP also recommended that the ill worker not come back to work until all symptoms were gone for at least seventy-two hours. The reason that symptoms must be cleared for this long is that norovirus infections have a long viral shedding period. People can continue to shed virus (be contagious) even though they begin to feel well as symptoms first start to subside.

**Figure 10** — Handwashing

The common exposure for these three examples were a food (peanut butter), an event (wedding), and a place (restaurant). Sometimes, exposures can result in some people becoming ill while others do not. There are several reasons why this might occur, relating to the food, the preparation of the food, and the susceptibility of potential hosts to infection. Clues were provided as to why the peanut butter in the first scenario or the pasta salad in the second scenario were not equally contaminated with their respective etiologic agents. In the case of the restaurant outbreak, groups of individuals were exposed to norovirus did not all experience the same symptoms or become ill. Specifically, many people who visited the washroom during the evening did not become ill, and many people who ate the fruit during breakfast that the ill worker cut up also did not become ill. Differences in responses to exposure to an etiologic agent are termed host susceptibility. This will be explored in the next section.

## Host susceptibility, symptoms, and stages of foodborne illness

Why do some people become ill and others do not, even when they have eaten the same meal together? Why do some people have severe symptoms of illness, while others have mild symptoms or no symptoms at all? How and why does foodborne illness occur if only a few microbial cells are ingested? These are the questions we will answer in this next section.

### Host susceptibility to foodborne illness

Populations are made up of individuals. Some may be healthy, some may be ill, and some may simply not have a fully developed or functioning immune system. People with an underlying condition that affects their overall health are called members of a vulnerable population. This group can be described as "YOPI," because it includes the young, whose immune systems are not fully developed; the old, whose overall health status is declining for a variety of reasons; pregnant people, whose immune systems are suppressed during pregnancy; and the immunocompromised who have an underlying illness or condition that increases their risk for infection.

The images in Figure 11 show examples of individuals in a typical vulnerable population. The young are considered to be children less than five to six years of age. Very young children under the age of eighteen months have the highest risk, as their immune systems are not fully developed. The old are generally considered those above sixty years of age. People who are more than eighty years old also have a higher risk within this category. Pregnancy suppresses many immune system functions to allow the body to grow the fetus. This is a high-risk period for the mother and the developing baby. Some microbes and toxins can cross the placental barrier and infect the baby during its development. The image representing the immunocompromised shows a person with an intravenous (IV) tube in their arm, but not all immunocompromised individuals look ill. For example, a person with diabetes could be part of this group, as high blood sugar (called hyperglycemia) provide nutrients for microbes to grow. Approximately 10% of the Canadian population lives with diabetes. Other conditions that make people immunocompromised include cancers, some auto-immune diseases, such as lupus or Crohn's, and having an organ transplant. Even taking antacids can temporarily increase a person's risk, as antacids change the pH in the stomach and gut in a way that provides a more hospitable environment for foodborne pathogens to survive and multiply.


**Figure 11** — YOPI –vulnerable population

## Chronic symptoms of foodborne illness

Earlier in this module, we defined gastrointestinal illness (diarrhea, vomiting, and abdominal cramps) as a common feature of foodborne illness. There are many other symptoms that may also be associated with foodborne intoxications and infections. Symptoms can be **acute** or **chronic**. Acute symptoms are sudden, such as vomiting or breaking out in hives. Chronic symptoms are those that occur for a long time, such as persistent, watery diarrhea (described in the wedding outbreak above). Chronic symptoms can take a long time to develop but be initiated by a prior illness. Another term for this type of symptom is **sequelae**, which is a disease that results from a prior injury or illness. Some researchers have estimated that between two to three percent of people who have a foodborne illness will develop some type of chronic sequelae (Lindsay, 1997). Chronic sequelae are terrible for people who have had a prior foodborne illness. That is why foodborne illness must be taken extremely seriously: some people develop life-long medical conditions that affect their health and enjoyment of life.

Figure 12 lists the types of chronic sequelae that may develop after a foodborne infection, including those that affect the fetus when the mother experiences a foodborne illness. The term for foodborne illness that affects the fetus is a **congenital infection**. The pathogens linked to these sequelae include *Salmonella*, *Campylobacter*, *Shigella*, *E. coli* O157 and other pathogenic forms of *E. coli*, *Yersinia*, *Listeria* and *Toxoplasma* (congenital infections), *Giardia*, and norovirus. Many other pathogens not shown in Figure 12 are also implicated in chronic sequelae, but these are less commonly reported (Batch, Henke, & Kowalcyk, 2013). The last line of the table describes chronic sequelae symptoms that affect the fetus when the mother experiences a foodborne illness. The term for foodborne illness that affects the fetus is a congenital infection. *Listeria* and *Toxoplasma* can both cause serious congenital infections.

| Chronic sequelae | Associated etiologic agents |
|---|---|
| **Irritable bowel syndrome (IBS)** | *Salmonella*, *Campylobacter*, *Shigella*, *E.coli* O157, *Giardia* |
| **Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis** | *Salmonella*, *Campylobacter*, *Shigella*, *Yersinia*, *Giardia* |
| **Reactive arthritis** | *Cryptosporidium*, *E. coli* O157, *Giardia*, *Shigella*, *Yersinia* |
| **Hemolytic uremic syndrome (HUS)** | *E. coli* O157:H7, *Shigella* |
| **Guillain-Barré syndrome (GBS)** | *Campylobacter* |
| **Neurologic disorders** | *Toxoplasma gondii* and *Listeria monocytogenes* |
| **Rheumatoid arthritis** | *Salmonella* |
| **Necrotizing fasciitis leading to amputation** | *Vibrio vulnificus* |
| **Congenital neurological issues (e.g., hearing and vision loss, epilepsy)** | *Listeria monocytogenes*, *Toxoplasma gondii* |

**Figure 12** — Chronic sequelae commonly reported after a foodborne infection

People may also develop further complications following sequelae such as HUS, that lead to further complications such as hypertension, cardiovascular disease, diabetes, pancreatitis, and others. Much is still unknown about the triggers for many of the chronic sequelae listed in Figure 12, and researchers are now beginning to track prospective illness for those who have been confirmed to have had a prior foodborne illness. Many researchers are considering the overall economic impacts and changes to a persons quality of life that arise from both the acute stages of foodborne illness and chronic long term health consequences (Hoffman & Walter, 2019).

To hear personal stories of the effects of haemolytic uremic syndrome and Guillain-Barré, click on these sites and videos.

***E. coli* O157:H7 – Yet another client story**
https://www.marlerblog.com/legal-cases/e-coli-o157h7-yet-another-client-story/

**Suddenly Paralyzed, 2 Men Struggle To Recover From Guillain-Barre**
https://www.npr.org/sections/health-shots/2016/05/16/476494277/suddenly-paralyzed-two-men-struggle-to-recover-from-guillain-barre

Descriptions of the most common acute symptoms specific to foodborne pathogens are shown in Figure 13. Medically serious, acute symptoms that are blood-borne affect the entire body; these are known as systemic infections. Many pathogens can cause serious infections such as meningitis and septicemia, although these are rare compared to the occurrence of diarrhea. Pathogens that cause systemic infections include *E. coli*, *Salmonella*, and *Listeria*. Figure 13 also includes the typical onset and duration times for the illness, which is important for differential diagnosis, as they are pathogen specific. Types of foods commonly associated with the pathogens are also included. More information about the pathogens may be found in the supplemental text for this module.

| | Etiologic agent | Onset | Symptoms | Duration | Foods associated |
|---|---|---|---|---|---|
| BACTERIA | *Bacillus cereus* intoxication | 0.5–6 hrs | Sudden onset of severe nausea and vomiting; diarrhea may be present | 24 hrs | Rice, noodles, meats and sauces; in dried and processed foods |
| | *Bacillus cereus* infection | 6–24 hrs | Abdominal cramps, nausea, watery diarrhea | 24–48 hrs | |
| | *Campylobacter* | 2–5 days | Diarrhea (may be bloody), fever, nausea, vomiting | 2–10 days | Raw and undercooked poultry, raw milk, contaminated water |
| | *Clostridium botulinum* intoxication | 12–72 hrs | Vomiting, diarrhea, blurry vision, difficulty swallowing and breathing, descending paralysis | Days to months | Low-acid home-canned foods, garlic in oil, fermented fish and meat, smoked fish |
| | *Clostridium botulinum* infection (infants) | 3–30 days | Weakness, floppy-baby syndrome, difficulty feeding, low muscle control | Weeks to months | Honey, dust, home-canned vegetables and fruit, corn syrup |
| | *Clostridium perfringens* | 6–24 hrs | Abdominal cramps, nausea, watery diarrhea | 24–48 hrs | Stews, gravies, temperature-abused foods (chili, casseroles) |
| | *E. coli* O157:H7 and other shigatoxigenic *E. coli* | 2–10 days | Severe abdominal cramps, bloody diarrhea (common), vomiting, blood in urine, muscle pain | 5–10 days | Raw and undercooked beef, raw or unpasteurized milk and juice, vegetables (leafy greens, sprouts), contaminated water |
| | *Listeria monocytogenes* | 3–70 days | Fever, muscle aches, nausea, diarrhea† | Days to weeks | Soft cheese, raw milk, deli meats, ice cream, smoked fish, fruits, vegetables |
| | *Salmonella* | 6–72 hrs* | Diarrhea, fever, abdominal cramps, vomiting | 4–7 days | Eggs, poultry, raw milk, and juice, vegetables (leafy greens, sprouts), fruits, nuts |
| | *Shigella* | 1–3 days | Abdominal cramps, fever, diarrhea | 4–7 days | Foods infected by food handlers (raw vegetables, salads, sandwiches), raw water contaminated with feces |
| | *Staphylococcus aureus* intoxication | 0.5–8 hrs | Sudden onset of severe nausea and vomiting; abdominal cramps, fever, and diarrhea may be present | 24–48 hrs | Temperature-abused foods and foods infected by food handlers, such as eggs, meats, custards, sauces |
| | *Vibrio parahaemolyticus* | 1–2 days | Watery diarrhea, abdominal cramps, nausea, vomiting | 2–5 days | Raw and undercooked seafoods, particularly bivalves (oysters, clams, mussels) |
| | *Yersinia enterocolytica* | 3–7 days | Appendicitis-like—fever, cramps, diarrhea, and vomiting | 1–3 weeks | Undercooked pork, raw milk, tofu, contaminated water |

| | Etiologic agent | Onset | Symptoms | Duration | Foods associated |
|---|---|---|---|---|---|
| VIRUS | Hepatitis A | 15–50 days (average: 28–30 days) | Diarrhea, dark urine, jaundice, and flu-like symptoms (fever, headache, nausea, and abdominal pain) | Weeks to months | Foods infected by food handlers, shellfish and foods contaminated with feces in raw water |
| | Norovirus | 24–48 hrs | Severe vomiting in 50% of illnesses, diarrhea, fever, abdominal cramps, nausea, fatigue | 1–3 days | Raw and undercooked seafoods, particularly bivalves (oysters); foods infected by food handlers or contaminated water; raw frozen fruits (raspberries), icing, salads |
| PARASITE | *Cryptosporidium* | 2–10 days | Watery diarrhea, stomach cramps, upset stomach, fever | Weeks to months | Foods infected by food handlers or contaminated water |
| | *Cyclospora* | 1–14 days | Watery diarrhea, appetite and weight loss, stomach cramps, vomiting, nausea, fatigue | Weeks to months | Fresh imported fruits and herbs (berries, cilantro, basil) |
| | *Giardia* | 1–2 weeks | Diarrhea, cramps, gas | Days to weeks | Foods infected by food handlers or contaminated water |
| | *Toxoplasma gondii* (congenital infection) | 5–23 days | Fever and mild flu for mother; child will develop congenital symptoms—impaired eyesight, mental retardation, seizures | Chronic sequelae may develop | Passed from mother to child through contaminated water or handling of cat feces |
| | *Trichinella* | 1–2 days; 2–8 weeks | First days—acute nausea, vomiting, diarrhea, fever and abdominal cramps; weeks later—muscle soreness, rash, weakness, cardiac and neurological symptoms | Months | Pork; raw and undercooked game meat (bear, walrus, seal), including cold fermented sausages—occurs in omnivore species |

* Low dose *Salmonella* infections have been documented to take up to sixteen days.

† Pregnant women may only experience mild flu-like symptoms. Blood-borne invasive infections (meningitis, septicemia) may occur. Serious complications for fetus, including abortion.

**Figure 13** — Clinical symptoms, onset, and duration for etiologic agents causing foodborne illness (adapted from American Medical Association *et al*., 2004, and Heymann, 2008).

## Stages of disease development

Pathogenesis is the ability of a pathogen to cause disease in a host. The interaction between a host and a pathogen is dynamic, since each modifies the responses and functions of the other. For example, if the host's immune system is compromised, even a small number of pathogens might cause disease, whereas the same exposure would not cause illness in a person with a healthy immune system. The number of pathogens needed to cause disease is known as the infectious dose. This varies for each type of pathogen and for each host.

Those who work in the food industry must be aware that consumers have different health statuses. Foods sold to vulnerable populations must be prepared to reduce the risk of microbial pathogen exposure.

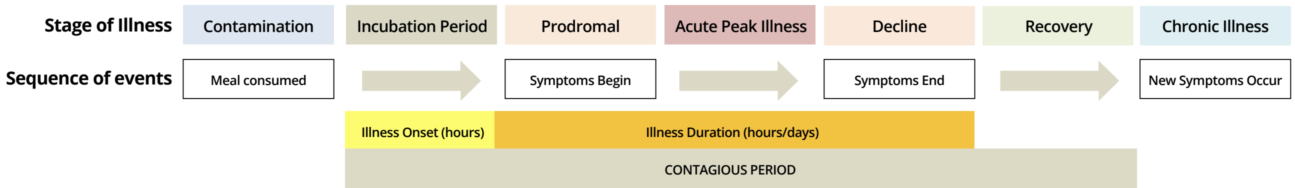



**Figure 14** — Stages of foodborne illness

Generally speaking, an acute infectious disease progresses through five stages.

1. The first stage is the **contamination** of the host with a microbial agent such as a virus, a bacteria, or a parasite. For a foodborne illness, the contamination event is when a meal contaminated by a foodborne pathogen is consumed.

2. The second stage is the **incubation** period, when the invading pathogen first acquires nutrients, evades immune detection, and then enters the logarithmic phase of its growth. During this stage, you might not even know you are infected. The gut is a very acidic environment, with pH between 2 to 3. When foods are first eaten, most microbes will be destroyed in the gut. However, if large numbers of microbes are present, once food moves out of the gut and into the digestive tract, the pH increases to above 6, allowing most microbes to survive and grow.

3. The **prodromal** stage follows, associated with the host presenting the first symptoms of infection, such as upset stomach, abdominal cramps, dizziness, and headache. At this stage, the case is experiencing symptoms and is termed **symptomatic.** The illness onset is defined as the time from when the suspect meal was first consumed to when the first symptoms began.

4. The **peak** of the disease is when clinical presentation of the most severe symptoms occurs, such as severe vomiting, diarrhea, chills, and aches. This is the acute phase of illness. Following the acute stage of the illness, the numbers of infectious pathogens begins to **decline,** and the symptoms become less severe. Once most symptoms have resolved (ended), this would mark the end of the duration of symptoms. The duration of an illness is pathogen-specific, although there is variation within any population. When these numbers are reported as an average, the median and range of times for illness onset and illness duration are often reported.

5. The **recovery** stage is associated with alleviation of most symptoms and host clearance of the pathogen (etiologic agent of illness). Often, people will continue to report extreme fatigue. This is also when people can pick up a secondary infection, as their immune system is depressed by having been engaged in fighting off the initial infection. A secondary infection is different from chronic illness and sequelae. Chronic sequelae may occur years after the initial infection.

During the period spanning the contamination stage to the recovery stage, an individual can transfer the pathogen to others. This is called the contagious period. Transfer of infectious agents can be from unwashed hands, termed "fecal-oral." This means that pathogens present in the feces are transferred, usually via poor hand-hygiene, to a food or other surface that allows for the pathogen to be ingested. For example, in the case of *Salmonella*, it is possible to harbour the bacteria for weeks or even months. Sometimes, pathogens can be present in the host and not cause any symptoms. Someone who has an infection but does not have symptoms is termed **asymptomatic**.

Norovirus is highly infectious and has been demonstrated to be transferred via air (aerosol), as it can be suspended in droplets following severe vomiting. In the restaurant outbreak example provided earlier, it is possible that individuals who went into the washroom after the staff member vomited were exposed to airborne droplets.

## Food attribution

Food attribution is the study of finding out what types of foods are sources of foodborne illness. One of the questions asked during food attribution research is, "At what point in the food chain does a pathogen originate?" This often happens at the farm level; where failure to control the pathogen occurs, contamination may take place or be amplified during processing or at home. Figure 15 illustrates a point of attribution between markets and preparation/consumption. Points of attribution can occur anywhere between the reservoir and exposure, depending on where the pathogen originates, at source, or via cross-contamination.

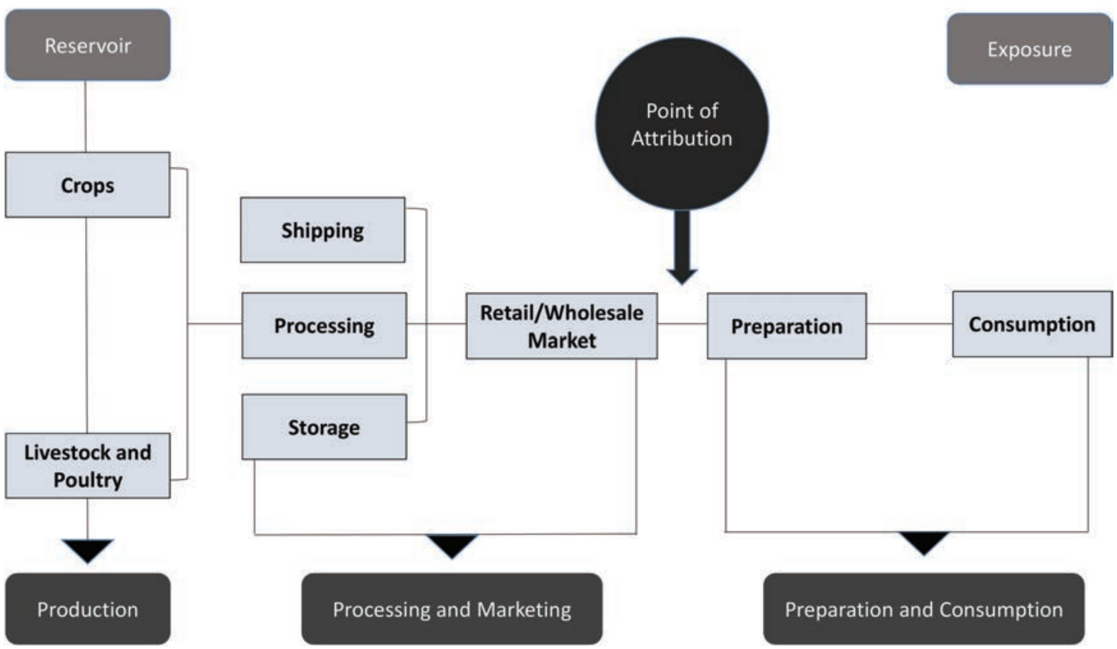



**Figure 15** — Attribution points along the food chain

Health and food agencies in the United States reviewed foods that contributed to illnesses, hospitalizations, and deaths over a ten-year period (1998–2008). They attributed illness to aquatic, land (beef, poultry, eggs, milk), and plant (grains, nuts, fruits, vegetables etc.) commodities. Produce was the source for most illnesses (46%) over this time period. Land-based commodities (42%) were also important, along with aquatic foods (6%).

Recent research focused on attribution for four pathogens: *Salmonella*, *Listeria*, *Campylobacter*, and *E. coli* O157. The 2017 report found that sprouted vegetables were attributed to the majority of *Salmonella* illnesses (at 17%; slightly more than chicken, at 14%). Vegetable crops were attributed to the majority of *E. coli* O157 illnesses at 46% (beef caused fewer illnesses, at 26%). As expected, dairy products were attributed to the majority of *Listeria* illnesses (48%), followed by fruits at 29%. Chicken (48%) was attributed to the majority of *Campylobacter* illnesses, followed by seafood sources (12%). For the full report, visit the CDC pages and reports.

**Foodborne illness source estimates for 2017 for *Salmonella*, *Escherichia coli* O157, *Listeria monocytogenes*, and *Campylobacter* using multi-year outbreak surveillance data, United States**
https://www.cdc.gov/foodsafety/ifsac/pdf/P19-2017-report-TriAgency-508.pdf

**Attribution of Foodborne Illness: Findings**
Attribution over a ten-year period
https://www.cdc.gov/foodborneburden/attribution/attribution-1998-2008.html

**Annual Reports on Foodborne Illness Source Attribution Estimates**
Attribution to four pathogens
https://www.cdc.gov/foodsafety/ifsac/annual-reports.html

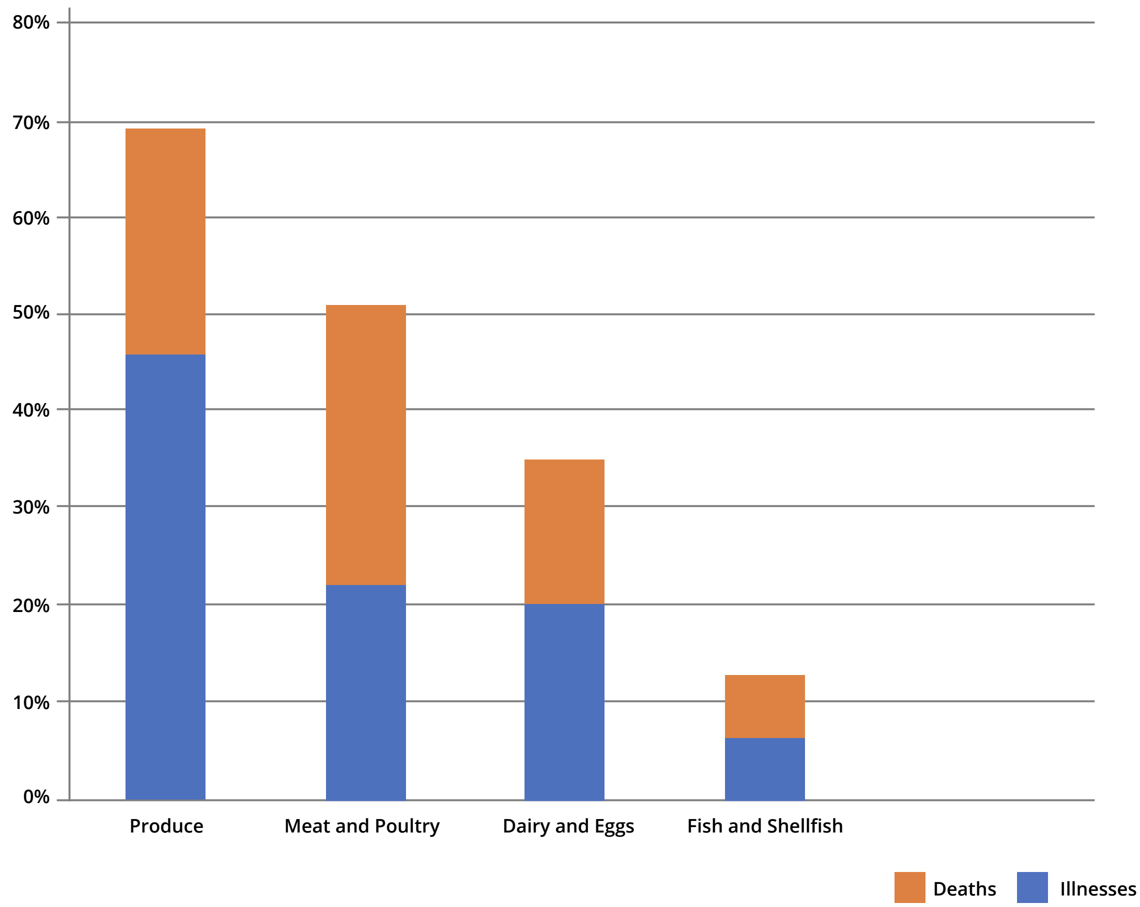



**Figure 16** — Food attribution in the U.S. over a ten-year period

In Canada, source attribution has been studied by FoodNet Canada, in collaboration with many other agencies. In this work, overall prevalence of pathogens is compared among four areas: human cases, at the farm, in water, and from retail foods. In the latest published infographic from the Government of Canada (provided at the following link) poultry and poultry sources were described as important sources for *Salmonella* and *Campylobacter*. This is a little different from U.S. data, as not all food commodities are being examined. To learn more about FoodNet research, refer to the following site.

**Infographic: Farm to Fork: FoodNet Canada 2017 and 2018 Results**
https://www.canada.ca/en/services/health/publications/food-nutrition/infographic-foodnet-canada-annual-report-2017.html

https://www.canada.ca/en/services/health/publications/food-nutrition/infographic-foodnet-canada-annual-report-2018.html

**Figure 17** — How do these commodities become contaminated?

## Fruits and vegetables

Fresh produce and canned, frozen, and juiced fruits and vegetables may acquire pathogenic microbes from environments where they are grown, during transport, and during secondary processing. Fruits and vegetables may be exposed to *E. coli* from manure-based fertilizers during run-off or from irrigation (ditch) water. They may be exposed to *Listeria*, *Bacillus*, and *Clostridium* that are naturally present in soil. *Bacillus* and *Clostridium* are problematic because they are spore-formers, are environmentally resistant, and can survive normal cooking processes. *Salmonella* is carried by birds and can contaminate dried-surface nut crops. *Salmonella* is also present in animal feces. Contaminated water may contain Hepatitis A virus, *Cyclospora*, *Cryptosporidium*, and other pathogens. Infected workers can also contaminate fruits and vegetables with norovirus, hepatitis, and fecal pathogens.

**Figure 18** — Honey can contain spores of *Clostridium botulinum*.

## Dairy, eggs, and honey

*E. coli*, *Salmonella*, *Campylobacter*, and *Staphylococcus aureus* can enter milk during the milking process. This can occur from gut pathogens in the cow (e.g., *E. coli*) or if the cow has an udder infection (e.g., mastitis and *S. aureus*). Pasteurization will kill many of these bacteria. However, post-pasteurization temperature abuse will allow spore-formers and residual microbes to grow in this nutrient-rich food. For example, Gopal et. al (2015) found that aerobic spore-forming spoilage microbes such as *Sporoscarcina* and disease-causing *Bacillus cereus* can multiply rapidly when milk temperature is elevated, and are difficult to remove from dairy equipment when they reside in biofilms (note: biofilms will be covered in the next lesson). Cheese or other products made from unpasteurized (raw) milk can also harbour these pathogens. The pathogen most commonly associated with eggs is *Salmonella*. Cracked or dirty eggs should be thrown away, as bacteria may enter the protein-rich yolk. Washed eggs should not be kept at room temperature for more than two hours, and all foods made with eggs should be refrigerated. Raw and pasteurized honey contains spores of *Clostridium botulinum*, a known risk for infants with underdeveloped immune systems.

**Figure 19** — Soft mould-ripened cheese is made only with pasteurized milk in British Columbia.

## Poultry

Chicken and other birds may carry *Salmonella*, *Campylobacter*, *E. coli*, or *Staphylococcus aureus*. Farmed chickens are grown in close contact, allowing microbes to easily spread from bird to bird. Prevalence of *Salmonella* on the farm can be related to irrigation water, housing practices, and wild birds contaminating outdoor areas (including transmission of avian flu to domestic flocks). Bacteria can be spread during transport in shared poultry cages, as well as during slaughter. Poultry are washed in large tubs of water that can spread bacteria from one carcass to another, as well as other slaughter and cleaning processes such as removal of gizzards, de-feathering, and sectioning.

## Meat

Ground meat has the potential to support the growth of harmful pathogens such as *E. coli*. Once beef is ground, there is a larger surface area, a higher redox potential (available oxygen), and greater opportunity for *E. coli* to spread from the outside of the meat surface to all portions of the ground meat. Pinned and tenderized steaks also have an increased risk of pathogens being carried from the exterior of the meat to the interior of the otherwise sterile meat muscle. Cattle that carry *E. coli* can spread the bacteria during transport, and microbes can contaminate meat during the slaughter process.

Other pathogens that can infect meat include *Yersinia enterocolitica*, found in raw or undercooked pork. Ready-to-eat and deli meats may become contaminated with *Listeria*.

### Seafood

*Campylobacter*, *Salmonella*, Norovirus, Hepatitis A, and *Vibrio spp*. are all pathogens that may contaminate seafoods. Shellfish often become contaminated when their environment contains human and animal waste. Scombroid intoxication occurs when fish with high levels of naturally occurring muscle histidine (e.g., tuna, mackerel, bluefish, and mahi-mahi) are temperature abused. Bacteria present on these fish will convert the histidine to histamine. The toxins are not destroyed by freezing, cooking, smoking, curing, or canning. Naturally occurring marine bacteria such as *Vibrio* can be present in bivalve shellfish through the normal shellfish filter-feeding process. Vibrio will grow rapidly at temperatures above 10oC. W*hen* raw or undercooked shellfish is consumed, infection can occur. Other naturally occurring marine biotoxins can also contaminate bivalves leading to paralytic, diarrhetic, and amnesic shellfish poisoning.

## Food allergy and food intolerance

Food allergies are a result of the body having an immune reaction to a part of the food. These can be medically serious, causing swelling in the throat and tongue, trouble breathing, and symptoms of anaphylactic shock. People with severe allergies must carry epinephrine (adrenaline), which helps to reverse the symptoms. Food-allergy responses are divided into two categories, based on whether they are immunoglobulin (IgE)-mediated or not. IgE allergies are the most severe. In Canada, there are ten priority allergens and food groups.

1. Crustaceans and molluscs
2. Eggs
3. Fish
4. Milk
5. Mustard
6. Peanut
7. Sesame
8. Soy
9. Tree nuts
10. Wheat and triticale

Other protein-containing foods may also cause allergies and allergenic reactions; for example, lupin. Sulphites in foods also cause allergen-like reactions.

Food intolerances differ, as these occur due to the body not being able to absorb food nutrients. Examples of food intolerances include lactose intolerance, from the inability to digest the milk sugar lactose; and and gluten intolerance to protein found in wheat, barley, rye, oats, and triticale. In comparison, celiac disease is not food intolerance. Celiac disease is an auto-immune disease affecting approximately 1% of the population. The inability to absorb gluten causes damage to the small intestine and many severe symptoms.

The Government of Canada requires all foods containing allergens, glutens, and added sulphites to be declared and appropriately labelled.

To learn more about food allergies and food intolerance, refer to these sites:

**Food Allergy Canada**
https://foodallergycanada.ca/food-allergy-basics/food-allergies-101/what-are-food-allergies/priority-food-allergens/

**Government of Canada – Food Allergies and Intolerances**
https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-allergies-intolerances/food-allergies.html

**Government of Canada – Allergens and gluten sources labelling**
https://www.canada.ca/en/health-canada/services/food-allergies-intolerances/avoiding-allergens-food/allergen-labelling.html

## Lesson D Quiz

1. Which phrase best describes pathogenic microbes?
   a. They harm or cause disease in a host organism.
   b. They trigger an allergic reaction when ingested.
   c. They promote digestive health and remain in the gut.
   d. They provide nutrients essential to the fermentation process.

2. What is the acronym YOPI used as a reminder of?
   a. Proper handwashing procedure
   b. Safe methods for storing food in coolers
   c. The people most susceptible to foodborne illnesses
   d. The steps in cleaning and sanitizing preparation equipment

3. Which definition applies to microbial foodborne infection? Select all that apply.
   a. Illness results from eating food which requires bacterial growth during its preparation.
   b. Illness results from eating food in which illness causing pathogens are present. The pathogens multiply in tissues in the body.
   c. Illness results from eating food that has been contaminated with chemicals (toxins) during preparation.
   d. Illness results from eating food with pre-formed toxins from bacteria, fungus, algae or other microbes. The toxins are present in the food before it is ingested.

4. Which definition applies to microbial foodborne intoxication? Select all that apply.
   a. Illness results from eating food which requires bacterial growth during its preparation.
   b. Illness results from eating food in which illness causing pathogens are present. The pathogens multiply in tissues in the body.
   c. Illness results from eating food that has been contaminated with chemicals (toxins) during preparation.
   d. Illness results from eating food with pre-formed toxins from bacteria, fungus, algae or other microbes. The toxins are present in the food before it is ingested.

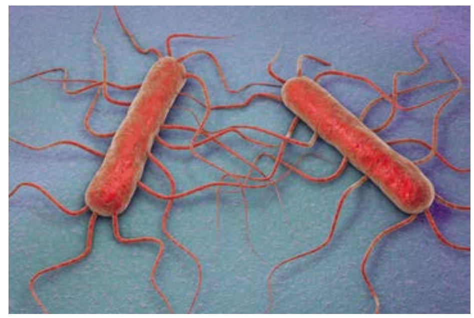
5. Which etiologic agent has the longest onset period?
   a. *E. coli*
   b. *Salmonella*
   *c. Hepatitis A*
   *d. Listeria monocytogenes*

6. Which etiologic agent may produce loss of muscle control as a symptom?

***E. coli***

***Campylobacter***

***Clostridium botulinum***



***Listeria monocytogenes***

7. Which etiologic agent is most commonly associated with eggs?
   a. *Shigella*
   b. *Salmonella*
   c. *Bacillus cereus*
   d. *Clostridium perfringens*

8. What makes some groups of people more vulnerable to foodborne illnesses than others?
   a. They may have inadequate standards of personal hygiene.
   b. They may be more susceptible due to age or pre-existing condition.
   c. Their knowledge of food safety may be lacking or misguided.
   d. They don't have a food thermometer to ensure food is cooked or a refrigerator thermometer to ensure perishable foods are cold enough.

9. Which statement describes irritable bowel disease?
   a. It is an acute condition caused by exposure to allergens.
   b. It is a chronic condition caused by viruses passed on by food handlers.
   c. It is a chronic condition which may be the result of a foodborne infection.
   d. It is an acute condition which may be the result of improper sanitation in kitchens.

10. Which pair of statements accurately describes the difference between food intolerance and food allergy?

a.

| Food intolerance | Food allergy |
|---|---|
| It occurs when the body is unable to absorb food nutrients. | It occurs when the body reacts to nutrients it has absorbed. |

b.

| Food intolerance | Food allergy |
|---|---|
| It increases the likelihood of contracting foodborne illness. | It may turn into a chronic condition as a result of a foodborne illness. |

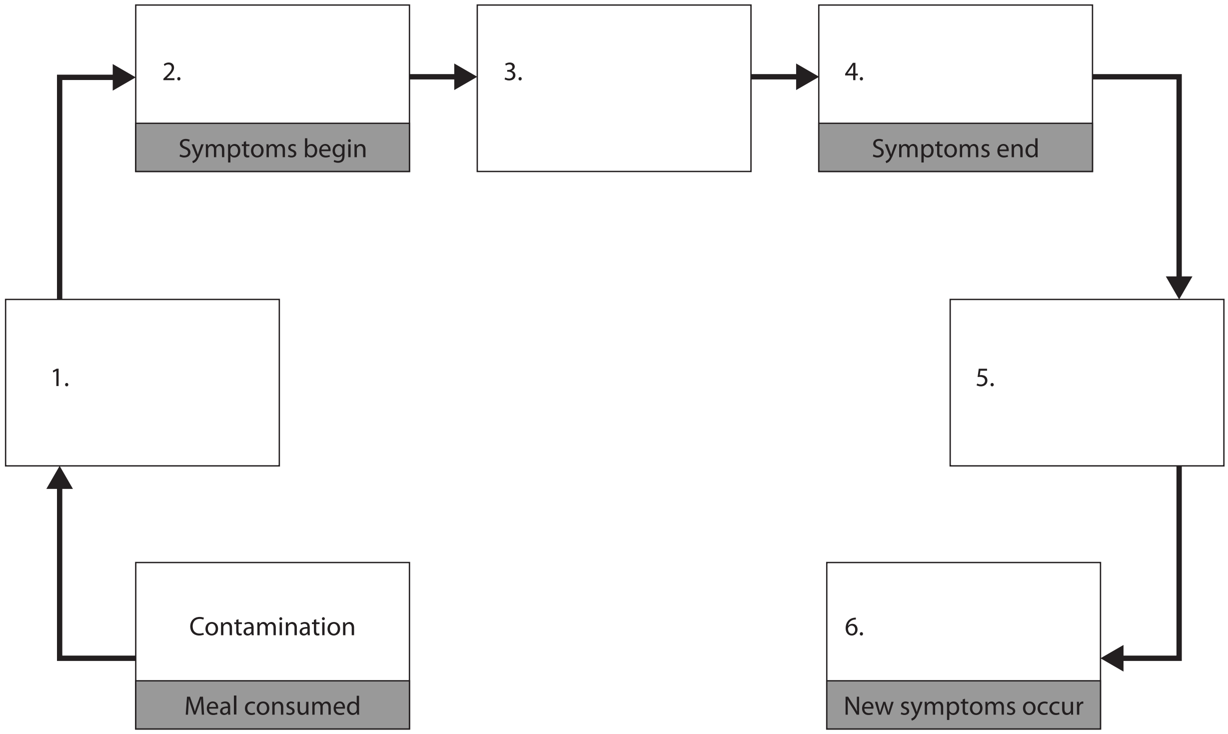
c.

| Food intolerance | Food allergy |
|---|---|
| It is caused by viruses present in the digestive system. | It is caused by bacteria present in the food. |

11. Why should food service workers understand how foodborne illnesses are identified and controlled?
    a. Food service workers are the most common source of pathogens in prepared food.
    b. Food service workers are usually the first ones to be informed of a foodborne illness.
    c. Food service workers are not responsible for management and control food safety.

12. In recent years, what have been the top three most common types of food causing foodborne illness?
    a. Fish and shellfish, meat and poultry, dairy and eggs
    b. Produce, meat and poultry, fish and shellfish
    c. Dairy and eggs, fish and shellfish, produce
    d. Meat and poultry, canned foods, dairy and eggs

13. Select the correct order for the stages of illness during food poisoning.




  a. Decline
  b. Chronic illness
  c. Prodronal
  d. Recovery
  e. Acute peak illness
  f. Incubation period

14. Which question would not be useful in an interview to inform a differential diagnosis?
  a. What were the last few meals you ate?
  b. Is anyone else you know also ill with the same symptoms?
  c. How often do you wash your hands?
  d. Have you traveled recently?

Check your answers with the answer key provided at the end of this textbook.

# Lesson E: Sanitation, Personal Hygiene, Pests, and Cross-Contamination

## Introduction

Microbes are everywhere in the environment. Microbes are found on us and in us.

Review the four-page comic book from the World Health Organization at the following link.

**Did You Know that Superbugs Can Be Found in Food?**
http://apps.who.int/iris/bitstream/handle/10665/259466/WHO-NMH-FOS-FZD-17.7-eng.pdf?sequence=1

While this comic book is concerned with the use of antibiotics and spread of superbugs, it does a great job of graphically illustrating the basics of how microbes from the environment spread to our food supply. Microbes are found in fecal matter, on our skin, in soil and water, on our pets (e.g., dogs, cats), in animals raised for food (e.g., chickens, cows), and in other farmed foods (from lettuce in the field to oysters in the ocean).

Of the "WHO Five Keys to Safer Food" given in the comic (see the last page), the most important one is "Keep clean." The words "sanitation" and "personal hygiene" in the title of this lesson reflect this key. Two other keys refer to issues of cross-contamination: "Separate raw and cooked" and "Use safe water and raw materials." The transfer of microbes from one place to another is called "contamination." Cross-contamination can occur when microbes on one substrate or surface are transferred to another substrate or surface when sanitation or other barriers fail to stop the movement.

In this lesson, we will discuss how to properly clean and sanitize equipment and surfaces, what should be in a sanitation plan, the importance of personal hygiene in food handlers, and the impacts of illness on our food supply.

### Learning outcomes

Upon completion of this lesson, learners will:

- Define the components of a food-premises sanitation plan
- Describe cleaning and sanitation methods for food premises and kitchenwares
- Identify pest issues and how to control their impacts on food premises
- Discuss the importance of personal hygiene for food handling
- Identify how food-worker illness contributes to foodborne illness
- Identify cross-contamination risks in food-supply chains and during food processing
- Discuss ways to prevent cross-contamination

## Readings and resources

**Disinfectants and sanitizers for use on food contact surfaces**
http://www.ncceh.ca/sites/default/files/Food_Contact_Surface_Sanitizers_Aug_2011.pdf

**Basic Elements of Equipment Cleaning and Sanitizing in Food Processing and Handling Operations**
http://ucfoodsafety.ucdavis.edu/files/26501.pdf

**Cleaning and sanitation program**
http://www.inspection.gc.ca/food/general-food-requirements-and-guidance/preventive-controls-food-businesses/cleaning-and-sanitation-program/eng/1511374381399/1528206247934

**Pest control**
http://www.inspection.gc.ca/food/general-food-requirements-and-guidance/preventive-controls-food-businesses/pest-control/eng/1511206644150/1528205213795

**Youtube: eFoodhandlers Inc. Basic Food Safety (watch all six videos for an overview of the basics)**
https://www.youtube.com/watch?v=GgzO5_YQDIl&list=PLuZ86vZDT5-kjEBqTfzDExJWjC5Epvmnh

## Terminology

This lesson uses quite a few acronyms, such as C&S (clean and sanitize). These are included as part of the key terms list that follows.

**asymptomatic carrier**
bactericidal
**biofilm**
broad-spectrum
**clean and sanitize (C&S)**
clean in place (CIP)
clean out of place (COP)
close-to-food-contact-surface (C-FCS)
**communicable illness**
**cross-contamination detergents**
**disinfectants**
**exclusion policy**
food-contact surface (FCS)
fumigation
fungicidal
harbourage
horizontal transmission
insecticidal
microbicidal
mycobactericidal
non-food-contact-surface (N-FCS)
persistent
pesticidal
reservoir
residue testing
**sanitation**
**sanitize**
**standard operating procedure (SOP)**
**verification**
vertical transmission
virucidal

## Activity: Why is this lesson important?

To reinforce the importance of this lesson, let's start with an activity.

Go to the Public Health Act Food Premises Regulation at this link:
http://www.bclaws.ca/civix/document/id/loo82/loo82/11_210_99#section1

For each of the following issues, is there a requirement or requirements in the Food Premises Regulation? Answer yes or no for each.

- Sanitation procedures
- Employee hygiene
- Control of illnesses (communicable diseases)
- Pest control
- Protection of food from contamination
- Storage of chemicals

Check your answers with the answer key provided at the end of this textbook.

# Sanitation

In this lesson, the term sanitation includes the actions of cleaning and sanitizing (C&S) surfaces, kitchenwares, and food premises.

Figure 1 outlines one approach you might consider to develop a sanitation plan. The plan will identify the "what" and "how to" for cleaning and sanitizing tasks, as well as the "when" and "how often."

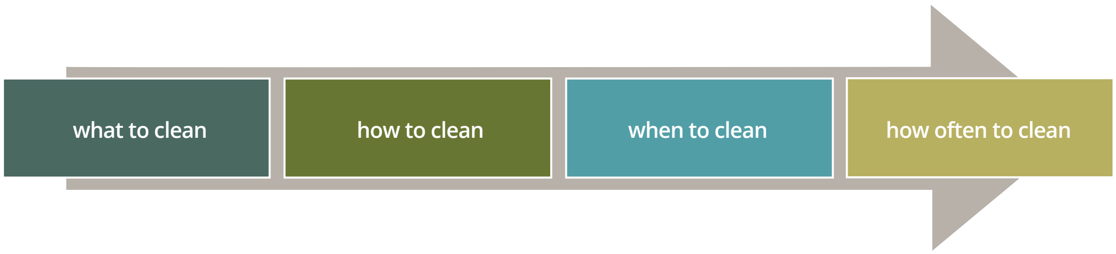



**Figure 1** — Components of a sanitation plan

## Sanitation plan components

A **sanitation plan** is more than just a list of steps to clean a surface or piece of equipment. It must also lay out:

- The item or items to be cleaned "what to clean"
- Tasks for deep cleaning and their frequency "how to clean"
- Cleaning and sanitizing products to be used "how to clean"
- Equipment and utensils to be used for sanitizing "how to clean"
- Methods to verify the sanitizer is at the correct concentration "how to clean"
- At what time(s) of day sanitation should occur "when to clean"
- How often sanitation is to occur "how often to clean"
- Verification procedures to ensure sanitation has occurred that meets the standards of your operation. These will likely involve sanitation schedules and log-sheets that staff sign once a sanitation task is performed.

The "what to clean" component can be divided into several areas, such as:

- Kitchenwares (e.g., dishes, utensils, cloths)
- Equipment (clean in place [CIP] and disassembled or clean out of place[COP])
- Food-contact surfaces (e.g., cutting boards)
- Structural items (e.g., shelving, lighting)
- Rooms (e.g., floors, walls)

The chemicals and methods used to clean and sanitize these areas may differ (the "how to clean" component). Generally, an outcome-based approach is used, with specific criteria for selected activities involving food-contact surfaces and kitchenwares. For example, only food-grade detergents and sanitizers can be used on food-contact surfaces and kitchenwares. The next section provides details on how detergents and sanitizers reduce microbial levels.

The last two components, "when to clean" and "how often to clean," both involve time, but "when" refers to the optimum time during a twenty-four-hour period or a food-production period, while "how often" is the frequency, such as five times per day or once per year. Rather than a specific time period, these parameters may instead depend on operational critical limits for some operations or in some premises. For example, a sanitation plan might state that "The packaging line will be shut down for a 4 hour cleaning period when a conveyor belt ATP test exceeds one million, or when the line has been operating for more than 24 hours."

Appendix D: Sample Cleaning Schedule and Sanitation Plans contains an example cleaning calendar and sanitation plan.

If the "what," "how to," "when," and "how often" of a sanitation plan is still confusing, think of your and your family's personal dental care as an example. Each person has daily dental care regimens of brushing and flossing their teeth ("what"). For tooth brushing, the frequency ("how often") is twice per day for everyone, in the morning and at bedtime. The specific "when" of bedtime varies—Mom and Dad brush their teeth at 11 p.m., but Junior brushes his at 7:30 p.m. Perhaps flossing happens once per day, at bedtime. In addition, toothbrushes may get replaced every two months (think equipment maintenance), and dental visits may be twice per year (deep cleaning and verification). This sanitation plan for family dental care does not discuss the "how to" for brushing or flossing, but it does lay out the daily times and the frequency. If you wanted to verify that your child had brushed or flossed, you would look for evidence. For example, is the toothbrush wet? Do the child's teeth look clean?

## Clean and sanitize

The term **"clean and sanitize" (C&S)** is used when describing how to properly decontaminate a food-contact surface. "Disinfect" and "disinfecting a surface" do not mean the same as the term "clean and sanitize" and are not used in the world of food. Sanitizers are not the same as disinfectants: these differences are shown in Figure 2.

| Product | Description |
|---|---|
| Disinfectants | • Used in hospitals<br>• Require a drug identification number (DIN) from Health Canada<br>• Contain chemicals that kill bacteria and/or viruses<br>• Can be used on non-food-contact surfaces (e.g., medical equipment), with some able to be used on food-contact surfaces |
| Sanitizers | • Used in food premises<br>• Do not require a DIN unless they make a disinfectant claim<br>• Reduce the numbers of, but may not kill, microbes on surfaces<br>• To be recognized by Health Canada, must reduce bacteria by 5 logs in 30 sec<br>• Used on food-contact surfaces (there are also non-food-contact sanitizers) |

**Figure 2** — Differences between disinfectants and sanitizers

The important differences to remember are that disinfectants must have a DIN and are usually used at much higher concentrations than sanitizers. Many sanitizers may be used as a disinfectant at higher concentrations. For example, bleach can be used as a disinfectant at concentrations from 1000–5000 ppm and as a sanitizer at 100–200 ppm. However, many disinfectants (such as formaldehyde or glutaraldehyde) cannot be used as a sanitizer because they are toxic and therefore unsuitable for food-contact surfaces.

The terms "cide" and "cidal" are Latin, and mean "killer" or "the act of killing" respectively. They are used as suffixes, and are placed at the end of nouns to describe what is being killed. They can be found in seven terms used in this lesson:

| Term | Meaning |
|---|---|
| microbicidal | killing of microbes |
| bacteriocide or bactericidal | killing bacteria |
| virucidal | killing viruses |
| fungicidal | killing of fungi |
| insecticide or insecticidal | killing of insects |
| mycobactericidal | killing of mycobacteria |
| sporicidal | killing of bacterial spores |
| pesticide or pesticidal | killing of pests |
| rodenticide | killing of rodents |

Throughout this section on sanitizers, personal hygiene, and pests, we may refer to these terms to identify the ability of sanitizers, chemicals, or other agents to control microbes and pests.

The purpose of C&S procedures is to control microbial activity. Cleaning involves removing any visible debris—food scraps, crumbs, grease, or soil—that those invisible microbes can feed on. Sanitizing is an attempt to remove or reduce invisible hazards (i.e. pathogenic microbes) to numbers tolerable in a food establishment.

This next video provides a good overview of C&S procedures.

**Basic Food Safety: Chapter 5 "Cleaning and Sanitizing" (English)**
https://www.youtube.com/watch?v=RAFMIXPq9BE

The basic steps to carrying out C&S are shown in Figure 3.

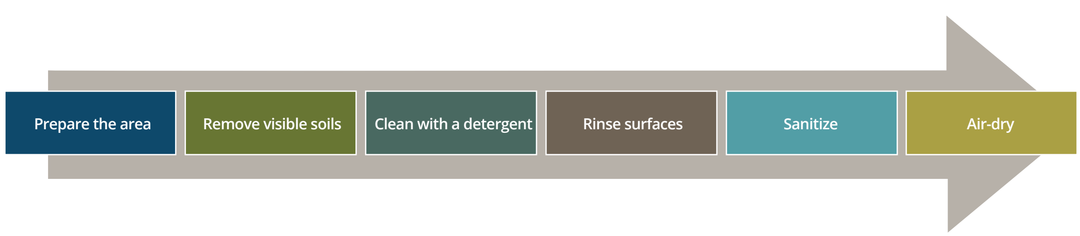



**Figure 3** — Cleaning and sanitizing steps

The first step is to prepare the area by either covering foods or putting them away. Move items off the surface of the area to be cleaned and sanitized, then remove the soils. Soils include bits of food, grease, dust, blood, dirt, cooked foods, and so on that are left behind on the area. Soils can fall off fruits and vegetables, arise from cutting up meats or other foods on a counter, or be bits of packaging fibres. Any food debris in soils can contain fats, carbohydrates, proteins, and minerals, so it can potentially be sources of nutrients for microbes.

Soils can include:

- Fats, oils, greases
- Proteins
- Carbohydrates and starches
- Minerals and salts (e.g. lime scale build-up from hard water)
- Corrosions (e.g. from rusting steel or corroded aluminum)
- Adhesives, inks, dyes, rubber marks
- Algae and fungi (in moist areas)

The first step of cleaning and sanitizing is to remove visible soils, which simply means to sweep up or wipe off any visible contaminants. The second step is to clean the area or object with a **detergent** (sometimes referred to as a cleaner). To begin cleaning, a detergent is mixed or dissolved in water. The soils are then washed and rinsed off the area or object.

The purpose of a detergent is to dissolve any soils that are insoluble in plain water, such as oils and fats. Not all detergents can remove all types of soils: there are different chemical categories of detergents, and each category is used for different purposes. The hardness and pH of the water also affects the type of cleaning agent chosen and the solubility of some soils. Soft water is preferable. Some soils can be dissolved at a neutral pH, whereas others require acid or alkali conditions. For example:

**Soft and hard water explained**
Most water supplies contain the minerals magnesium and calcium. Soft water has a very low mineral content, whereas hard water has much higher content.

Both soft and hard water have their benefits. Hard water is preferred for its taste and health benefits, and soft water is preferred for washing and bathing, as it lathers better.

- Proteins are less soluble under acidic conditions, therefore an alkaline cleaner would be better to clean meat grinders, meat slicers, or counter tops where chicken has been cut up. Because pH plays a major role in controlling microbial growth, it is not surprising that the pH of a detergent is also important. Alkali detergents (pH above 7) are better at dissolving fats and proteins.
- Household cleaners that are used to remove deposits of calcium, lime, and iron oxide (rust), such as CLR, are acid cleaners with a very low pH of 2.1.

## Before and after cleaning photos

Figure 4 shows the walls of a smoked meat processor, before and after cleaning. The sticky residue on the walls was caused by smoke, heat, and a mixture of protein, fat and oils.

**Figure 4** — Smoked meat processor before cleaning

Figure 5 shows the walls of the same smoked meat processor after cleaning. A heavy-duty alkaline caustic cleaner was used to remove the protein residue. It was applied as a heated foam to the walls. Mineral deposits on the wall that are present may be removed with an acid-based cleaner.

**Figure 5** — Smoked meat processor after cleaning

Figure 6 shows meat plant equipment biofilm build-up. This biofilm is a combination of hard water scale and mineral deposits that make it difficult to remove protein residues, fats and oils. Note that this type of biofilm is different from microbial biofilms which are covered later in the lesson.

**Figure 6** — Biofilm build-up on meat plant equipment before cleaning

Figure 7 shows the meat plant equipment after cleaning. The right-hand side shows how cleaning with an acid cleaner removes scale build-up from hard water deposits. Routine cleaning with alkaline foam will remove daily build-up of protein deposits after cleaning.

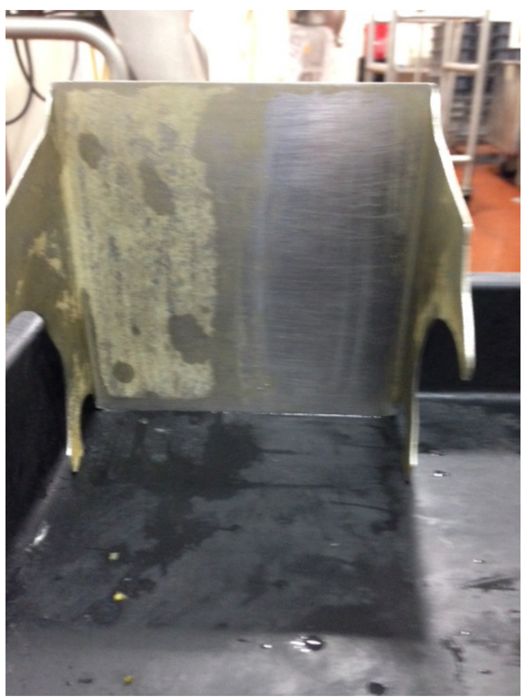


**Figure 7** — Meat plant equipment after cleaning

Detergents can be described using many different chemistry terms, including saponification, anionic, cationic, hydrophilic and hydrophobic.

If you are interested to learn more about the chemistry behind detergents, extra suggested readings follow.

**Cleaning and Disinfection in Food Processing Operations**
http://safefood360.com/resources/Cleaning.pdf

**Explorations of everyday chemical compounds: detergent (useful infographics)**
https://www.compoundchem.com/?s=detergent

**How to Study the Chemistry of Detergents**
https://www.wikihow.com/Study-the-Chemistry-of-Detergents

### Cleaning methods

Whichever detergent is used to clean with, consideration must also be given as to what is being cleaned. This is particularly important for equipment that may not be able to be moved or could take considerable effort to disassemble for cleaning. Cleaning and sanitizing may involve flushing out food products from pipes and equipment. Any portions of pipes that cannot be accessed or properly flushed, cleaned and sanitized can create problems. Foods can accumulate in these areas allowing microbial growth and biofilm formation on the inside of the pipe. These areas are referred to as dead zones or dead ends and should be avoided.

There are essentially three ways to clean equipment:

1. Mechanical cleaning, or clean in place (CIP), for equipment that does not need disassembling
2. Manual cleaning, for equipment that must be completely disassembled for cleaning
3. Clean out of place (COP), for equipment that must be partially disassembled.

**Figure 8** — Rusted equipment

Figure 8 shows rusted equipment traced back to long-term use of improper detergent. The specialized equipment shown in this photo is very expensive to replace.

Dry cleaning and dry sanitizing. Other considerations for cleaning include the type of facility and equipment. A wet-wash clean and sanitize would not be suitable for processing plants that manufacture dry or powdered products. For example, a conveyor belt should be able to be cleaned by sweeping, scraping, and using dry cleaning methods. Dry cleaning methods in food plants can include using compressed air to dislodge debris and soils from tight spaces, steam brushing, vacuuming, and dry ice blasting. Dry sanitizing can include methods such as using alcohol wipes or using approved surface sanitizers that dry rapidly and are usually applied as a spray or mist.

### Biofilms

Biofilms are communities of microbes that adhere to each other, forming layers (i.e., films) that also attach to surfaces. Some bacterial cells have externalized structures (called flagella, pili, or fibrils) that help them attach to surfaces. Once the bacterial cells are attached to a surface, they excrete a sticky substance that helps them hold on, stick together, and protect themselves from the environment. Bacteria that do this are also known as EPS- (extracellular polymeric substances) producing strains. This protective, sticky, polysaccharide layer makes biofilms more difficult to remove, which gives enough time for the EPS-producing bacteria to multiply and for other microbes to attach to the layer. **Bacteria only require a few minutes to start attaching to surfaces**. If bacteria are not removed using effective C&S methods, they will build up into biofilm layers that become very difficult to remove. Figure 9 shows the stages of biofilm layer development.

**Biofilms in the kitchen**
Harmful bacteria can form biofilms on surfaces such as kitchen sponges and cutting boards and lead to contamination and cross contamination of foods.

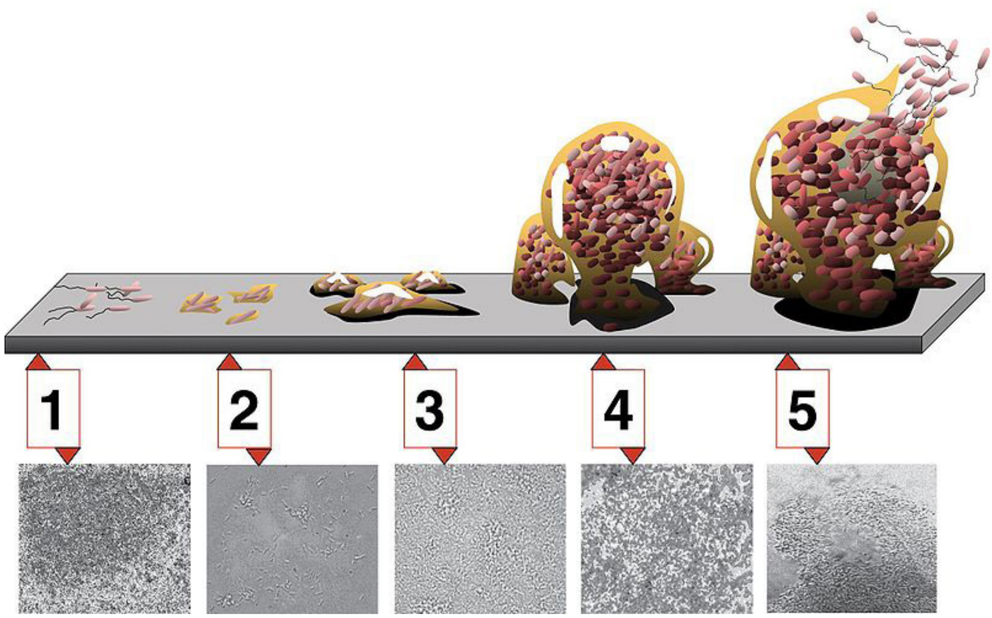



**Figure 9** — 5 stages of biofilm development

Figure 9 demonstrates the 5 stages of biofilm development. The stages are as follows:

- Stage 1: initial attachment
- Stage 2: Irreversible attachment
- Stage 3: Maturation I
- Stage 4: Maturage II
- Stage 5: Dispersion to other locations

Each stage of development is paired with a photomicrograph of a developing *Pseudonomonas aeruginosa* biofilm.

Earlier, we described brushing your teeth as an example of a sanitation plan. Dental plaque is a type of biofilm too. If not removed frequently, plaque build-up becomes tartar, a solid biofilm that becomes increasingly difficult to remove from teeth over time, and can lead to possible gum infection. Biofilm build-up in food-processing plants may also harbour pathogens that can contaminate foods. Biofilms can form on floors, in walls, in pipes, in drains, inside plastic piping, and inside equipment. This is a particular problem in equipment and in areas that are difficult to clean, such as depicted in Figure 10 (soils found behind the panel and Figure 11—the dead-end pipe with food batter). These are known as **harbourage sites** for bacterial growth (Carpentier & Cerf, 2011). The types of surfaces affected by biofilms include stainless steel, rubber, Teflon, and plastics. The biofilms become a **reservoir** for pathogenic bacteria. The same strain of bacteria can be identified in the same processing plant for years if it is not removed through an effective C&S regime. For example, *Listeria monocytogenes*, an issue in meat and dairy processing plants, is known to form persistent strains. Studies have shown that more than one type of pathogen can co-exist in a biofilm, and that pathogen growth may be enhanced or inhibited because of other bacterial species that are present.

The key feature of biofilms is that the bacteria within a biofilm can be more resistant to cleaning and sanitizing agents than the bacterial cells themselves (Wang, 2019).When choosing an appropriate detergent, sanitizer, and method, the nature of the food-preparation or processing activity must be considered as well as the surface to be cleaned and sanitized. Detergents were mentioned earlier in the lesson. Detergents include acids, alkalis, and phosphates, and some also include agents (known as chelation agents) that prevent soils from settling out of solution and leaving deposits on dishes or surfaces before they can be rinsed away.

Once cleaning and rinsing away of soils has occurred, the final step is sanitation. Sanitizers, like detergents, are chosen to suit the surface and the microbes of most concern. Sanitizing can occur through use of chemicals (i.e., sanitizing agents) and by heating or steaming kitchenwares or equipment.

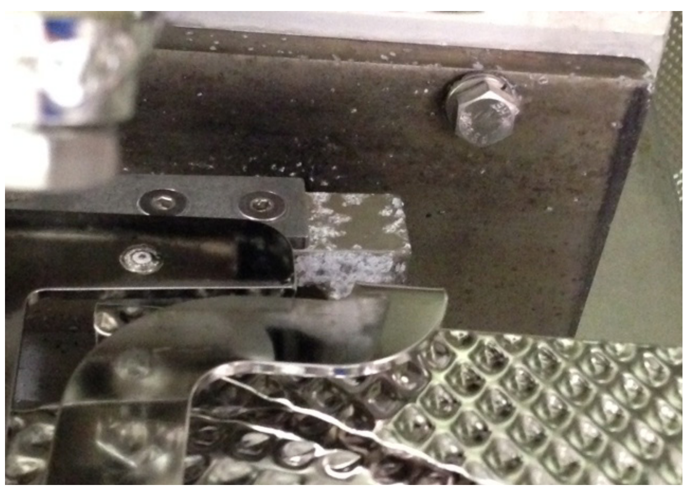


**Figure 10** — Biofilm formations missed during cleaning

Biofilm formations can be easily missed after cleaning equipment parts that are made up of different types of surfaces (Figure 10 and Figure 11). Figure 11 shows food batter found in the dead end of a food distribution pipe, after a clean-in-place procedure was conducted. The pipe should be clear and clean with no food batter left behind. In this case, food debris continually contaminated batches of food until the dead end pipe was removed from the distribution line.

**Figure 11** — Food batter in food distribution pipe dead end

The temperature of water or steam to sanitize kitchenwares using the three-sink method or in dishwashers is given in guidance for food premises. In British Columbia, water in dishwashers should be between 66C to 82°C, depending on the type of dishwashing machine. Lower temperatures may be used if a chemical sanitizer is used. If you're interested in these details, see the following resource document.

**Commercial Dishwashing Specifications: Guideline for Mechanical Warewashing in Food Service Establishments**
http://www.bccdc.ca/resource-gallery/Documents/Guidelines%20and%20Forms/Guidelines%20and%20Manuals/EH/FPS/Food/GuidelinesMechanicalWarewashinginFoodServiceEstablishmentswebformatJan2013.pdf

## Chemical sanitizers

What sanitizer should you use? Sanitizers should be "**broad-spectrum,**" which means they are effective at killing a wide range of bacteria; i.e., able to kill Gram-positive and Gram-negative bacteria. They should have some residual activity, meaning that they continue to work for a short period of time after being applied. But, they should not stay too long in the environment and should not contribute to development of resistance in bacteria and other microbes. They should be easy to use, fast acting, have low toxicity (to human users), and not be too expensive. For example, bleach is an excellent sanitizer in the bathroom or for floors but is not the right choice for some equipment because it is very corrosive. Factors impacting the effectiveness of sanitizers include:

- pH of the water
- Exposure time
- Temperature
- Concentration

Whatever sanitizer is chosen, use it according to the manufacturer's instructions. Not using a high enough sanitizer concentration might render it relatively useless but using too high a concentration could be toxic. To verify, check the concentration with indicator paper.

Chemical sanitizers fall into these basic groups:

1. Chlorine-based (i.e., bleach, either sodium or calcium hypochlorite)
2. Iodine-based (iodophors)
3. Peroxyacetic acid (PAA, also called peracetic acid and hydrogen peroxide [$H3O_2$])
4. Quaternary Ammonium Compounds (QUATS)
5. Other sanitizers (e.g., carboxylic acid or chlorine dioxide)

## Chlorine-based sanitizers

Chlorine-based sanitizers are available as liquids and in solid powder or granular forms. Bleach, likely the most common sanitizer known, is chlorine-based. Bleaches are also sometimes referred to as hypochlorites, after the chemical composition of this sanitizer, sodium hypochlorite.

***Pros*:** Chlorine-based sanitizers (such as bleach) are powerful oxidants, fast-acting, microbicidal, and inexpensive. They are bactericidal, fungicidal, and virucidal.

***Cons*:** These chemicals can be an irritant to the eyes and skin, and corrosive to stainless steel surfaces and equipment. Bleach does not perform well in the presence of organics such as food particles or biofilms, in elevated light or temperature conditions, or in extreme pH environments. Long contact-times may be required to inactivate bacterial spores.

***How to use:*** On food-contact surfaces, chlorine-based sanitizers can be used without further rinsing if the final concentration is low enough. No-rinse bleach concentrations range from 50 ppm in mechanical dishwashers (and for glasses) to no more than 200 ppm as an upper limit of bleach for clean-in-place items. A reasonable target for food contact surfaces (for example, counters) is 100 ppm. Household bleach concentrations (listed on the back of the bleach bottle) are usually at 5.25% (i.e., 52 500 ppm) but may range from as low as 3.75% to as high as 7.0%. If the concentration is not listed, assume the bleach is at 5.0%. Note that bleach degrades over time as sodium hypochlorite (NaOCl) breaks down into water-soluble ions.


**Figure 12** — Domestic bleach bottle with no concentration given on packaging. Domestic bleach is assumed to be have a 5.25% chlorine.

In British Columbia, a bleach dilution calculator can be used to calculate how much concentrated bleach solution should be added to water to get the desired final concentration:

**Chlorine Dilution Calculator**
http://www.foodsafe.ca/dilution-calculator.html

## Activity: Bleach dilution calculator

Use the bleach dilution calculator to determine how many millilitres of 5% concentrated bleach would need to be added to ten litres of water to give a 200 ppm no-rinse solution of bleach.

Check your answers with the answer key provided at the end of this textbook.

### Iodine-based sanitizers

Iodine may be more recognizable as iodine drops in a first-aid kit used for treating wounds (as an antiseptic agent). Iodine-based sanitizers, also called iodophors, contain iodine and an additional chemical that helps the iodine stay in solution.

***Pros:*** Iodine-based sanitizers are fast acting, relatively inexpensive, and environmentally friendly. They are used in the beer and beverage industry to clean glassware, and as the sanitizer for foot baths in food-processing environments. They are bactericidal, fungicidal, and virucidal.

***Cons:*** Iodine-based sanitizers work best in slightly acidic conditions and are ineffective at alkaline pH levels. Iodine solutions may stain plastics, clothing, and equipment, and can affect the flavour of food at higher concentrations. They do not perform well on biofilms and are ineffective sporicides.

***How to use:*** The recommended concentration is 12.5 ppm to 25 ppm for a one-minute contact time.

### Peroxyacetic acid (PAA) and hydrogen peroxide

Peroxyacetic acid (PAA) and hydrogen peroxide can be used as separate sanitizers, but are most effective when they are used together in a single formulation. Breakdown products for these chemicals are not harmful, being composed of acetic acid (vinegar), oxygen, water, and hydrogen peroxide (used to clean earrings and piercings).

***Pros:*** PAA and hydrogen peroxide are fast acting, work well in colder temperatures, are effective sporicidal agents, and are environmentally friendly. In combination (PAA and peroxide), they are effective at sanitizing biofilms. They are bactericidal, fungicidal, mycobactericidal, and viricidal.

***Cons:*** PAA and hydrogen peroxide work quickly, but also break down quickly, leaving little residual activity. Like chlorine products, these agents perform poorly in the presence of organics. They can be corrosive to some metals but can be used on stainless steel. Hydrogen peroxide performs poorly on biofilms.

***How to use:*** The recommended concentration for PAA and peroxide mixtures is 100 ppm to 200 ppm; surfaces must be rinsed when levels exceed 300 ppm. Hydrogen peroxide is used at concentrations of 80 ppm to 600 ppm; surfaces must be rinsed at concentrations exceeding 1100 ppm.

### Quaternary ammonia compounds (QUATs)

Quaternary ammonia compounds (QUATs) are commonly used as sanitizers in the food industry. QUATs are stable in different pH solutions and can be used to dissolve organic residues to disinfect, to clean, and to soften fabrics—and they are antimicrobial. Ammonia is different from quaternary ammonia in its chemical structure and is less stable. Ammonia is a toxic gas and is quite corrosive, although it does work well as a cleaning agent. Liquid household ammonia solutions are often used to control mould and to clean walls, floors, and garbage cans.

***Pros:*** QUATs work well in alkaline conditions and warm water, and are a good cleaner as well as sanitizer. They are not corrosive to metals. They are bactericidal, fungicidal, and virucidal (for some viruses).

***Cons:*** QUATs have reduced effectiveness in hard water, are less effective against Gram-negative bacteria such as *E. coli* and *Salmonella*, and have poor sporicidal activity. QUATs also have poor virucidal activity against non-lipid containing viruses, such as norovirus or hepatitis.

***How to use:*** Typical QUAT concentration for food-contact surfaces is 200 ppm; rinsing is required at concentrations above 200 ppm. There are a wide variety of QUAT sanitizers, and each must be used according to the manufacturer's instructions.

### Other sanitizers

Other sanitizing agents can be used to control microbial issues and may be selected based on the intended purpose. Carboxylic acid sanitizers or fatty acid sanitizers work well at lower pH levels, have a broad range of activity (although they are less effective with yeasts and moulds), and do not create much foaming during use.

Chlorine dioxide is a very effective sanitizer at low concentrations (5 ppm), in alkaline conditions, and in cold water. However, the solution must be prepared on site with expensive equipment to produce the form it is used in—a gas. It would be useful to clean insides of large storage tanks, for example.

Carboxylic acid sanitizers and chlorine dioxide are not commonly used in restaurants or in food stores. Acid-anionic and fatty acid sanitizers are two other classes of sanitizers. These sanitizers are non-corrosive and can be used in acidic conditions.

### Sanitizer verification

How do you know whether your chemical sanitizer level is adequate? The best way is to check sanitizer levels daily with a test strip. Test strips come with a colour-coded key and can instantaneously describe whether a sanitizer is at the correct concentration. Figure 13 shows employees checking sanitizer levels with test strips. In both checks shown, the colour on the test strip shows that the sanitizer is at an acceptable concentration; i.e., at 100 ppm for bleach and 200 ppm for QUATs. Food premises are expected to verify the concentration of sanitizer in solution, regardless of whether the sanitizer comes pre-mixed, is measured out and diluted manually, or if an automated sanitizer delivery system is used. In the case of an automated sanitizer delivery system, testing is necessary because errors in the delivery system settings, plugs in lines ,or mis-measures may occur.

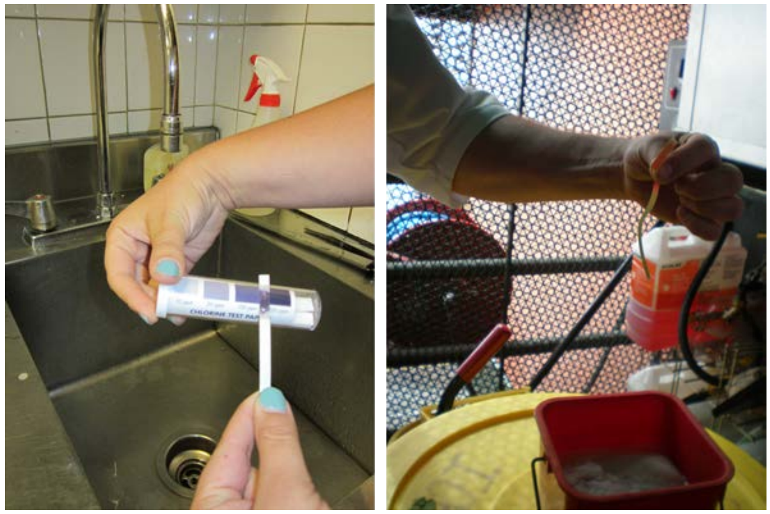


**Figure 13** — Verification of sanitizer concentration at premises: bleach (left) and QUATs (right)

## Sanitation programs and monitoring within a processing plant

Verification of sanitation within a processing plant is more complicated than simply measuring the concentration of sanitizer. At the end of Lesson 2, we introduced how indicators are used to test for food quality and food safety. The same principle is applied here, except that verification of sanitation involves checking the environment for possible indicators of contamination. To establish whether the sanitation program is meeting objectives, plants will test surfaces for residues, protein, adenosine triphosphate (ATP), bacteria, or other parameters. Residue testing for allergens, for example, is critically important to prevent illnesses. Testing for bacteria or residues in plants can prevent cross-contamination. When testing for bacteria or bacterial indicators to ensure sanitation activities in a processing plant are adequate, the plant must first decide on a threshold for the bacteria or indicator.

Over time, plants can establish trends for one or more of these parameters. When numbers start to trend higher over the expected or desired level, this is an indication that something in the sanitation program needs adjustment. For example, ATP tests of conveyor belts with direct food contact must not exceed 10 000 units. If the level goes over that amount, immediate sanitation remediation must occur. In this example, if ATP counts are much higher than the norm, this might mean the plant requires a deep clean or that the category of sanitizer needs to be adjusted, as microbes have built up biofilms that can be removed only with a different sanitizer from the one currently in use.

In processing plants that produce ready-to-eat foods, the presence of the bacteria *Listeria monocytogenes* is a particular concern. For example, *L. monocytogenes* is able to survive in refrigerated storage, and so is a hazard of concern in meat and dairy plants that produce deli meats or cheese. For products with a long shelf life, the presence of these bacteria in the processing environment can result in **post-processing** contamination. What this means is that any *Listeria* present on food-contact surfaces after the final critical control step (CCP) or kill step can contaminate foods before packaging. How can processing plants verify that *Listeria* is not present on food-contact surfaces or within their plant? As presented in Figure 14, areas within a processing plant are assigned a zone according to whether the area is a food-contact surface (FCS), close-to-food-contact surface (C-FCS), or a non-food-contact surface (N-FCS). One method employed for verification is to swab each FCS, C-FCS, and N-FCS for the presence of any species of *Listeria*. The term "swab" is used here as a verb to describe the activity of collecting samples from surfaces, which can be done using an actual swab or a sponge to wipe and sample a particular surface.

| Type of surface | Examples of areas | Zone designation |
|---|---|---|
| Food-contact surfaces (FCS) | Packaging table, slicers, utensils, carts | 1 |
| Close-to-food-contact surfaces (C-FCS) | Legs of packaging table, walls in processing rooms, wheels of carts | 2 |
| Non-food-contact surfaces (N-FCS) | Drains and hallways | 3 |
| | Entrance and delivery areas, loading docks | 4 |

**Figure 14** — Examples of area and zone designations for areas within a food-processing environment

*Listeria* is known to reside in cracks and poorly welded seams, in wet areas such as cooling fans and condensers, and in hard-to-reach areas and equipment. Although not all *Listeria* species can cause illness, the presence of any is an indicator that sanitation procedures are not removing all the bacteria from a particular surface.

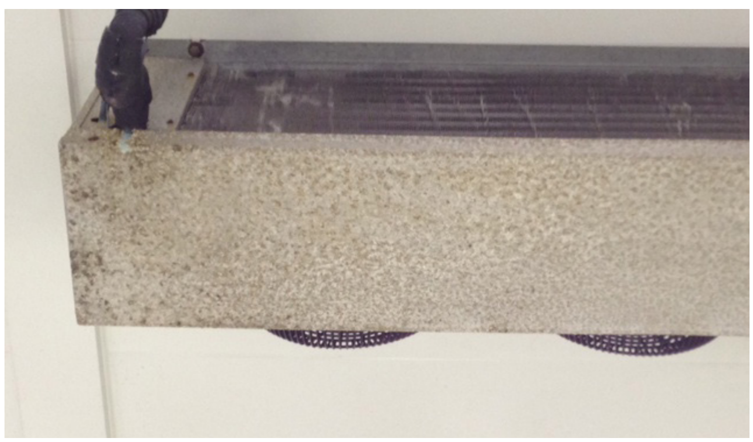


**Figure 15** — Dirty chillers and drip trays are harbourage sites for moulds and *Listeria*. Fans will spread these microbes on air currents though-out the processing plant.

Figure 16 shows an example of soil and food debris found on the inside of an equipment panel cover. The front of the panel was clean, but a reservoir for contamination in the plant was present on the other side. A swab was taken of the inside of the cover, and coliforms were found at levels that were "too numerous to count."

**Figure 16** — Soil and food debris found behind the panel cover of this equipment

The level of concern upon detecting the presence of *Listeria innocua* (a non-pathogenic *Listeria* species) will depend on the zone in which it is found. In a sample from a drain in zone 3, it is almost expected to occasionally find *Listeria* spp. In zone 1, however, if any species of *Listeria* spp. is found, including *L. innocua*, then the food produced may be at risk of contamination. Of greatest concern in zone 1 would be finding *L. monocytogenes*. Processing plants will plan specific activities and take specific actions depending on detection of the presence of *Listeria* spp. in the processing environment. These might include testing food from the batches made in the time period of sampling, increased swabbing, or increased cleaning and sanitizing activities.

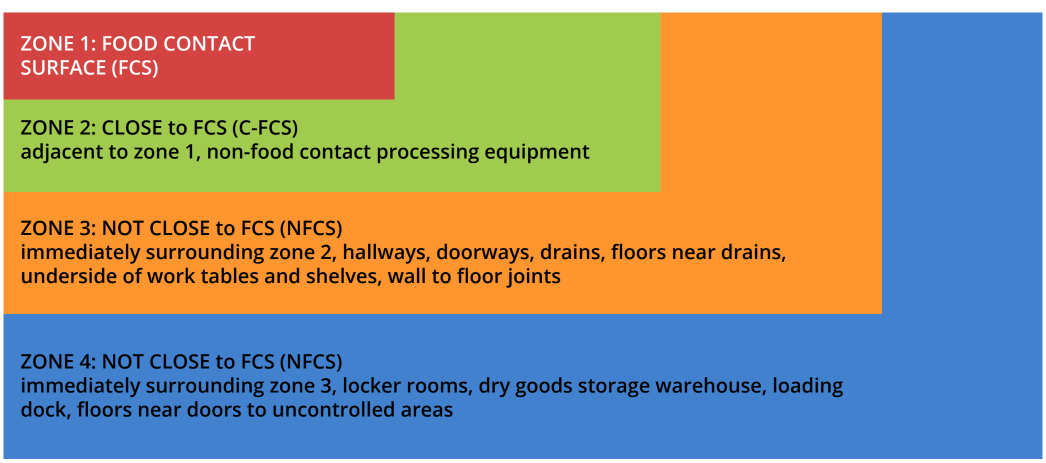



**Figure 17** — Zone concept diagram for environmental monitoring

To learn more about specific responses, consult the operational procedure used by CFIA inspectors when *Listeria* is detected in the ready-to-eat meat processing environment. This spells out how they must act, along with expected activities of the processor.

**Operational procedure: Control response plan for the detection of Listeria in the ready-to-eat processing environment**
http://www.inspection.gc.ca/food/compliance-continuum/guidance-for-inspectors/srrp/listeria-in-the-ready-to-eat-processing-environmen/eng/1541188608710/1541188608990

## Personal hygiene

The objective of personal hygiene is to minimize the risk of contaminating food or transferring illness to customers by personnel who have direct or indirect contact with food and customers. Personal hygiene involves personnel maintaining a good standard of cleanliness and hygiene, and conducting themselves in an appropriate manner when at work.

A personal hygiene program should include the following components:

1. A dress code for the food premises that includes a description of protective clothing for each task. In a restaurant setting, protective clothing might include an apron for food servers and gloves and hair nets for food handlers who prepare foods. In a food-processing environment, a distinction between street clothes and work clothes might be necessary. Appropriate footwear, shoe coverings in certain areas of a processing plant, or proper work boots may be needed. Head coverings such as hard hats and hair nets, beard nets, etc. might also be necessary.
2. Procedures for managing hair and personal items such as jewelry and cell phones while on shift.
3. Handwashing requirements that include rules regarding when to wash hands.
4. Rules related to the health status of employees, including the management of illness and exclusion from food handling and customer contact in the event of illness. Signs and symptoms of illness to be considered include:
    - Jaundice
    - Diarrhea
    - Vomiting
    - Fever
    - Sore throat
    - Skin lesions
    - Ear, eye, or nose discharges
    - Respiratory illness and cough
    - Abdominal cramps
5. Management processes for visitors to the food premises.

Lists of basic do's and don'ts for food handlers are as follows.

Don'ts for food handlers:

- Don't handle food if you are sick
- Don't sneeze or cough over unprotected foods
- Don't wear the same gloves for different tasks (e.g., to cut up a chicken and prepare salads)
- Don't handle food with open wounds (keep them covered)
- Don't wear street clothes in the work site
- Don't chew gum or smoke while preparing food
- Don't use your work apron to clean or dry your hands
- Don't wear jewelry
- Don't wear nail polish
- Don't carry pens, phones, or other objects that can fall into food
- Don't use food-preparation sinks for handwashing
- Don't smoke or spit in food-handling areas

Do's for food handlers:

- Do keep your hair tied back and wear a hair net and/or beard net
- Do wear a clean uniform and change your work clothes daily
- Do wear a clean apron
- Do keep nails short
- Do change gloves frequently
- Do wash your hands in designated handwashing sinks
- Do cough into your shoulder or sleeve

## Handwashing

Handwashing is the single most important way to prevent the spread of pathogenic microbes. There is a reason why there are so many reminders to wash your hands. A review of eleven articles about outbreaks among food workers revealed five that were dedicated to handwashing. A few of the facts from these articles, along with the abstracts from all eleven, are given in the supplementary materials for this lesson.

The fecal-oral route is the most common way for foods to become contaminated. Approximately one hundred times more bacteria reside under fingernails than on other sites on the hand (Todd, Greig, Bartleson, & Michaels, 2008c). Jewelry and long fingernails may puncture and damage gloves, but improper glove use, such as failing to change gloves between tasks, has led to more outbreaks (Todd et al., 2008c; Todd, Michaels, Greig, Smith, & Bartleson, 2010). Studies have shown that toilet paper does not always act as a barrier, and the most effective handwashing is with nailbrush, soap, potable water, friction when washing, and thoroughly drying hands with single-use paper towels (Todd et al., 2008c). Alcohol-based hand sanitizers should not be used in place of handwashing. However, in some situations when washing and drying hands is not possible, alcohol-based sanitizers may work for lightly soiled hands. They do not work against most viruses or spore forms of bacteria (Todd, Michaels, Holah, et al., 2010).

Compliance with handwashing is an issue requiring multiple strategies that include positive (reward) or negative (penalty) incentives via peer pressure and organizational culture (Todd et al., 2010).

While we cannot eliminate all hazards, we can minimize risk. Incentivize employees to follow the best practices and create a strong culture of food safety in every environment along the food-production chain.

Food handlers need to wash their hands after:

- Using the bathroom
- Eating
- Smoking
- Touching the mouth, nose, clothes, or hair
- Using a phone
- Taking out the garbage
- Handling raw food, including fresh vegetables
- Petting an animal
- Coughing or sneezing
- Using chemicals to sanitize
- Before starting work and after breaks
- Using the cash register
- Handling foods with allergens


**Figure 18** — How to wash your hands

## Glove use

People often think that wearing gloves will limit or avoid cross-contamination. But, this is only true if gloves are changed frequently between activities. Even when gloves are worn, microbes present on hands or the gloves can be spread when the gloves are removed from hands. If the same gloves are used to cut chicken and also to prepare a salad, the worker's hands may be protected from microbes, but the gloves that were contaminated by raw chicken may transmit pathogens to the salad and to other surfaces and equipment in the kitchen.

**Figure 19** — Glo-germ illuminated by ultraviolet (UV) light on a glove representing areas of contamination on the glove.

In the video, food safety specialist Jasmina Egeler and chef Gilbert Noussitou discuss the pros and cons of wearing of gloves and demonstrate how, once gloves in the kitchen are contaminated, they can easily transfer microbes to food.

**Video: Gloves in the kitchen**
https://www.youtube.com/watch?v=KFq3JoWWelY

The video shows how microbes on your hands (the Glo Germ powder) gets transferred to your gloves. Hands must be washed before gloves are put on. And gloves must be changed often, as often as you would need to wash your hands. In the photo below, the food handler is cutting raw poultry with raw hands. After this task, the food handler must wash their hands to avoid spreading any microbes from the raw poultry to other areas of the kitchen.

**Figure 20** — Bare hands cutting raw poultry


**Figure 21** — How to properly take off gloves

## Food-worker illness and foodborne illness outbreaks

Pathogens that can be transferred by ill workers through food or environmental surfaces can be viral, bacterial, or parasitic. Based on a review of 816 outbreak reports by Greig, Todd, Bartleson, & Michaels (2007), the pathogens most commonly associated with ill food workers are:

- Norovirus
- *Salmonella*
- Hepatitis A
- *Staphylococcus aureus*
- *Shigella,*
- *Streptococcus*
- *Cyclospora*
- *Giardia*
- *Cryptosporidium*

Other agents linked to ill food handlers include Rotavirus, *Yersinia*, *E. coli*, and *Campylobacter*. Ill food handlers have been linked to a variety of foods, from catered deli sandwiches and desserts (norovirus infection and salmonellosis) to canned mushrooms (S. aureus enterotoxin) (Greig et al., 2007). Within the outbreaks reviewed, larger outbreaks of 3000 or more ill people were associated with mass gatherings over several days, such as festivals (Todd, Greig, Bartleson, & Michaels, 2007a). The majority of outbreaks linked to food handlers occurred in restaurants and catered events: other outbreak venues included hospitals and daycares; during travel on ferries, planes, and trains; in camps; and from food handling in food processing (Todd et al., 2007a).

The two most common ways that food handlers cause foodborne illness are (1) by directly infecting a customer, and (2) via fecal contamination of foods that were temperature abused, allowing microbes to grow (Todd, Greig, Bartleson, & Michaels, 2007b). The most common cause is bare-hand contact with food and failure to wash hands. Other issues highlighted in the review included inadequate cleaning of equipment and cross-contamination (Todd et al., 2007b). Pathogens are most often transmitted through bare-hand contact with food or other surfaces, with infectious doses as low as 1 to 100 bacteria, virus, or parasites (Todd, Greig, Bartleson, & Michaels, 2008a, 2008b).

Appendix E: Journal of Food Protection Abstracts contains abstracts for 11 papers on food handler outbreaks summarized from information collected between 1927 and 2006.

### Exclusion policy for ill workers

All businesses should have guidance on managing illness in the workplace, which should clearly explain the expectations for staff about working while ill. Not all illnesses are equal—for example, working with a headache is uncomfortable but will not make someone else sick, while working with a bad cold or flu may spread the microbes to another co-worker. Some types of illnesses are highly contagious (known as communicable illness) and can be transferred directly to other people or through handling of food and water. In food premises and other high-risk settings such as hospitals and daycares, ill workers are required to be excluded for specific time periods to minimize the risk of spreading illness to others. Depending on the occupation of the ill worker and the type of illness acquired, specific recommendations are made on when workers may safely return to work. This guidance may include a time period, such as within 48 to 72 hours after symptoms are resolved or may, for example, require an ill food-handler to submit a stool sample for testing to ensure the illness is over. This can be problematic for asymptomatic carriers of foodborne pathogens, such as *Salmonella*. For more information about exclusion policy set out by the province of BC, consult the following guidelines.

**Communicable Disease Control Enteric Cases and their Contacts: Exclusion from High Risk Settings**
http://www.bccdc.ca/resource-gallery/Documents/Guidelines%20and%20Forms/Guidelines%20and%20Manuals/Epid/CD%20Manual/Chapter%201%20-%20CDC/Enteric%20Exclusion%20guidelines.pdf

## Pest issues in food premises

Pests in processing plants, food stores, and restaurants—collectively referred to as food premises—will be briefly reviewed in the context of food microbiology. Pests on farms, which can damage crops and livestock and cause serious losses and animal disease, will not be covered here.

Issues resulting from pests in food premises may be categorized into several groups:

- Rodents, such as mice and rats
- Flies of various sizes
- Cockroaches
- Pantry pests, such as beetles, weevils, moths, and other insects (e.g., ants) that get into foodstuffs
- Mosquitoes in outdoor areas such as patios
- Birds

Mosquitoes do not cause transmission of foodborne disease. Mostly they are a nuisance pest in dining areas. However, they are reservoirs for bloodborne illness, and changing climate patterns are extending the ranges of mosquito-borne illnesses such as Lyme disease and West Nile virus in B.C. For this reason they should be controlled, but are not covered further here.

Birds are a significant reservoir for *Salmonella*, and bird feces can also carry other potential human pathogens such as Avian influenza (note: Avian influenza is not a foodborne illness). This is reviewed under environmental and animal reservoirs.

**Rodents**
https://www.cdc.gov/healthy-pets/rodent-control/index.html

## Rodents

Rodents of concern in BC include the house mouse (*Mus musculus*), the brown rat (Norway rat or sewer rat, *Rattus norvegicus*), and the black rat (roof rat, *Rattus rattus*). As well as by their appearance, they can be recognized by their feces, as shown in Figure 22.

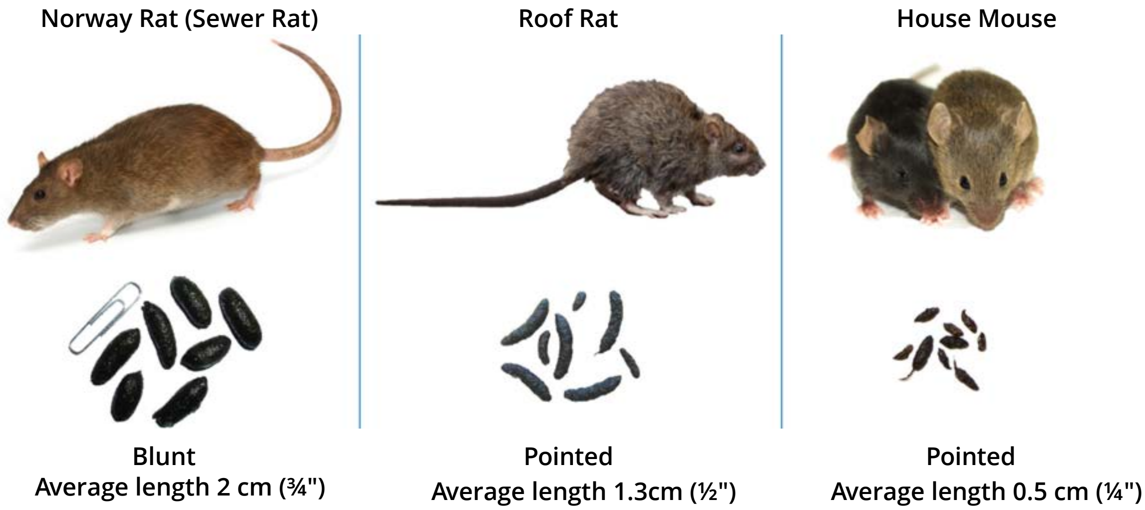



**Figure 22** — Rodents of concern in BC and identifying feces

These rodents breed quickly. They have five to ten litters per year, with a gestation period (i.e., the time from conception to birth) of approximately three weeks. Rodents infest premises where they can gain entry and there is a place to nest and an available source of food and water.

To avoid rodents, ensure adequate sanitation inside and outside the premises. Three activities are required to manage rodent issues:

1. Do not allow rodents access to food, water, garbage, and organic wastes. As shown in Figure 23 (the photo of the mouse that suffocated in the bag of flour), rodents can chew into paper bags and cardboard boxes. Keep foods in thick plastic or metal containers with lids.
2. Prevent entry into premises by plugging and removing all access points. These include under and around doors, particularly loading bay doors, small holes and cracks between walls and floors, and through drainpipes. Eliminate nesting sites inside and outside of the premises.
3. Control existing rodent problems with traps and consider hiring a pest management company. When a pest management company is engaged, always obtain a contract with a guarantee to eliminate pests.

Monitor pest presence to allow you to assess the number, frequency, and locations in your premises.

**Figure 23** — Suffocated (dead) mouse protruding from bag of flour in a restaurant dry-goods storage room

There are over thirty-five diseases of public health significance carried by rodents. Rodents can transmit disease when they bite, via urine and feces, and from cross-contamination from paws to foods and food surfaces. Foodborne illness such as salmonellosis and *E. coli* infection can be caused by these pests. Disease agents are transmitted when rodent feces are left behind or carried on the feet or bodies of the rodents as they walk over floors, equipment, dishes, foods and food surfaces (Centers for Disease Control and Prevention, 2010). Rodents also cause serious illnesses such as plague and fevers—rat-bite fever, Colorado tick fever, Omsk hemorrhagic fever, relapsing fever, Rocky Mountain spotted fever, and others. These more serious illnesses are rare in food premises as they are potentially transmitted through bites (fevers), rodent fleas (plague), or aerosol inhalation of dried feces (Hanta virus).

## Flies

Each housefly (Figure 24) can carry more than 1 000 000 bacteria on its body. Flies are known to be carriers of *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *E. coli* spp., *Listeria* spp., *Cronobacter* spp., and *Vibrio cholera* (Centers for Disease Control and Prevention, 2010; Facciolà et al., 2017). These microbes may be carried on the legs or body hairs of flies but are found most commonly in the gut. Flies ingest the microbes into their gut, where they multiply. As flies regurgitate food to aid in digestion, any microbes they regurgitate will contaminate foods and surfaces. When conditions are favourable, eggs of the common housefly (*Musca domestica*) can hatch within twenty-four hours of being laid. Eggs are laid in moist, nutrient-rich materials such as garbage or uncovered foods. After hatching, flies have a larval maggot stage that lasts three to four days, followed by a pupa stage before the adult fly emerges. A study of flies from restaurant garbage bins found over 20% of the flies collected were contaminated with pathogens: *Cronobacter* spp., *Salmonella* spp. or *Listeria* spp. Any single fly would have a 4.4% chance of containing one of these pathogens in their gut and a 2.2% chance of carrying them on their bodies (Facciolà et al., 2017).

**Figure 24** — Common housefly

The common fruit fly (*Drosophila* ssp.) is attracted to fermenting fruits and vegetables. Fruit flies must also be controlled in food premises, as they can potentially transfer harmful bacteria onto foods. Previously, fruit flies were thought to be only nuisance pests. However, bacteria from contaminated fruits, vegetables, and other food sources can accumulate on the bodies of the fruit flies. This was demonstrated in a study where bacteria that cause foodborne illnesses (*E. coli* spp., *Salmonella* spp., and *Listeria* spp.) were detected on the bodies, legs, and hairs of fruit flies (Black, Hinrichs, Barcay, & Gardner, 2018).

**Figure 25** — Uncontrolled garbage

Managing flies in food premises requires exclusion and sanitation controls.

1. Limit entry by screening doors and windows and, when possible, keeping doors and windows closed.
2. Use fly paper, fly traps, fly lights, or other tools to trap and kill flies.
3. Keep drains clean and sanitize regularly to prevent breeding.
4. Inside the premises, keep foods covered to prevent access.
5. Outside of the premises, keep garbage sealed and remove sources of standing water. Store garbage, recycling, and food waste containers away from the building. Keep the lids on and clean bins regularly.

## Cockroaches and pantry pests

Cockroaches are insects that do not usually fly but can walk very fast. They eat all types of human foods, dried blood, dead cockroaches, human feces, and the toenails of sleeping infants or ill persons. They are capable of transmitting pathogens that may cause diarrhea, dysentery, cholera, leprosy, plague, typhoid fever, and viral diseases. Microbes are carried in their gut and are spread when they leave behind feces or disgorge (throw up) partially digested foods (World Health Organization, 2003).

Cockroaches can also be an allergen source and may trigger asthmatic issues in heavily infested areas. The vomit they expel has a noxious odour, and their nocturnal habits and scratching noises are known to cause psychological distress in some individuals (these bugs are creepy!). The most common cockroach species in Canada is the German cockroach (*Blatella germanica*), shown in Figure 26.

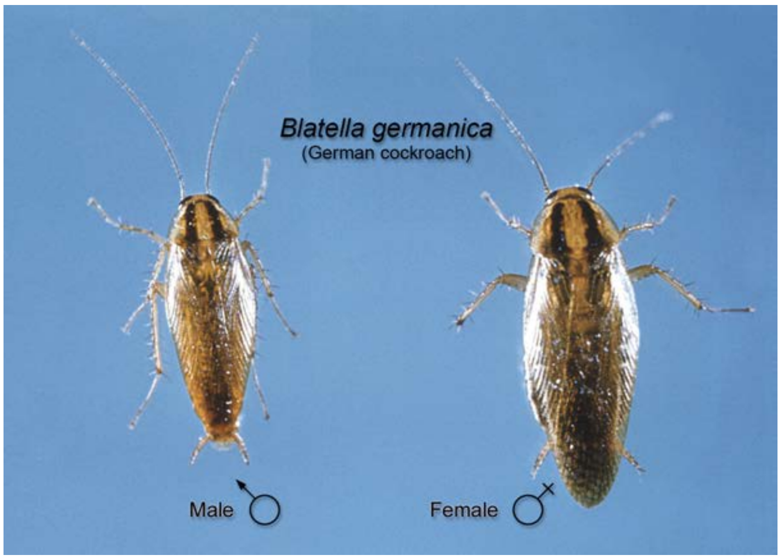



**Figure 26** — German cockroach

Similar to the management of rodents, the keys to preventing cockroach infestations are to keep premises clean, remove food sources, and prevent entry. Existing infestations are difficult to remove, as cockroaches have flattened bodies capable of hiding under skirting boards in kitchen areas, in and under cupboards, and between walls. Chemical treatments are available, but spraying surfaces may be insufficient to reach all areas; fumigation is required to access crevices. However, cockroaches are known to acquire resistance to chemical treatments. Limiting entry and sealing up cracks and crevices are important mitigation steps, along with preventing access to food sources (Centers for Disease Control and Prevention, 2009).

Pantry pests include meal moths, beetles, and weevils. These pests often appear with a new food shipment, residing in boxes or bags of nutrient-rich carbohydrate and protein products (e.g., crackers, oatmeal, peanuts, flour, and corn meal). These pests can cause a lot of damage, as they contaminate more foods than they consume. Unlike rodents, flies, and cockroaches, pantry pests do not carry or transmit disease-causing microbes.

The Indian meal moth (*Plodia* spp. and others) has been described as a pantry moth, a flour or grain moth, or a weevil. Meal moths can consume many types of foods, including corns, nuts, cereals, birdseed, flours, rice, pastas, spices and more (clothing). This pest does not carry disease-causing microbes or parasites, but can be very damaging to foodstuffs. They leave behind feces, cocoons, and webbing and often fly away from food sources during the pupation portion of their life cycle. This makes them difficult to eradicate, as they may not all be where the infested foods are located. Foods infested with this pest must be discarded. The moth can chew through cardboard and plastic. To ensure sealed foods in the pantry are not contaminated after an infestation, foods may be frozen for at least four days or heated in an oven to 60°C (130°F) for at least thirty minutes to destroy any eggs or larvae. When contaminated foods are removed, moths may be seen for up to three weeks following. If more moths are seen after this time, it is likely there is another food source that has been contaminated (University of Minnesota Extension, 2018).

Granary weevils (*Sitophilius* spp.) are reddish-brown beetles that do not fly, and are found exclusively in food storage areas. The larvae develop inside rice, corn, and other grains and may not be detectable when foods are first opened. When they occur, it is always due to prior contamination at the site of harvest and processing. Contaminated foods must be discarded.

Many different species of beetles can be found in dried foods, pastas, teas, dried flowers, nuts, beans, dried fruit, and other dried food items. Some beetle species can fly; foods contaminated with these pests must be discarded.

To control pantry pests, keep cupboards, surfaces, and floors clean of debris to eliminate food sources. Try to store foods in cool, dark, and dry environments that pests do not like. At home, be cautious of dry pet foods and bird seeds, as all pantry pests will consume these foods if available. Keep foods in sealed containers (University of Minnesota Extension, 2018).

## Cross-contamination overview

Cross-contamination refers to the movement of microbes from one substrate to another. This movement can happen in many different ways, as in the examples in Figure 27.

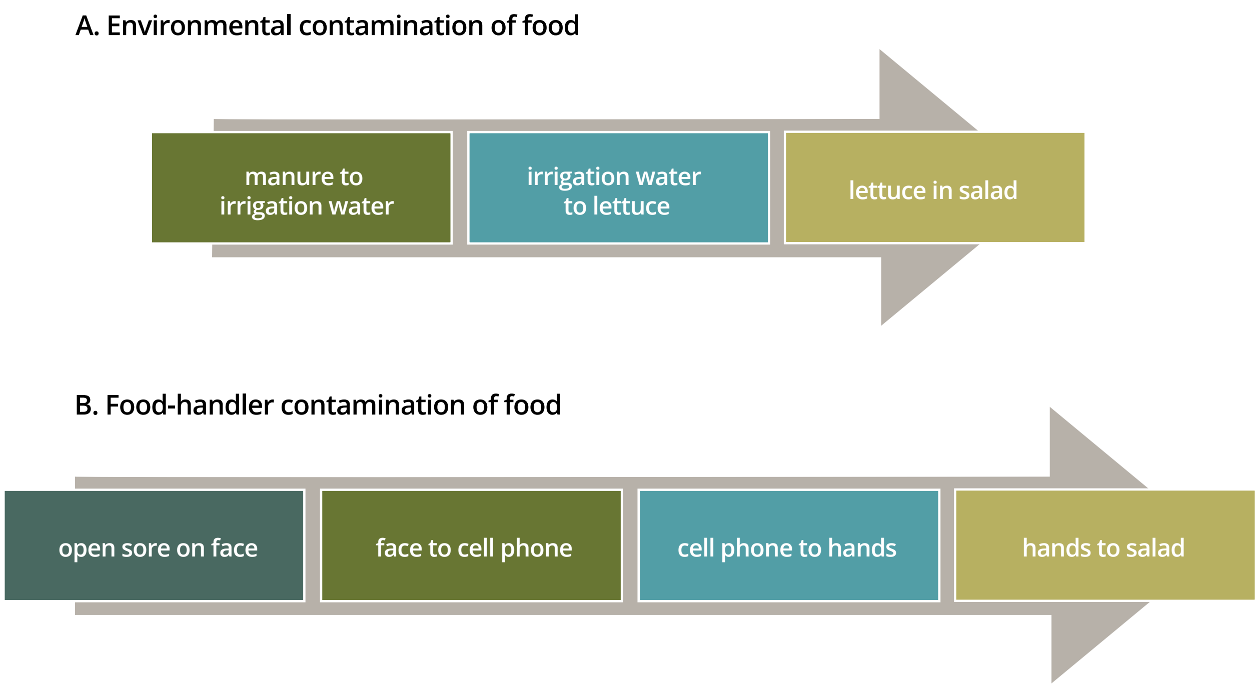



**Figure 27** — Examples of routes of cross-contamination

**News 21: How Safe is Your Food?**
http://foodsafety.news21.com/

**News 21: Farm to Fork**
http://assets.news21.com/2011/farmtofork/

**News 21: How Feces Get Into the Food Supply**
http://foodsafety.news21.com/2011/safety/inspection/feces/index.html

Wherever you go, you carry microbes with you, picking up more along the way and leaving some behind. Cross-contamination can be simply from one surface to another—such as hand contact with a cutting board to a counter—or from one location to another, such as when animals that have microbes on their hooves and hides are transported from a farm to a processing plant. In this section, we will briefly review reservoirs for contamination and the main modes of transmission for microbes, followed by some examples of cross-contamination from farm to fork.

## Environmental and animal reservoirs

Environmental reservoirs include soils, water, air (dust) and are interconnected with animal reservoirs comprised of wild and domestic animals, and pests. For example, irrigation water may be contaminated by either domestic or wild sources of animal feces. Such contamination may occur directly or indirectly through runoff from manure storage areas into water sources. Liquid dispersal of microbes is a particular concern: once microbes have entered a water system, that water may contaminate foods or may cause infections in animals that drink the water, potentially amplifying the problem. *Campylobacter* is a bacterial species found in the intestinal track of many wild animals. It is known to survive in water at 10°C and in soils for weeks (Facciolà et al., 2017). *E. coli* bacteria reside in the intestines of all warm-blooded mammals, such as humans, cattle, wild deer, and rodents. When pathogenic *E. coli* strains are excreted into the soils and water of agricultural growing areas, cross-contamination of produce plants may occur. It is suspected that contaminated irrigation water affects fresh produce through droplets left on the surface of the plant after watering, through adsorption into plant tissues via the growing roots of the plant, or when produce is washed with untreated irrigation water. Research into microbial attachment to growing plants has found the presence of flagella that allow this binding to occur (Rossez, Wolfson, Holmes, Gally, & Holden, 2015).

Environmental soil bacteria reservoirs of concern for food microbiology include *Clostridium* spp., *Bacillus* spp., *Listeria* spp., and others. *Clostridium* and *Bacillus* are spore-formers. Their spores are resistant to drying and cold and are able to survive in the environment for long periods. *Clostridium* and *Bacillus* are always considered as a hazard in foods grown in soils, particularly in dried foods such as grains, pastas, and rice. *Listeria monocytogenes* can contaminate foods of both vegetable and animal origin, such as cabbage, sprouts, meats, and dairy products. As previously discussed in this lesson, *Listeria* are often associated with post-processing cross-contamination events; i.e., from a biofilm or unsanitary surface in the food-processing plant to foods prior to packaging, such as deli meats, cheese, salads, and ice-cream.

Both birds and insects are known animal reservoirs for *Salmonella* bacteria. *Salmonella* is always considered a hazard in foods grown and dried outdoors where they may be exposed to bird droppings or insect activity, such as tree nuts and cocoa seeds. Air (dust) may contaminate foods when there is risk for cross-contamination in dry environments. For example, this is evident in food-processing areas where flour is manufactured. Recent pathogenic *E. coli* flour outbreaks in British Columbia and elsewhere in Canada suggest that exposure to contaminated flour caused many illnesses (Canadian Food Inspection Agency, 2018; Public Health Agency of Canada, 2017). Transmission vectors for contamination were not identified. However, the grain could have been contaminated by cattle feces during heavy rains through run-off, or the milled grain could have been contaminated by pests such as mice or rats.

## Human reservoirs

Many bacterial pathogens normally live in the intestinal track of healthy animals, including humans. The intestinal tract is a warm, wet, and nutrient-rich environment. Most of the microbes that live in our intestinal tract die when they are excreted as waste, because they are not aerobic bacteria. Illnesses occur when pathogenic bacteria are excreted, find a suitable food substrate to colonize or reside on, and then are ingested by people who become infected. There are an estimated 1000 different species of bacteria that live in a healthy human gut, but only a few species that cause foodborne illnesses and outbreaks. These include *E. coli*, *Salmonella*, and *Campylobacter* bacteria, all of which can live for extended periods outside the body.

If on-site sewage disposal systems (e.g., septic tanks) or municipal sewer systems are not functioning properly, microbial contaminants from human feces may enter water supplies, including marine waters used to grow fish and shellfish. Norovirus, for example, has been linked to several outbreaks involving consumers of raw oysters. Humans are the only known reservoir of norovirus strains that infect humans, and these strains do not infect oysters. The only way norovirus particles could enter a raw oysters is through cross-contamination from human sewage. Other high-risk sewage disposal practices such as use of night soil (human feces) to fertilize crops or improper sewage disposal practices can also lead to potential cross-contamination of water and food.

Intestinal gut bacteria are not the only microbes that cause foodborne infections. *Staphylococcus aureus* is an ordinary inhabitant in 30% of the human population: these people are carriers of these bacteria but are not ill themselves (i.e., they are asymptomatic). *S. aureus* is found mainly in the nasal cavity and on hands, and is easily spread through fluid and hand contact with foods. When infected wounds are not covered, fluids are expelled from the nasal cavity (i.e., sneezing), or poor handwashing leads to food contamination, these bacteria can quickly grow in potentially hazardous foods. *Staphylococcus aureus* bacteria produce enterotoxins that cause rapid onset of vomiting and diarrhea.

> Over 10 million *S. aureus* can be found in a single drop of pus from an infected cut.

### Modes of transmission

The most common mechanism for microbial transmission of food pathogens is the fecal-oral route. This only means that the source of microbes in the feces is consumed by the host. People don't knowingly consume poop! So how does fecal material end up in our food and drinking water?

Microbes and other contaminants can be transmitted via three methods:

1. **Surface-to-surface** (e.g., using the same cutting board to cut up chicken and vegetables for salad)
2. **Liquid** (e.g., contaminated water)
3. **Airborne** (aerosol) dispersal (e.g., sneezing or coughing)

Transmission can be categorized as either **direct** or **indirect,** based on whether the pathogen source and food came into direct contact or whether there was one or more intermediate step or steps between the pathogen and food. Examples of contamination routes via each of the three methods follow.

### Surface-to-surface transmission

When deli-slicers are not properly cleaned and sanitized, microbes present on the slicer can grow. One very problematic microbe in the food-processing environment is *Listeria*. It has unique characteristics that allow it to survive well at refrigeration temperatures. When fully cooked and ready-to-eat meat products are run through deli-slicers, any *Listeria* on the surface of the deli-slicer can transfer to the surface of the meat.

In 2008, Maple Leaf deli meats were linked to fifty-seven illnesses and twenty-two deaths. *Listeria monocytogenes* was the cause of the illnesses, and an independent review commissioned by the federal government led to a series of recommendations, such as "sanitation methods should be validated and implemented." Although the processing plant had been doing environmental monitoring for *Listeria* and had found positive results, no additional cleaning and sanitizing nor reporting of these findings (referred to as trend analysis) occurred. Another of the recommendations was to "simplify and modernize federal legislation and regulations," which led to the Food Safety Modernization Act that came into force in 2019 (Weatherill & Expert Advisory Group, 2009).

### Liquid transmission

Examples of fecal-oral pathways as described earlier can involve manure. Cattle defecating in pastures may be carriers of *E. coli* O157:H7. If the feces are washed away by a heavy rain, the microbes may be transported to a water source such as a river that supplies irrigation water to a local farm. Contaminated water may be directly ingested if the bacteria penetrate a drinking water well, as occurred in Walkerton, Ontario, in Canada's worst waterborne outbreak. When water is directly contaminated, activities related to food handling—such as cleaning of surfaces and equipment, rinsing of produce, and using water as an ingredient—become a risk, as well as the simple act of just drinking the water.

After four days of light rain, on May 12, 2000, an extreme rainfall event of 134 mm overwhelmed Well 5 of a municipal water supply in the town of Walkerton, located one hour north of Toronto, Ontario. This caused Canada's worst waterborne disease outbreak, in which 2300 people suffered from gastroenteritis, sixty-five were hospitalized (with twenty-seven cases of haemolytic uraemic syndrome) and seven people died. The bacteria responsible for these illnesses were *E. coli* 0157:H7 and *Campylobacter*, from a farm near Well 5. This tragedy could have been averted if multiple barriers and controls were implemented (Ritter et al., 2002). Water-quality monitoring (testing), water treatment (e.g., chlorination), well maintenance, animal access to sources of water, education and training of staff, and communications from the water suppliers to health and to the public were all factors in this outbreak.

**Figure 28** — Cow near an open irrigation water source

### Airborne transmission

A single sneeze can carry up to 50 000 000 *Streptococcus* bacteria, which can disperse up to half a metre away (Todd et al., 2008b). Norovirus is also highly infectious and can easily spread through vomit particles. When contamination occurs in a central area with high traffic, such as a bathroom, several people can be exposed to norovirus particles that remain in the air for several hours and in the environment. Direct contamination from one person to another is called person-to-person contamination. If it involves food, then it is called foodborne contamination.

Two outbreaks related to sneezing and nasal fluid discharges involved contaminated egg salad. In one outbreak, over 600 people were infected at a single charity event. In the other outbreak, sixty-one cases of *S. pyogenes* occurred at a military base. In both of these outbreaks, infected food handlers contaminated the egg salad—a highly potentially hazardous food and excellent environment for bacterial growth—and was followed by temperature abuse of the egg salad that allowed the bacteria to grow into high numbers (Todd et al., 2007b).

**Figure 29** — Chef sneezing in the kitchen, showing the spread of aerosols

Appendix F: Food Production Chain contains examples of cross-contamination issues that may occur in six settings:

- Outdoor farms
- Indoor farm production areas
- Slaughterhouses and meat plants
- Fruit and vegetable processing plants
- Transportation to kitchens
- In a kitchen

## Activity: Creating transmission pathways

Create a transmission pathway for each of the methods above, via surface-to-surface, liquid medium, and airborne dispersal, with *Salmonella* as the pathogen. In your transmission pathways, you should use crickets (grasshoppers) at least twice, but may use other transmission agents as well. Use tomatoes or crickets as foods or ingredients of concern that have become contaminated. Create a minimum of three of the six possible transmission pathways, having at least one direct and one indirect pathway.

| | Direct | Indirect |
|---|---|---|
| **Surface-to-surface** | | |
| **Liquid** | | |
| **Airborne** | | |

Check your answers with the answer key provided at the end of this textbook.

## Storage issues and cross-contamination in food premises

In the kitchen, there can be any number of locations for microbes to inhabit, depending on the culinary environment. Microbes can also be purposely introduced during food fermentation, such as when we add yeast to make bread rise or lactic acid bacteria to ferment meats. These microbes are also spoilage agents and problematic when found in foods not intended to be fermented.

Microbes can thrive in virtually every en4vironment. When microbial growth is uncontrolled, pathogens pose a risk to the food industry and the food-production process. In the following three situations, cross-contamination and movement of microbes from one area to another can be prevented as noted.

1. **Problem:** raw meat, poultry and fish are not separated from vegetables, fruit, and ready-to-eat foods.

   **Prevention:**
   - Vertically separate stored meats at the bottom of the refrigerator so that raw meat juices don't drip on other foods. OR,
   - Horizontally separate meats on separate shelving units or different coolers, and store foods in sealed containers or protective bags.

2. **Problem:** meat grinders or meat slicers on the counter are next to where vegetables are also prepared. The mechanical action of grinding and slicing will create the potential for aerosolized particles or bits of raw meats or microbes to land onto the neighbouring vegetables.

   **Prevention:**
   - Place equipment for different tasks in specific areas. Use refrigerated prep tables where fruits and vegetables can be kept in cold storage below the table. Designate specific cutting boards for fruits and vegetables in a workspace on top.
   - Ensure work tables are made of stainless steel—these surfaces are smoother than other materials (e.g., wood will have cracks), are easier to clean, and harder for microbes to colonize and form biofilms.

3. **Problem:** food handlers transport microbes from one place to another during different tasks.

**Prevention:**

- Control personal hygiene with frequent handwashing and by changing soiled aprons or designating specific aprons to work with raw versus ready-to-eat foods.
- Locate cooking equipment (grills, ovens, fryers, stoves) on one side of the food-preparation area, often along a wall. Separate holding stations can be used to keep food hot or cold, such as using steam tables to keep ingredients above 60°C before adding them to dishes.

# Lesson E Quiz

1. What is the relationship between cleaning and sanitizing?
   a. Cleaning is important to remove debris before sanitizing occurs.
   b. Sanitizing uses harsher chemicals than cleaning.
   c. Sanitizing is done more frequently than cleaning.
   d. Cleaning is unnecessary in areas that are sanitized.

2. What type of cross-contamination is possible in each of the following photos?

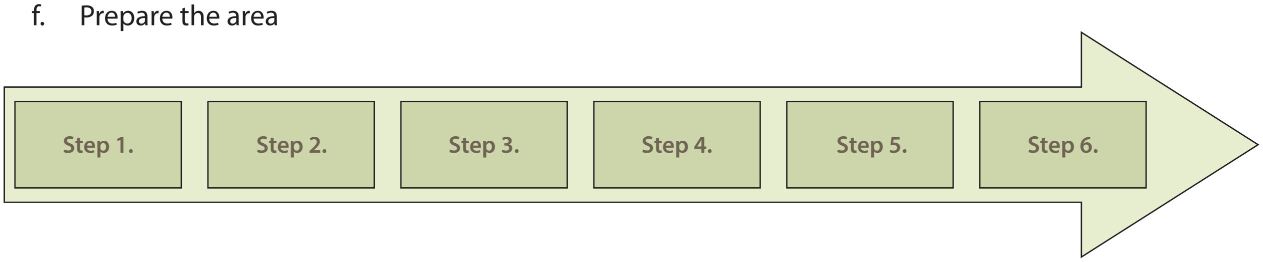
a. Physical contamination
b. Chemical contamination
c. Biological contamination

a. Physical contamination
b. Chemical contamination
c. Biological contamination

a. Physical contamination
b. Chemical contamination
c. Biological contamination

3. What does the term "verification" refer to in the sanitation program?
   a. Verification ensures that cleaning and sanitizing is assigned to qualified staff.
   b. Verification ensures that cleaning and sanitizing is done on a regular schedule.
   c. Verification ensures that cleaning and sanitizing is effective at reducing pathogens.
   d. Verification ensures that cleaning and sanitizing is meeting the minimum regulations.

4. Identify the correct order for the steps involved in cleaning and sanitizing.
   a. Rinse surfaces
   b. Clean with a detergent
   c. Remove visible soils
   d. Air-dry
   e. Sanitize
   f. Prepare the area



5. Which factor makes timely sanitizing important?
    a. Harmful bacteria form biofilms that are more resistant to sanitization.
    b. Kitchen surfaces and equipment cannot be used while they are being sanitized.
    c. Sanitizer effectiveness is affected by water acidity, temperature and concentration.

6. What are the two most important ways for food handlers to prevent the spread of pathogenic microbes in this list?
    a. Ensure that hand washing is done properly and frequently to prevent contamination of food
    b. Ensure that clothing standards are adequate and adhere to code
    c. Ensure that correct sanitizers are used and results are monitored
    d. Ensure that food-grade gloves are worn by workers and changed as often as necessary to prevent contamination of food

7. Which description matches the best way to eliminate houseflies often found in kitchens?
    a. Block entry, enclose garbage and implement a pest control monitoring program in outdoor areas.
    b. Can be best prevented by limiting access to food and water.
    c. Limit access by using window screens and keeping doors closed.

8. Which description matches the best way to eliminate rodents often found in kitchens?
    a. Block entry, enclose garbage and implement a pest control monitoring program in outdoor areas.
    b. Can be best prevented by limiting access to food and water.
    c. Limit access by using window screens and keeping doors closed.

9. Which description matches the best way to eliminate cockroaches often found in kitchens?
    a. Block entry, enclose garbage and implement a pest control monitoring program in outdoor areas.
    b. Can be best prevented by limiting access to food and water.
    c. Limit access by using window screens and keeping doors closed.

10. Why is it important for a business to have a written employee illness policy?
    a. So employees know if they will get paid if they take sick leave
    b. So that appropriate actions are taken by supervisors and employees when communicable illness occurs in the work site
    c. So businesses can reduce their liability if patrons get foodborne illness
    d. So businesses can get a rate reduction on employee medical insurance policies

11. Which of the following situations could lead to cross-contamination in a commercial kitchen?
    a. A hand washing station is located next to a cupboard containing dry ingredients.
    b. A worker preparing ground meat shares a prep surface with another worker cutting cucumber for the salad bar.
    c. A worker stops sweeping in the receiving area to help a delivery driver unload.

12. Which statement expresses the main idea about food microbiology in this unit?
    a. Microbial growth that causes food contamination begins with worker carelessness.
    b. Stopping microbial growth requires that all food handlers use proper sanitizing procedures.
    c. Food handlers can reduce the chances of foodborne illness being caused by microbial growth.

13. What is not part of verifying the effectiveness of a sanitation program?
    a. Testing for bacteria or residues
    b. Monitoring results for trends
    c. Training cleaning staff

14. Identify the pathogens most commonly associated with human reservoirs. Select all that apply.
    a. Norovirus
    b. *Cryptosporidium* spp.
    c. *Salmonella* spp
    d. *Staphylococcus aureus*
    e. *Bacillus cereus*
    f. *Clostridium botulinum*

15. Identify the pathogens most commonly associated with environmental soil reservoirs. Select all that apply.
    a. Norovirus
    b. *Cryptosporidium* spp.
    c. *Salmonella* spp
    d. *Staphylococcus aureus*
    e. *Bacillus cereus*
    f. *Clostridium botulinum*

16. Identify the pathogens most commonly associated with animal (zoonotic) reservoirs. Select all that apply.
    a. Norovirus
    b. *Cryptosporidium* spp.
    c. *Salmonella* spp
    d. *Staphylococcus aureus*
    e. *Bacillus cereus*
    f. *Clostridium botulinum*

Check your answers with the answer key provided at the end of this textbook.

# Lesson F: Microbial Barriers for Food Preservation

## Introduction

Preserving foods for later use was developed as a necessity to extend the nutritional and caloric value of foods by preventing food deterioration. Preservation techniques were likely established empirically; i.e., through practical firsthand observations. The most common techniques involved drying, fermenting, salting, and smoking. Today, we have the benefits of refrigeration, canning, and other packaging techniques to extend the shelf life of foods. In this lesson, we will define food barrier technologies, also known as hurdle technologies, and discuss how they are used to limit or eliminate microbial growth, thereby improving the shelf life and safety of foods. Note that the terms barrier and hurdle are used interchangeably in this lesson.

Now follow the link to watch a video introduction to hurdle technology:

**Hurdle Technology**
https://www.youtube.com/watch?v=AjE6zvNWqE8

### Learning outcomes

Upon completion of this lesson, learners will be able to:

- Describe what is meant by a food barrier or hurdle
- Provide criteria for pH and water activity hurdles
- Identify and list hurdles used to limit microbial growth
- Describe production methods where hurdle technologies are employed
- Describe types of preservatives and packaging used to extend shelf life

### Terminology

Key terms given in bold

active packaging
antimicrobial
antioxidant
**barrier**
**biofilm**
canning
**controlled atmosphere**
D-value
dehydration
freeze-drying
hermetically sealed
homeostasis
**hurdle**
**hurdle technology**
**high temperature, short time (HTST)**
**irradiation**
**modified atmosphere packaging (MAP)**
**pasteurization**
metabolic exhaustion
**organoleptic**
**reduced oxygen packaging (ROP)**
retort
somatic
sublimation
**ultra-high temperature (UHT)**
**vacuum packaging**

## Factors affecting microbial growth

Our early ancestors may not have understood the science behind food safety, but they knew the advantages of extending the shelf life of food, and were likely all too familiar with the discomforts of eating spoiled or contaminated food. They learned that doing things to foods such as changing the temperature (by chilling or cooking), reducing the available water (by drying, salting, or smoking, as shown in Figure 1), and fermenting (by using starters containing fermentative microbes to outcompete food spoilage microbes) extended the shelf life and allowed storage of foods for extended periods of time. Their innovations in disrupting the growth of undesirable microbes by altering the environment of the food form the basis of modern-day hurdle technology.

**Figure 1** — Hot smoking of trout filets

What is a **hurdle**? Figure 2 shows three hurdles on an athletic field. Each hurdle is a **barrier**. The idea behind hurdle technology is that adding multiple low-level barriers or hurdles, either sequentially (one hurdle, then another hurdle) or concurrently (multiple hurdles applied at the same time), will reduce the numbers of foodborne pathogens in foods. Hurdles applied in either of these ways will be more effective than one massive hurdle or the sum of several individual hurdles (Mukhopadhyay & Gorris, 2014; Ross, Griffiths, Mittal, & Deeth, 2003). The goal is to reduce microbe populations without negatively impacting the quality of the food being treated. Food quality in this context is a measure of the food's **organoleptic** status (its sight, taste, and odour) and its nutritional content (Khan, Tango, Miskeen, Lee, & Oh, 2017).

**Figure 2** — Hurdles on an athletic field

Lesson B covered factors that affect microbial growth in foods. These included:

- Intrinsic factors—nutrients, pH, $a_w$, redox potential ($E_h$), and antimicrobials;
- Extrinsic factors—temperature, time, atmosphere, and relative humidity;
- Food processing—slicing, mixing, washing, packing, pasteurization, smoking, handing including addition of additives and preservatives; and
- Other microbes in the food—competition, growth rate, mutualism, antagonism, commensalism, changes in intrisic factors

Factors that reduce microbial growth may be considered a barrier. Figure 3 shows a series of barriers used in the production of a food, with each step representing a different type of barrier. For the first step (bottom left), when a food is cooked, the heat treatment will reduce the numbers of microbes in a food. For the last step (top right), chilling a food to a sufficiently low temperature will prevent microbes from multiplying. When all of the steps shown in Figure 3 are used during preparation and packaging of a food, these multiple steps or hurdles increase the difficulty for microbes to successfully colonize the food.

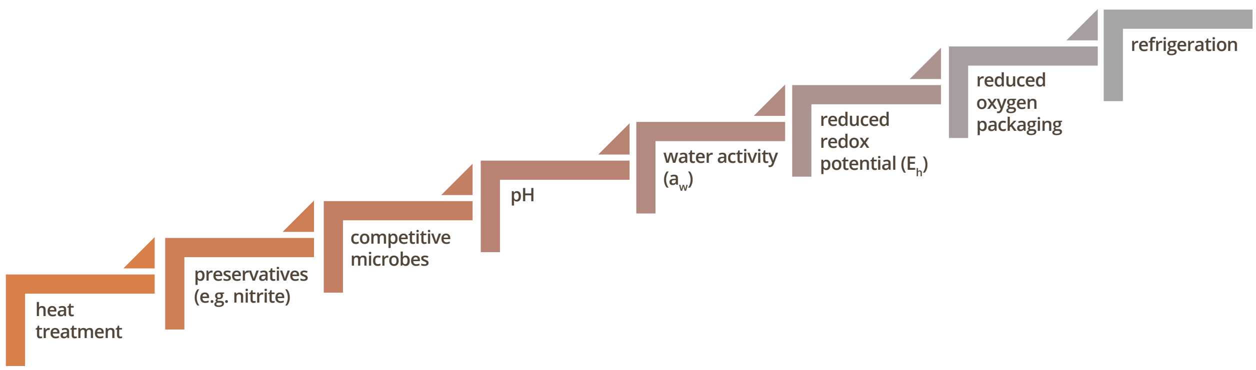



**Figure 3** — Hurdles or barriers to microbial growth

Microbes attempt to overcome these barriers, but responding to each environmental change (i.e., hurdle) introduced requires energy. The more energy microbes spend on restoring their internal balance, or homeostasis, the less they have for growth (Leistner, 2000). The growth of the microbes will slow, moving from the log phase (active growth) to the lag phase (slow growth), or even to the stationary phase (no growth). The presence of too many hurdles will force microbes to use energy to maintain their current state, until all their energy and resources are used up. Lesson C presented an example of this: the overgrowth by competitive microbes, fermentative LAB. LAB are able to outgrow and outcompete other microbes during fermentations, in the process also creating a second hurdle by creating a highly acidic environment.

Once resources are used up, many microbes reach a state of metabolic exhaustion and enter the death phase (Leistner, 2000). However, some species of microbes do not enter into a death phase, which instead react to external environmental stressors by becoming more resilient. These are the species that form environmentally resilient spores (*Clostridium* spp.), or produce specialized proteins, such as sticky proteins that bind bacteria into a protective, specialized structure called a **biofilm**. Therefore, it may be more effective for food preservation and pathogen control to combine a series of subtle hurdles rather than imposing a single, strong hurdle, in order to prevent driving such microbes from forming spores or biofilms (Leistner, 2000).

Let's examine some of these barriers in more detail, starting with the simplest, pH and water activity ($a_w$).

## Activity: Intrinsic microbial barriers: pH and $a_w$

Complete the three questions that follow:

1. Is the following statement true or false?
   "Foods with a pH of 4.6 or less or a water activity of 0.85 or less are unable to support the growth of microbes (they are called non-potentially hazardous foods)."

2. Review Table 3-A in the document at the following link.

   **Appendix 3: Bacterial Pathogen Growth and Inactivation**
   https://www.fda.gov/downloads/Food/GuidanceRegulation/FSMA/UCM517405.pdf

   Name one pathogenic bacteria able to grow when $a_w$ is less than 0.85, and two bacteria able to grow when pH is less than 4.6.

| Pathogenic bacteria able to grow at $a_w$ less than 0.85 | Pathogenic bacteria able to grow at pH less than 4.6 |
|---|---|
| | |
| | |

The key concept to take away from this short exercise is that a single barrier is NOT an absolute barrier to growth of all microbes, including pathogenic species. That is why multiple barriers are used to control the risks of pathogenic microbes in foods. The two barriers of pH and $a_w$ can work together to control growth of potential pathogens. When these two barriers are applied together, it is not necessary to have both pH less than 4.6 and $a_w$ less than 0.85. When used in combination as multiple hurdles, greater values (i.e., pH > 4.6 and $a_w$ >0.85) are effective.

3. The following activity presents an example of the previous strategy in the manufacture of fermented meats. Follow the link and review the section "4.11.1.1 Requirements for Shelf Stable Fermented Meat Products"

   **Meat Hygiene Manual of Procedures: Chapter 4—Meat Processing Control and Procedures**
   https://epe.lac-bac.gc.ca/100/206/301/cfia-acia/2011-09-21/inspection.gc.ca/english/fssa/meavia/man/ch4/4-5-11-4-4e.shtml#a4-11

   Fill in the blanks to describe the conditions shelf-stable fermented meats must meet according section 4.11.1.1 of the linked document:

   The pH of finished product is 4.6 or less, regardless of $a_w$.

   The $a_w$ of finished product is 0.85 or less, regardless of pH.

   The pH is ______ or lower at the end of the fermentation and the $a_w$ is _____ or lower in the end product.

Check your answers with the answer key provided at the end of this textbook.

There are three other required conditions mentioned in section 4.11.1.1 of the linked document that fermented meats must meet to be considered shelf-stable and able to be stored at room temperature. The first two involve a curing agent (salt) and a chemical preservative (nitrite). The third one involves temperature control during the process (degree-hours). The requirements for pH, $a_w$, and other hurdles like temperature control must be evaluated for each food depending on the process, packaging, and whether the food is intended to be cooked before consumption. These conditions or hurdles are described in the following section.

## Extrinsic microbial barriers: Freezing and refrigeration

During transportation from the farm and factory to the grocery store and restaurant, foods may be exposed to undesirable temperatures, which increases the risk of food damage, food spoilage, and growth of harmful microbial pathogens (Ryan, 2013). The pathway foods take from farm to fork is called the cold chain. Each destination along the path is considered one link in the chain. As foods travel from the farm to packing plants and distribution and transport centres, temperature control hurdles are employed to keep harmful bacteria out of temperature conditions where they would enter log phase growth; i.e. ,the temperature danger zone between 4° and 60°C. Any time the temperature of foods enter this range, we say there is a break in the cold chain. Why is this important?

Simply stated: **breaks in the cold chain increase food risk.**

### Refrigeration

When food is left outside adequate refrigeration temperatures for even a few hours, bacteria will reproduce rapidly and potentially reach dangerous levels. This can happen when:

- Refrigeration during transport malfunctions
- Reefer units are turned off during transportation
- Food pallets are left out on the loading dock
- Raw meat is cut up or ground and left on a counter
- Raw meat is hung in a market place at room temperature
- Cooked food is left to cool on a counter for too long
- Food is left to thaw on a counter
- Food in refrigerators are stored at temperatures above 4°C
- Many, many other situations occur

Sometimes, the quality of fresh produce (e.g., lettuce) is compromised because the refrigerated produce freezes during transport, particularly during Canadian winters. This freezing will not kill any harmful microbes and will cost producers and retailers in damaged product. Once the produce thaws, microbes can easily take advantage of damaged leaves and diminished surface protection and begin to grow. It should be emphasized that chilling, freezing, or mild heating does not destroy microbes, but simply keeps most microbes in the lag phase or stationary phase of growth. Given that the number of bacteria can double approximately every 20 minutes under optimal conditions, food should not be left in the temperature danger zone (between 4°C to 60°C) for more than two hours.

## Freezing

Freezing food makes water unavailable to microbes, thereby controlling their growth. However, once a food is thawed, the water availability (and temperature) increases, allowing microbes to grow and reproduce. While freezing will not kill bacteria or viruses, the freezing process will kill and control the risk of parasites when done correctly. Fish that is to be consumed raw or only partially cooked requires prior freezing for parasite control. (A few exemptions exist for some types of fish, such as tuna, depending on their diet.) For parasites to be killed, fish must be frozen under one of the following three conditions:

- −35°C for 15 hours
- −20°C for 7 days
- −35°C until solid and then stored at −20°C or below

All sushi-grade fish and fish used in ceviche must undergo freezing for parasite control, as it is consumed raw. Parasites common in local Pacific fish that can cause foodborne illness include roundworms (*Anisakis simplex*, *Pseudoterranova decipiens*) and tapeworms (*Diphyllobothrium* spp.). To learn more about these parasitic worms, consult the fish safety note from the BC Centre for Disease Control in the following link:

**Illness-Causing Fish Parasites (Worms)**
http://www.bccdc.ca/resource-gallery/Documents/Educational%20Materials/EH/FPS/Fish/Illness-Causing%20Fish%20Parasites%20Nov13.pdf

There are many species of worms that may infect fish, but not all worms will cause illness. For example, Figure 4 shows anasakid parasites in recreationally caught smoked sockeye salmon, which was reported to cause gastric illness in one family. Even though the sockeye salmon was smoked, the worms were not killed in the process. In contrast, Figure 5 shows *Henneguya salminocola* worms in pink salmon, seen in a raw fish purchased from a retail store. Although the *Henneguya* worms look unsightly, they will not cause foodborne illness.

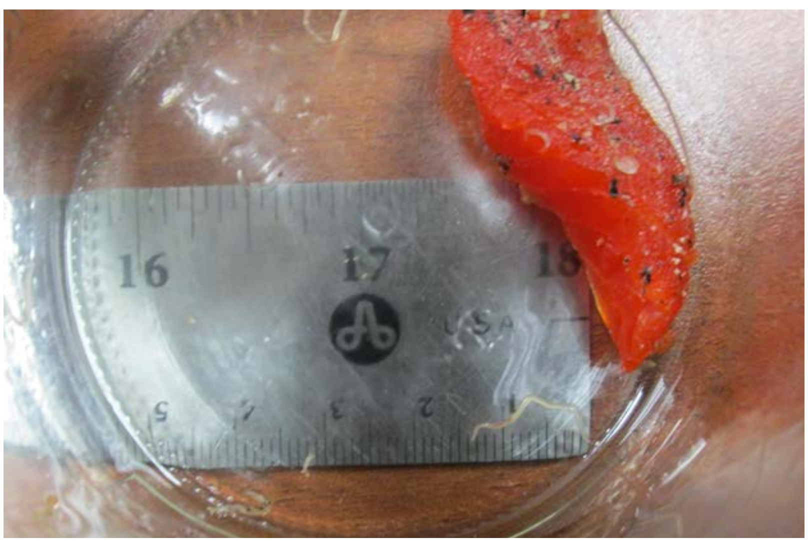



**Figure 4** — Anasakid worms in sockeye salmon


**Figure 5** — *Henneguya* sp. in pink salmon

Some meats, if they are not to be eaten fully cooked, require freezing for parasite control, such as pork and wild-caught bear meat. This is to control for the risk of *Trichinella*, the parasite that causes trichinosis. There have been multiple trichinosis outbreaks in BC associated with smoked wild-caught bear meat (McIntyre et al., 2007).

From an industry perspective, liquid nitrogen is a better, faster way to freeze foods. See this short video at the following link for an explanation.

**Fresh Clips: Liquid Nitrogen and $CO_2$**
https://www.youtube.com/watch?v=N8_ucKSvi9M

## Extrinsic microbial barriers: Storage and packaging

Spoilage and growth of pathogenic microbes may also occur in foods that are stored or packaged prior to sale. The terminology used to describe storage and packaging methods is shown in Figure 6. Storage facilities with controlled atmospheric conditions limit the growth of microbes by optimizing extrinsic microbial barriers, e.g., air temperature, humidity, or oxygen levels. Packaging can also provide a microbial barrier through exclusion of all air (vacuum packaging), reducing oxygen and increasing nitrogen and carbon dioxide gas content (modified atmosphere packaging) and controlling gas content inside the packaging using devices called scavengers (controlled atmosphere packaging).

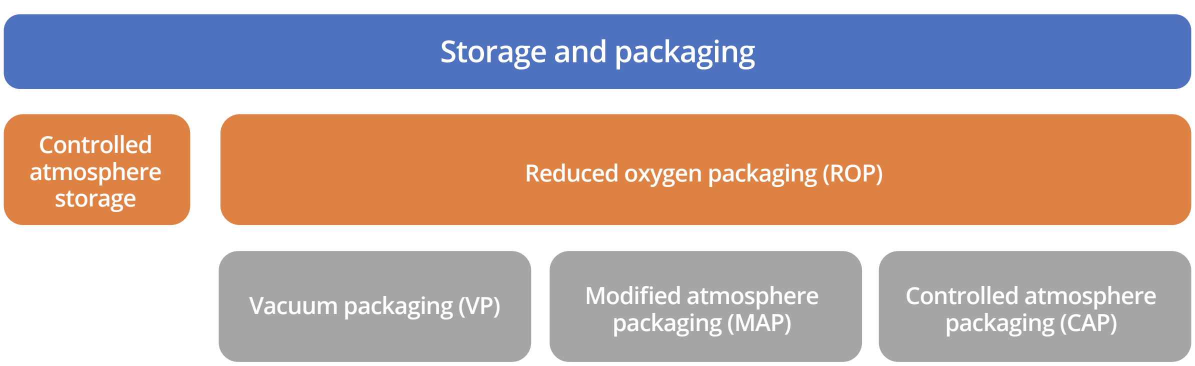



**Figure 6** — Storage and packaging terminology

## Controlled atmosphere storage

Have you ever wondered why you can eat apples and potatoes that were grown last year? Prolonged storage of fruits and vegetables is made possible by keeping them in storage buildings that have controlled atmospheres. Within these structures, the air is kept at low temperatures and with a composition that allows the produce to stay fresher for extended periods of time. Normally, air has a composition of 78% nitrogen, 21% oxygen, and only 0.03% carbon dioxide, with levels rising past 0.04% in recent years from global warming. In the controlled atmospheres of storage buildings, the carbon dioxide level is usually kept at around 8% to 10%, which limits the growth of spoilage microbes, particularly psychrotrophic and aerobic bacteria.

The videos available at the following links can help explain how this process works.

**A Living Fruit: Picked and Still Breathing**
https://www.youtube.com/watch?v=Rav6uV9G844

It's important to realize that temperature, humidity, oxygen, and carbon dioxide are all monitored and controlled to keep fruits and vegetables fresh longer. The video at the following link focuses on the system designs and high-tech equipment used to maintain fruit fresh longer.

**CA Controlled Atmosphere Keeps Fruit Fresher Longer**
https://www.youtube.com/watch?v=pLMsesdQqmw

## Reduced atmosphere packaging

In recent years, the consumer trend toward wanting foods with lower amounts of preservatives and additives has led to innovations in packaging. Perhaps you have noticed products such as chips and bagged vegetables in puffy packaging with added air space. **Reduced oxygen packaging** (**ROP**) refers to all types of packaging in which some or all of the normal atmospheric air is excluded (Figure 10). In modified atmosphere packaging (MAP), some of the atmospheric air is excluded and replaced with other gases, usually carbon dioxide. Vacuum packaging (vac-packed) excludes all air by reducing air pressure. Controlled atmosphere packaging is similar to controlled atmosphere storage, in that the packaging maintains an initial, particular gas mix.

To learn more about the three methods of reduced oxygen packaging consult the United States Foods and Drugs Administration Food Code (2017) at the following link. Note that the 2017 Food Code separates out two types of vacuum-packaged foods, cook chill and sous vide packaging (see page 49 online or page 18 of the PDF document for definitions). Their inclusion is for the specific process for which they are used. To find the pathogens (there are more than one) that are impacted by Reduced Oxygen Packaging, consult Annex 6—Food Processing Criteria on page 679 (online) or page 623 (PDF).

**Food Code**
https://www.fda.gov/food/guidanceregulation/retailfoodprotection/foodcode/ucm595139.htm

### Vacuum packaging (VP)

This type of packaging removes ALL air from the package prior to sealing. Most spoilage microbes require oxygen (i.e., they are aerobic), so when oxygen is absent, they cannot grow. This type of packaging is therefore excellent to limit their growth. However, removing all the oxygen from packaging will increase the risk for one important anaerobic bacteria.

## Activity: Vacuum packaging fill in the blank

Name this bacteria: ___________________________________

Many foodborne pathogens are facultative anaerobes, and can survive in reduced oxygen packaging environments. Name at least three other bacteria of concern mentioned in the food code:

_______________________________________________________________________________________________

_______________________________________________________________________________________________

_______________________________________________________________________________________________

### Modified atmosphere packing (MAP)

**Modified atmosphere packaging** (MAP) is designed to hinder the growth of microbes by denying them oxygen via the introduction of unnatural levels of gases such as carbon dioxide around the food. The ratio of nitrogen, oxygen, and carbon dioxide used varies according to the foods being packaged. Packaging types may also vary; they may be impermeable or semi-permeable, depending on whether the food is of a type that respires (breathes), such as fresh produce.

The type of packaging used may also depend on the food's fragility; for example, potato chips are an easily crushed food. But, from a microbiological viewpoint, potato chips are very low-risk food because the $a_w$ is low. Inert nitrogen gas is added to potato chip bags solely to puff up the package and prevent the chips from crushing, and to prevent them absorbing moisture from the air.

Carbon dioxide gas is bacteriostatic (stops bacteria from reproducing) and able to inactivate many microbes, so it is often used in MAP to control unwanted growth of microbes. The brief video at the following link gives a great overview of how MAP is used in the food industry.

**About Modified Atmosphere Packaging**
https://www.youtube.com/watch?v=Hgra42ZrTqQ

### Controlled atmosphere packing (CAP)

With respect to microbial hurdles, results similar to controlled atmosphere storage in buildings can be achieved within a package. **Controlled atmosphere** packaging involves the use of chemical scavengers that keep carbon dioxide and oxygen levels at a constant level within the package, and is also called active packaging. The controlled gas levels stop oxidation and extends the nutritional value of fresh fruits. A sachet might be added to the package, similar to the desiccant sachets added to packaging to remove moisture from dried foods. In this process oxygen is absorbed and delays natural degradation. The value for this type of packaging is the ability to avoid preservatives that people don't wish to have on their fresh fruit or vegetables (for e.g. sulfites).

This next video shows how whole fruits are cut and packaged to extend shelf life. Strict hygienic conditions are used to eliminate risks of contamination. We enjoy fresh packaged tropical fruits because of these novel fruit packaging methods. This video incorporates marketing as well as food security into their rationale for why this is important: elderly people and those with health issues may not be able to slice and prepare different types of fresh fruit without assistance. Using active packaging will help countries gain access to other markets they would not normally be able to transport fresh cut fruit to.

The video in the following link was produced by Easy Fruit, a company that uses a type of active packaging called an active tray, that employs polymers added to plastic packaging. The video is a bit long, but the visuals will help you understand how this work is done, how packaging can extend shelf life, and what hygiene levels are needed.

**EASYFRUIT: Active Packaging to Extend the Shelf Life of Fruit**
https://www.youtube.com/watch?v=Aig9rZIFBho

View the following link, which although industry focused, is very informative regarding the rationale behind active packaging.

**Packaging: Oxygen Scavenging**
https://www.youtube.com/watch?v=LmTakJLP0kc

## Processing microbial barriers: Adding chemical preservatives

When you read the ingredient list on a package of prepared foods, you may find many of the unfamiliar and challenging-to-pronounce names (Figure 7). Some of these may be vitamins such as thiamine, riboflavin, and niacin, which are added to enrich the nutritional content of a product. Others may be chemical additives that help to stabilize the product to keep it from separating or changing in consistency. Many of them will be chemical preservatives, which are different from added vitamins and stabilizers. Preservatives can be divided into two groups:

1. Antimicrobials, which inhibit the growth of microorganisms
2. Antioxidants, which preserve foods against the chemical process of oxidation

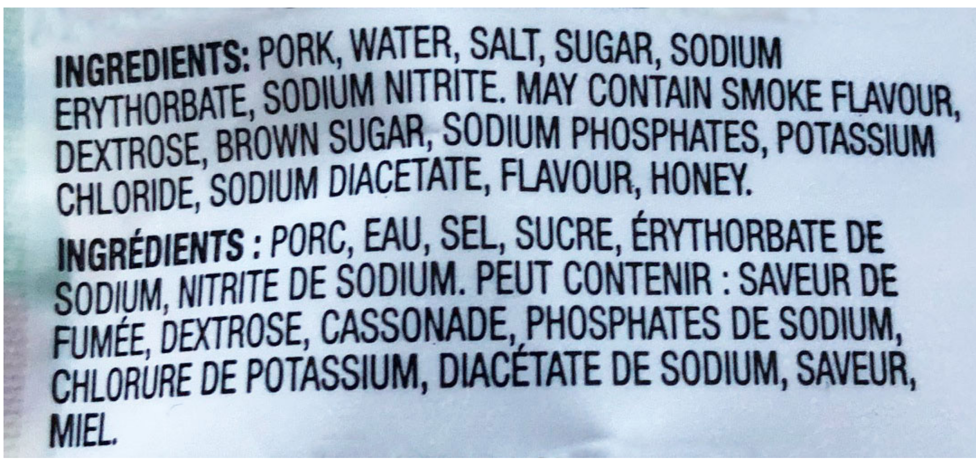



**Figure 7** — Preservatives listed on a typical food label

### Antimicrobials

Common antimicrobial preservatives include sorbate, benzoate, sulphates, phosphates, nitrites, and nitrates, among other chemicals. Some examples of the use of antimicrobial preservatives are:

- Potassium sorbate may be added to baked goods to prevent mould.
- Sodium benzoate works by preventing the metabolism of sugar, the main source of energy for many bacteria. Soft drinks may contain benzoate to prevent growth of yeasts and moulds.
- Curing agents for fermentation of meats will contain nitrates and nitrites to restrict the growth of spore-producing bacteria such as *Clostridium botulinum*. However, too much of these chemicals can cause toxic poisoning and they are also considered carcinogens.
- Chitosan, a type of sugar made from crustacean shells, has broad antimicrobial effectiveness against Gram-positive bacteria.

### Antioxidants

Antioxidants are utilized to induce a hurdle to microbial growth, as well as to preserve product quality, prevent rancidity, and confer product stability. Common food antioxidants include ascorbate and the hydroquinone family of chemicals. Hydroquinones are derivatives of benzene, a petrochemical and a carcinogen. The use of hydroquinone preservatives is hotly debated by nutritionists, food microbiologists, and toxicologists, as the effects of chronic, long-term exposure to these benzene derivatives is unknown.

### Using both antimicrobials and antioxidants

Sometimes, antimicrobial and antioxidant preservatives are used together in a food product. For example, the addition of nisin, an antimicrobial toxin that kills Gram-positive bacteria, combined with antioxidants and other preservatives can prolong the shelf life of cheeses, meats, and beverages. Sometimes the introduction of a chemical or additive can impose multiple hurdles. Some preservatives are both antimicrobials and antioxidants. For example, when ascorbate is added to a food, it confers both an antimicrobial and antioxidant hurdle. Since ascorbate (also called ascorbic acid or vitamin C) is found naturally in many fruits, it is preferred as a more natural preservative.

Preservatives add one or more hurdles or barriers to undesirable microbial growth. Other production methods can also be a barrier to microbial growth. In Topic 2 of this lesson, we discussed how specific levels of pH and $a_w$ are microbial barriers in fermented meats. We also mentioned the use of a curing agent and a chemical preservatives to meet conditions for shelf-stable fermented meats. In the rest of the lesson, we will discuss how various production methods are significant hurdles to microbial growth, starting with the most basic: cold-chain temperature control.

To see a complete list of permitted preservatives for foods in Canada, consult the list from Health Canada at this link:

**List of Permitted Preservatives (Lists of Permitted Food Additives)**
http://www.hc-sc.gc.ca/fn-an/securit/addit/list/11-preserv-conserv-eng.php

In the rest of the lesson, we will discuss how various production methods are significant hurdles to microbial growth.

## Processing microbial barriers: Curing and smoking

Preserving meats and fish often involve the production methods of curing and smoking. In ancient times, drying, curing, and smoking were commonly used to prevent spoilage of meats and fish due to oxidation or rancidification. Today, these methods are employed to enhance flavour, colour, and texture.

### Curing

Examples of common cured products include bacon, ham, dry sausages, candied salmon, and jerky. Traditional curing involves the introduction of various salts, including sodium chloride (NaCl) and sodium or potassium nitrite ($NaNO_2$ and $KNO_2$), along with sugars such as glucose and sucrose. Other chemical agents, such as phosphates, potassium sorbate, ascorbate (vitamin C), erythorbate, monosodium glutamate, lactates, and various spices, may be added during curing to introduce and enhance flavours and improve colour. Sweet ingredients other than sugars may also be added, such as maple syrup, honey, molasses, and corn syrup.

Any one of the ingredients mentioned in the preceding paragraph, either by itself or in combination with other ingredients, may be referred to as a curing agent. However, this term has a specific meaning when used by regulators assessing fermented meats.

**Definition of curing for edible meat products**

According to the Canadian Food Inspection Agency, Curing means, in respect of an edible meat product, that salt together with at least 100 parts per million (ppm) and not more than 200 ppm of sodium nitrite, potassium nitrite, sodium nitrate, or potassium nitrate or any combination thereof, was added to the meat product during its preparation."

**Meat Processing Controls and Procedures (archived: Government of Canada)**
https://inspection.canada.ca/en/preventive-controls/meat/nitrites

Curing prevents microbial growth by lowering the amount of available water ($a_w$), and by reducing the pH. Salts and sugars will pull water out of microbial cells until those cells can no longer function. The cells may not die right away, but are inactive. Curing with salts and sugars can be done wet—by making a water and salt/sugar brining solution that foods are soaked in—or dry—by rubbing curing agents onto foods or mixing curing agents into foods.

"The dose makes the poison" is a saying well-known to toxicologists, who know that most substances can be toxic if too much is ingested, including water! This point is particularly important for salt curing agents, especially nitrite and nitrate. Although these are both permitted additives, the amount that can be added to foods is tightly regulated (generally from 100 to 200 ppm). Consuming too much of either of these additives can result in a condition called methemoglobinemia, when nitrite and/or nitrate converts hemoglobin in the blood to methemoglobin, greatly reducing the blood's oxygen-carrying capacity. Bologna, wieners, and other cured meats have caused this condition when too much nitrite is added (Orgeron, Martin, Caraway, Martine, & Hauser, 1957). To avoid errors in measuring nitrite/nitrate, most operators will purchase commercial mixes of this curing agent, composed of nitrite and/or nitrate premixed with table salt. The premixes come with instructions on the amount of the curing agent to be added to meat. Common names for curing mixes containing sodium nitrite and/or sodium nitrate are Prague Powder #1 and Prague Powder #2.

## Smoking

Wood smoke contains chemical products of combustion, such as formaldehyde and acetic acid, that lower the pH of smoked foodstuff, thereby acting as antimicrobial agents. Since smoking involves heat, this process also removes moisture (Figure 8).

**Figure 8** — Smoking ham

In addition to altering the acidity of foods and removing moisture, smoking also adds chemical compounds that act as preservatives .For example, phenol and other phenolic compounds in wood smoke are both antimicrobials and antioxidants, and so slow bacterial growth and the chemical decomposition of fats known as rancidification. However, this class of chemicals is also carcinogenic.

The process of smoking also involves the extrinsic factors of temperature, time, and relative humidity. Smoking Foods smoked at temperatures above 45°C are generally regarded as hot-smoked, with most hot-smoking processes taking place between 50°C and 80°C. Foods smoked at temperatures below 45°C are generally regarded as cold-smoked, with normal cold-smoking temperatures being between 20°C and 30°C. Although smoking may reduce microbial loads as described above, the addition of smoke is not used solely to control microbial risk. Smoking is carried out to add flavour. Food safety and microbial risks during smoking operations are assessed based on temperature, time, and humidity.

Safety assessments of cold-smoked products will include an evaluation of the amount of time foods are kept at temperatures above 10°C. For example, with fermented meat products, temperatures above 15.6°C are a concern for the formation of enterotoxin-producing *Staphylococcus aureus*. The amount of time a product spends at these temperatures must be evaluated for safety. Some cold-smoked foods, such as cold-smoked salmon, must be refrigerated and sold immediately or frozen, as they have a very short shelf life. Hot-smoked foods must be processed under temperature and time conditions that will ensure there is a 6 to 7 log reduction in pathogen levels.

Relative humidity during the process is another important extrinsic factor to measure. If conditions are too dry, thermal processes (i.e., cooking) may not reduce microbial loads, or the outer layer of the food may harden and not allow effective reduction of the microbial load through thermalization or drying.

## Processing microbial barriers: Canning and pickling

Canning and pickling are food-preservation techniques that involve storage of foods in containers. At some point in production, canning always involves thermal processes to create hurdles to microbial growth. Pickling may also involve thermal processes, but always involves changes in pH as a microbial barrier.

### Canning

Canning is a commonly practiced method of preserving foods that uses several hurdles: heat treatment, increases in atmospheric pressure, vacuum sealing, and, sometimes, addition of preservatives. Canning revolutionized food preservation in the early 1800s. It was developed when

Napoleon Bonaparte offered a cash prize for an invention that would allow prolonged food storage, so he could feed his armies. The prize was won by the Frenchman Nicolas Appert, for his method involving glass bottles that were heated and sealed.

**Figure 9** — Home-preserved foods in sealed glass jars

In addition to the glass jars with metal seals shown in Figure 9, foods may be canned in metal cans, aluminum pouches, or any other airtight packaging or container that can be subjected to heat and pressure. Foods with low pH (i.e. less than a pH of 4.6), called high-acid foods, include berry jams, apple sauce, and tomatoes. These foods can be canned using a boiling water canning method, without added pressure. Tomatoes may also be processed in a pressure canner and usually need to be acidified before boiling water or pressure canning methods are employed (USDA, 2015). Foods with high pH (alkaline foods with a pH greater than 4.6), are called low-acid foods. Low-acid foods must be pressure canned as botulism can occur in low-acid foods, such as meats and vegetables. Home canning should always be done to approved methods. Consult the resource below for more information.

**USDA Complete Guide to Home Canning**
https://www.healthycanning.com/wp-content/uploads/USDA-Complete-Guide-to-Home-Canning-2015-revision.pdf

*Retort* is a term used to describe the process or the equipment used in high-pressure steam canning operations. *Retort pouches* refers to a type of food packaging that is a flexible, allows foods to be hermetically sealed inside the package (i.e., airtight), and can be commercially sterilized and stored unrefrigerated for long periods. Retort pouches are made from a combination of thin layer of a metal such as aluminum, nylon or puncture-resistant plastic, and other materials. Retort pouches may contain food that is ready-to-eat, or may be designed to allow the food to be reheated, such as a boil-in-the-bag product. Microwavable retort pouches are manufactured with plastic films rather than metal, and can still withstand high-temperature sterilization.

Commercially sterilized foods are subjected to temperatures greater than the boiling point of water (>100°C). The only way to raise temperature of food to greater than 100°C is to increase the atmospheric pressure. To ensure food is properly sterilized, commercial producers must use specific conditions of temperature, pressure, and time that are known to kill *Clostridium botulinum*, an anaerobic microbial hazard of most concern in canned foods. Recall that in Lesson B, we introduced the term *D-value*, which refers to a single log reduction (or a 90 % reduction) in the population of a specific microbe. Commercial canning operations require a 12 log reduction, which is obtained under temperature and time conditions of 121°C for 2.5 minutes; this is known as a 12D bot cook. These stringent conditions will destroy any *Clostridium botulinum* spores that are present during the canning process. If these conditions are not met, these bacteria to begin growing and producing the botulism toxin in the anaerobic environment of the final canned product.

## Activity: Fill in the blank

A 12D process means that there is reduction of bacteria in the food by __________%

(Hint: a 2 log reduction or 2D process means that there is a 99 % reduction in bacteria.)

Check your answers with the answer key provided at the end of this textbook.

Bulging cans and bulging or leaking retort pouches are an indication that gas is being created inside the closed, sealed container. This is a sign of active microbial metabolism and so that the canning process failed. A failure could occur when a puncture or pinhole arises in the package, or when the proper combination of pressure, temperature, and time was not achieved during the canning process.

There are several kinds of damage to metal cans that indicate that the integrity of the can, and thus the safety of the food inside, is compromised. View the following link to see examples of unacceptable food can damage.

**Unacceptable Food Can Damage**
http://www.bccdc.ca/resource-gallery/Documents/Educational%20Materials/EH/FPS/Food/UnacceptableFoodCanDamage1page.pdf

Determining the safety of a canning operation and process is complicated. Factors that need to be examined include the type of equipment used, the type of containers and their dimensions, the food content, and what measurements of the product must be made to ensure that adequate process time and temperature are achieved. See the following links for examples of some of the information required from a canning operation.

**Guidance for Industry: Submitting Form FDA 2541 (Food Canning Establishment Registration)**
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-submitting-forms-food-canning-establishment-registration-and-food-process-filing

**Food Process Filing for Low-Acid Aseptic Systems**
https://www.fda.gov/media/93959/download

## Pickling

Pickling foods involves soaking them in acidic solutions to help prevent spoilage. Pickling often occurs in vinegar, which is a 5% solution of acetic acid. Vinegar has a pH of 2.4, which is a hurdle to the growth of many microbes.

A few pathogens are resistant to acidic conditions, including *E. coli* O157:H7. This pathogenic microbe is known to be associated with raw beef (it is the cause of hamburger disease) and can also be present on fruits and vegetables that come in direct contact with sources of fecal waste, such as contaminated irrigation water. When pickling fruits and vegetables, the addition of vinegar may not be sufficient by itself to eliminate pathogenic *E. coli* (Lee, 2004). Another process condition to consider is time. *E. coli* is not likely to grow or to survive in acidic products after the food is held for a period of time. An outbreak of *E. coli* among Korean schoolchildren occurred when kimchi (an acidified fermented cabbage dish) was served within days of its preparation. If the kimchi was held longer, the *E. coli* would have had time to die off (Cho et al, 2012). Combination hurdle processes are most effective in inactivating harmful microbes in pickled products. For pickles (i.e., pickled cucumbers), these processes include the addition salt to the vinegar, heating to 74°C for 15 minutes followed by rapid cooling, and possibly the addition of preservatives such as sorbic acid (Lee, 2004).

## Processing microbial barriers: Pasteurization

**Pasteurization** is a heating process that reduces the presence of potential pathogens to tolerable numbers, and is a foundational technique for controlling bacterial contamination. Pasteurization involves heating food, usually liquids, to a specific temperature for a specified length of time. The liquid is then sealed in packaging (e.g., a bottle or a pouch) and cooled quickly. The heat kills many bacteria and the sealing of the packaging deprives any remaining bacteria of the oxygen they need to survive. When done properly, pasteurization can kill 99.999 % of spoilage microorganisms and is 90% effective in eliminating pathogenic bacteria.

Pasteurization is most commonly associated with milk and beverages. Early records from at least 500 to 900 years ago from China and Japan described how heating will preserve wine. This technique was refined in 1864 by Louis Pasteur (Figure 10), who made clear the relationship between microorganisms and food spoilage, and refined the process which today bears his name.

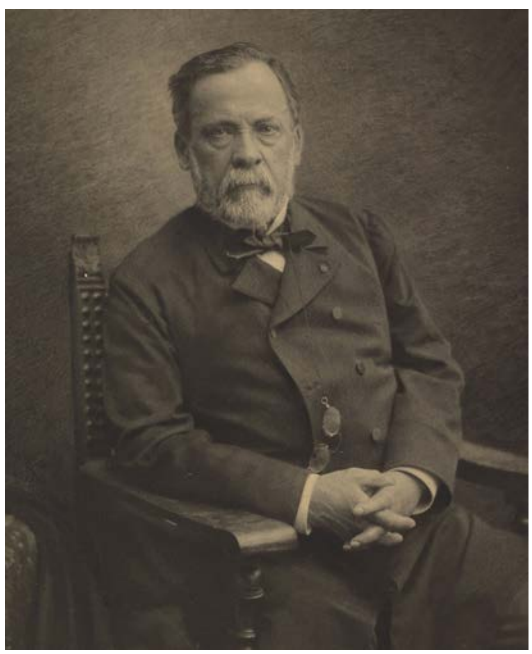


**Figure 10** — Louis Pasteur

### Types of pasteurization

Figure 11 shows the several different types of pasteurization methods used for milk. **High temperature, short time (HTST)** pasteurization involves heating milk to 71.7°C for 15 to 20 seconds, and is used for milk products to be stored at refrigeration temperatures. **Higher heat, shorter times (HHST)** pasteurization may also be used for these milk products. **Ultra-high temperature (UHT)** pasteurization involves heating milk to 135°C for at least one second, and is used for milk products that will be stored at room temperature, such as tetra pack boxed milk and the little creamers you find on tables in restaurants. UHT processing is done aseptically (under strict hygienic and sterile conditions). Always check the label on dairy creamers (and other dairy products), as many are processed using an HHST method or a modified UHT process that is not done under aseptic conditions. These processes allow for extended refrigerated shelf life, but not room temperature storage. Batch pasteurization is a process in which a lower temperature of 63°C is applied for 30 minutes (or equivalent).

| Name | Time | Temperature | Storage |
|---|---|---|---|
| HTST | 71.7°C | 15 sec | Refrigerated |
| HHST | 88.3°C<br>96.2°C | 1 sec<br>0.01 sec | Refrigerated |
| UHT | 135°C | >1 to 15 sec | Room |
| Batch | 63°C | 30 min | Refrigerated |

**Figure 11** — Methods of milk pasteurization

Pasteurization equipment can be specialized, and verification of temperature control is one important aspect of dairy operations. In Canada, milk is pasteurized using HTST methods, in which milk is heated to 72°C for 16 seconds and immediately cooled. Because of the higher fat content, cream and fluids used to make ice-cream are pasteurized using higher temperatures and longer times, as fat is a poor heat conductor (Alfa-Laval, 1995). In BC, these criteria are regulated under the "Milk Industry Standards Regulation" of the Milk Industry Act, available at this link:

**H.T.S.T. Pasteurization—General**
http://www.bclaws.ca/civix/document/id/loo89/loo89/464_81#section103

Raw fluid milk must comply with standards that limit the numbers of bacteria cells and **somatic** cells that may be present. If these are not met, penalties will apply to the operator. Somatic cells are any cells from the body of an animal. In the case of raw milk, somatic cells refer to white blood cells, and they are an indication of milk quality. When somatic cell counts are high, this indicates that a cow has mastitis, and so potentially harmful bacteria, such as *Staphylococcus aureus,* may be present.

The potential presence of *Staphylococcus aureus* is one reason why milk must be pasteurized before consumption. Other pathogens eliminated during pasteurization include: *Campylobacter, Coxiella* (causes Q-fever), *Mycobacterium tuberculosis*, *Salmonella*, *E. coli, and Listeria*. Raw milk is not allowed to be sold in Canada because of the high risk for the presence of pathogens. There have been many documented cases of illness and outbreaks linked to raw milk consumption in BC and elsewhere. Pasteurization of raw milk decreased infant mortality when it was introduced over 150 years ago, and sanitary milk handling remains crucial to reduce risks of illness (Currier & Widness, 2018). Studies of raw milk outbreaks in the United States have found that outbreaks linked to raw milk have increased in recent years, and that children are at the highest risk of illness (Costard, Espejo, Groenendaal, & Zagmutt, 2017; Langer et al., 2012). Over 80 % of the outbreaks occurred in states where raw milk is legal.

Raw milk illnesses are a growing problem in the US, particularly in states where raw milk is legal.

- The risk of an outbreak is 150 times greater for raw milk compared to pasteurized milk (Langer et al., 2012).
- Unpasteurized dairy products cause 840 times more illnesses and forty-five times more hospitalizations than pasteurized products (Costard et al., 2017).

More information about illnesses causes by raw milk consumption in BC and the United States is available at the following links.

**Table of British Columbia Raw Milk Events Described in Surveillance Reports**
http://www.bccdc.ca/resource-gallery/_layouts/15/DocIdRedir.aspx?ID=BCCDC-288-3332

**Dairy Safety**
http://www.bccdc.ca/health-info/prevention-public-health/dairy-safety

**Raw Milk Dangers**
https://www.cdc.gov/foodsafety/rawmilk/raw-milk-index.html

## Processing microbial barriers: Dehydration and freeze-drying

The previous discussion of canning and pasteurization outlined the use of thermal treatments as hurdles to microbial growth. These hurdles reduce microbial populations using heat. We will now consider the production-method hurdles of freeze-drying and dehydration.

Food dehydration and freeze-drying both share the same barrier of limiting water availability, but they achieve this barrier in different ways. Dehydration of foods involves heating to remove water, while freeze-drying, as the name implies, involves low temperature flash-freezing of foods and high vacuum pressure to remove excess moisture. Because dehydrated and freeze-dried foods have very little moisture in them, generally they can be stored at room temperature and are shelf stable, and very little microbial risk exists in these products.

### Dehydration

Drying foods is a common, but complicated, process. Heating converts liquid moisture at the surface of the food into water vapour, which then moves into the air. The water content of the food therefore decreases. This process can be sped up by using a fan or other mechanism to remove moist air. The relative humidity of the air during drying is important. When humidity is high, drying of foods is less effective. However, when humidity is too low, heat treatment is not as effective.

Dehydration uses heat to promote water loss. Drying of foods is also dependant on the size of food particles, with larger particles taking longer to dry. The greatest microbial risk during dehydration occurs at the beginning of the drying process, when temperatures are low. During this period, particularly when foods are entering the danger zone (temperatures from 20°C to 40°C), microbes may continue to multiply until the water activity ($a_w$) reduces sufficiently. Pathogen log reduction will usually only occur at temperatures exceeding 55°C. Therefore, foods dehydrated at temperatures lower than 55°C for extended periods are vulnerable to increases in microbial growth. Once $a_w$ drops low enough, any microbes still present in the food will no longer be able to multiply. The longer the period of dehydration during which the humidity remains low and heat high, the more the number of microbes will decline, as water is no longer available for their survival.

A novel way to dehydrate foods that allows for as much or as little moisture content as desired in the final food product is REVTM, or radiant energy dehydration. This technique was developed at the University of British Columbia, and has now been commercialized. The process and equipment used to create this hurdle, absence of water in the food product, is explained in the material available at the following link.

**How It Works: Dehydrate Products Faster with REV™**
https://www.enwave.net/how-it-works

### Freeze-drying

Freeze-drying results in higher quality products than dehydration. Only water is removed during freeze-drying, whereas during dehydration, minerals, vitamins, and other solutes may be transported outside of the food during the dehydration process. Freeze-drying removes water by a process called sublimation, in which water changes from a solid (ice) to a gas (water vapour) without ever becoming liquid water. To accomplish sublimation, food is pre-treated, rapidly frozen, and then pressurized. This process occurs rapidly, so that ice forms quickly and is quickly followed by release of the gas, allowing the food to keep its structure. Freeze-dried foods are usually vacuum-packed so they don't absorb water from the air. Two hurdles are imposed by freeze-drying: reduced activity of water from freezing and sublimation, and reduced access to oxygen. Similar to the process of dehydration, microbes are not immediately killed during freeze-drying. Instead, microbes will die off over time due to limited water availability.

## Production-method microbial barriers: Irradiation

The process of irradiating foods with ionizing radiation effectively and indiscriminately kills all microbes. There are three types of ionizing **irradiation** technologies currently used: gamma ray, electron beam, and X-rays. The high energy of the rays or beams is transferred to water and other molecules in the microbes and in the foodstuff. The energy creates transient reactive chemicals known as oxidants that damage the DNA of the microbes, the genetic blueprint for all of their metabolic activities. When a radiating dose is high enough, it will compromise a microorganism to a point where it can no longer function properly, and so is unable to grow or reproduce and dies. According to the U.S. Centre for Disease Control (CDC) and others who have completed extensive studies on safety of irradiated foods, this technique is a safe and effective technology that can prevent many foodborne diseases (Tauxe, 2001).

In 2017, Health Canada authorized the treatment of fresh and frozen raw ground beef with ionizing radiation (Figure 9). The irradiation of ground raw beef can eliminate *E. coli*, *Salmonella*, *Campylobacter*, and other pathogens. Currently only the following irradiated foods are permitted to be sold in Canada: potatoes, onions, flours, spices and seasonings, and raw ground beef. As well as removing potentially harmful foodborne pathogens, irradiation can also protect foods such as grains, onions and garlic from spoilage and prevent sprouting, thereby extending their shelf life.

However, consumers remain sceptical and concerned over safety of irradiated foods. Nutritional studies of irradiated foods have shown some decline in vitamin content, but declines also found in foods that are cooked (e.g., grilled, fried, or boiled). The higher the dose of radiation, the greater the impact on the taste and quality of the food.

To learn more about this topic, watch the video available at the following link. In the video, Dr. Rick Holley, a respected Canadian researcher, explains beef irradiation and addresses consumer perceptions in Canada.

**Beef Research School: What Is Irradiation?**
https://www.youtube.com/watch?v=QvJfOsYQe8w

**Scientific Opinion on the efficacy and microbiological safety of irradiation of food (European Food Safety Authority)**
https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2011.2103

Today, any food that has been radiated in the United States or Canada carries a special internationally recognized label called the *radura* (Figure 12), along with a statement that the food has been irradiated preceding the list of ingredients. While radiation cannot replace good sanitation practices or eliminate the dangers of cross-contamination, it could help prevent many outbreaks of food poisoning. Low-dose irradiation of foods (up to 1 kilo Gray unit or kGy) can prevent sprouting in tubers and bulbs, prevent insect loss in cereals, delay fruit ripening and extend shelf life of food stuffs. Moderate irradiation (between 1 and 10 kGy) is needed to remove non-spore forming pathogens in foods and dry spices, and high levels of irradiation (up to 50 kGy) are needed to make meat containing vacuum-packed foods shelf-stable and destroy bacterial spores (EFSA, 2011). The fact that this hurdle is not used universally is likely due to negative public opinion and to costs that limit implementation of this method of food preservation.


**Figure 12** — Canadian label for irradiated foods

To find out more about food irradiation and practices in Canada, visit the following links:

**Canada Food Inspection Agency: Food Irradiation**
Canadian Food Inspection Agency: Food Irradiation Fact sheet

**Irradiated Foods: Requirements**
Canadian Food Inspection Agency: Food Irradiation Requirements.

**Frequently Asked Questions Regarding Food Irradiation**
Health Canada's Frequently Asked Questions Regarding Food Irradiation

**Health Canada: Food Irradiation**
Health Canada Food Irradiation

## Processing microbial barriers: New technologies and traceability

Figure 1 at the beginning of this lesson showed a photograph of trout being smoked over a fire pit to illustrate the historical simplistic hurdles applied to preserve foods. Traditional food-preservation technologies include smoking, pasteurization, inclusion of additives, fermentation (described in Lesson C), and freezing and refrigeration. Technologies and equipment are now available that advance the science of hurdles. Foods can be processed using lower heats that result in minimal changes to the organoleptic and nutritional quality of foods.

Emerging technologies and descriptions of the principals by which they act as microbial barriers are presented in Figure 13. (Information in the table is derived from Khan et al., 2017; Li & Farid, 2016; and Valdramidis & Koutsoumanis, 2016.) Figure 13 also includes technologies used for traceability. Tracing is determining where foods are in the food chain (during production and distribution). Although not strictly a hurdle or barrier, tracing can help manufacturers determine where risk occurs within the food chain. Of particular importance is the ability to measure food temperatures throughout the cold chain.

| Name of technology | Description of hurdle* |
|---|---|
| **Non-thermal novel technologies** | |
| Bacteriophages | The best way to kill an unwanted bacteria is to give it a virus—that's the theory behind the use of bacteriophages. There are many of these viruses that can be added to packaging or directly to foods to suppress the growth of unwanted and harmful microbes. |
| Pulsed electric field | Short pulses of electricity are used to damage the protective outer layers of microbes. This technology can be used for liquid and solid foods. Levels of electricity are between 20 to 80 kV. |
| High-pressure processing | Foods are place is flexible packaging and high pressure >600 MPa1 is applied. It is often used in combination with mild heat to increase effectiveness in killing spore forming microbes. The pressure damages the microbes outer layers and proteins. |
| Ultraviolet light and pulsed light | Ultraviolet (UV) light is a low wavelength light (100 to 400 nm) that cross-links the DNA of microbes. Pulsed UV light and other types of light (infrared, white light) have better penetration, and can also inactivate spores. The light damages DNA and structures of microbes. |
| Cold plasma | Cold plasma is composed of electrons, ions, photons, and other charged particles, usually in a gas form, and is used to kill microbes. This technology is promising because gases inside of already packaged foods can be used in this process. |
| Ultrasonic | In this technology, sound waves are used to create shock waves in liquid foods that can inactivate microbes. Waves are effective at a level of < 2.5 MHz. |
| **Thermal novel technologies** | |
| Microwave and radiofrequency | These are both dielectric heating methods. Dielectric heats uses ionic and dipole fields (electromagnetic) within the food matrix, which allows for even heating. These heating methods are often used in combination with other hurdles; for example, to improve drying time. |
| **Traceability** | |
| TTI—time temperature indicators | These devices display a particular colour change when a specific temperature threshold is exceeded. |
| RFID—radio frequency identification | A tag is used to track the geolocation of a food and can send the data to a smart phone. Depending on the tag, it may also measure temperature, and it may work in real-time. |
| * Abbreviations for units of measure used in descriptions are: kV = kilovolt, for electricity; Pa = pascal, for pressure; nm = nanometre, for a wavelength of light; MHz = megahertz, for sound wave frequency | |

**Figure 13** — Emergent Hurdles for Improved Food Sanitation

## Activity: Hurdle technologies and lasagna

To illustrate the role of hurdles in food production, preparation, and food safety, let's consider the hurdle technologies actively involved in preparation of a lasagna dish. The ingredients you will use to make your lasagna as as follows:

***Lasagna Ingredients***

- Noodle layer
  - ◊ Packaged dry lasagna noodles
- Tomato and meat sauce layer
  - ◊ Canned tomato sauce
  - ◊ Spices and salt
  - ◊ Spicy dry ready-to-eat italian sausage
- Cheese and spinach sauce layer
  - ◊ Ricotta cheese
  - ◊ Powdered parmesan cheese
  - ◊ Eggs
  - ◊ Cream
  - ◊ Flour
  - ◊ Frozen spinach
- Topping of mozzarella cheese

## Part 1: Hurdle identification during food processing

In the table shown below, identify all the hurdles that would have been used by the manufacturers for the ingredients shown during food processing (**note:** not all hurdles are shown in this exercise). Check your answers with the answer key provided at the end of this textbook.

| **Choose hurdle for each ingredient** | **Lasagna noodles** | **Tomato sauce** | **Spices and flour** | **Italian sausage** | **Eggs and cream** | **Spinach** | **Parmesan, ricotta and mozzarella cheese** |
|---|---|---|---|---|---|---|---|
| Acidic pH | | | | | | | |
| Canning | | | | | | | |
| Curing | | | | | | | |
| Drying or dehydration | | | | | | | |
| Fermentation/microbes competing | | | | | | | |
| Irradiation | | | | | | | |
| Low water activity ($a_w$) | | | | | | | |
| Pasteurization, cooking or heating | | | | | | | |
| Preservatives | | | | | | | |
| Reduced oxygen packaging | | | | | | | |
| Refrigeration or Freezing | | | | | | | |

**Figure 14** — Hurdles used during processing of foods in given lasagna recipe

What hurdles do you plan on using when you make a lasagna? First, review the steps that follow for your lasagna preparation, and then think about what you will need to do to ensure no harmful microbes or spoilage agents have a chance to grow.

**Figure 15** — Lasagna

***Steps in lasagna preparation***

Step 1. Prepare the noodles.

Step 2. Prepare the tomato and meat sauce.

Step 3. Prepare the cheese and spinach sauce.

Step 4. Assemble. First put a layer of meat sauce down, then a layer of noodles, of cheese and spinach sauce, of noodles, and continue in this pattern until all the noodles and sauce are used.

Step 5. Top with mozzarella cheese.

Step 6. Bake the lasagna.

Step 7. Store the leftovers.

### Part 2: Hurdle identification while assembling lasagna

In the table shown below, identify all the hurdles that would have been used by the manufacturers for the ingredients shown during lasagna assembly. Check your answers with the answer key provided at the end of this textbook

| Step | 1. Prepare noodles | 2. Prepare tomato and meat sauce | 3. Prepare cheese and spinach sauce | 4. Assemble the lasagna | 5. Bake the lasagna | 6. Store leftovers |
|---|---|---|---|---|---|---|
| Pasteurization or heating | | | | | | |
| Cooling | | | | | | |
| Refrigeration | | | | | | |
| Freezing | | | | | | |
| Time | | | | | | |

**Figure 16**—Hurdles to be used during given steps in lasagna preparation

Check your answers with the answer key provided at the end of this textbook.

# Lesson F Quiz

1. Which expression describes the methods used to prevent microbial growth in food preparation?
   a. Processing factors
   b. Hurdles or barriers
   c. Controlled atmosphere
   d. Sensitivity and specificity

2. What is meant by the organoleptic status of a food?
   a. Its average shelf life
   b. Its sight, taste and odour
   c. Its storage requirements
   d. Its nutritional value and possible allergens

3. Which of the following is an intrinsic factor limiting microbial growth in food?
   a. Curing
   b. Acidity
   c. Freezing
   d. Sodium benzoate

4. For which **two** food preparation processes is heating to a specific temperature for a specific time most critical??
   a. Pickling
   b. Canning
   c. Smoking
   d. Dehydration
   e. Freeze drying
   f. Pasteurization

5. What is the advantage of using non-thermal novel technologies to control the growth of microbes in prepared food?
   a. They offer cost-effective solutions to increasing production costs
   b. They minimize changes to the taste and nutritional value of the food
   c. They eliminate all microbial growth in a single step during processing

6. What effect does freezing have on the microbes in food?
   a. It controls the growth of pathogens by lowering the pH of the food.
   b. It kills bacteria and viruses but does not prevent the growth of parasites.
   c. It pauses microbial growth by making water unavailable while food is frozen.

7. How does the expression "the cold chain" apply in the food industry?
   a. It is a reminder of the importance of temperature control from farm to fork.
   b. It is a reminder of the appropriate testing and verification procedures of freezer temperatures.
   c. It is a reminder of the minimum and maximum temperatures required to halt microbial growth.

8. Which description applies to vacuum packaging?
   a. A lack of oxygen prevents the growth of aerobic bacterium.
   b. Carbon dioxide and oxygen levels are kept constant to reduce oxidation.
   c. Nitrogen: carbon dioxide levels are increased from normal atmospheric air to suppress spoilage and bacterial growth.

9. Which description applies to controlled atmosphere packaging?
   a. A lack of oxygen prevents the growth of aerobic bacterium.
   b. Carbon dioxide and oxygen levels are kept constant to reduce oxidation.
   c. Nitrogen: carbon dioxide levels are increased from normal atmospheric air to suppress spoilage and bacterial growth.

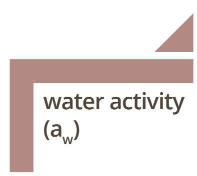
10. Which description applies to modified atmosphere packaging?
   a. A lack of oxygen prevents the growth of aerobic bacterium.
   b. Carbon dioxide and oxygen levels are kept constant to reduce oxidation.
   c. Nitrogen: carbon dioxide levels are increased from normal atmospheric air to suppress spoilage and bacterial growth.

11. Are these factors inhibiting microbial growth intrinsic or extrinsic?

a.


○ Intrinsic
○ Extrinsic

b.




○ Intrinsic
○ Extrinsic

c.


○ Intrinsic
○ Extrinsic

12. What should not motivate food producers to learn about the microbiology of food preservation?

a. Increased profits

b. Public health

c. Worker efficiency

d. Consumer opinion


e. Managing risks

f. Label design

13. Choose the false statement about pH, $a_w$ and microbes.
   a. Some microbes can grow at acidic pH of less than 4.6.
   b. Some microbes can grow at water activity of less than 0.92.
   c. Harmful microbes are unlikely to grow in fermented meats when the water activity is 0.92 or less and the pH is 5.0 or less.
   d. No microbes will grow in foods that have a pH less than 4.6 because these are defined as non-potentially hazardous foods.

Check your answers with the answer key provided at the end of this textbook.

# Lesson G: Assessing Risk in Food Recipes and Determining Control Points

## Introduction

Assessing risk in a food recipe is a multi-step process that requires the person making an assessment to have knowledge about the food, where it was grown, how the food is going to be made (the food process), and how to control the risks in the food (i.e., the control and critical control points). There are different tools and strategies designed for food assessments, such as food flow charts and templates that guide the user to identify hazards in foods and processes. Once the hazards and risks are identified, control strategies are described to reduce the risks from identified hazards. As described in Lesson F, microbial risks can be controlled with a wide variety of barriers or hurdles: cooking, refrigeration, adding preservatives, etc.

### Learning outcomes

Upon completion of this lesson, learners will be able to:

- Describe the principles of Hazard Analysis and Critical Control Points (HACCP)
- Differentiate between a control point and a critical control point
- Visualize risk in a recipe by designing a food flow-chart
- Evaluate process steps for risk
- Identify hazards and risks in a food-manufacturing process, and how to control them

### Readings and resources

**Conducting a hazard analysis**
http://www.inspection.gc.ca/food/requirements/preventive-controls-food-businesses/hazard-analysis/eng/1513283555932/1528205368359

**Food Safety Plan Workbook**
https://www2.gov.bc.ca/assets/gov/health/keeping-bc-healthy-safe/food-safety-security/food_safety_plan_workbook_sept6_2017.pdf

**Guide to Food Safety**
http://www.inspection.gc.ca/food/non-federally-registered/safe-food-production/guide/eng/1352824546303/1352824822033

### Terminology

Key terms given in bold

best-before date
CCP deviation response
**control point**
**corrective actions**
**critical control point**
durable life
environmental control
expiry date
**HACCP – hazard analysis and critical control points**
**hazard**
kill step
mitigate
**monitoring procedures**
operational control
prerequisite programs
**risk**
**verification procedures**
**records**
**deviation**

## Assessing risk

One way that **risk** is characterized is by determining the likelihood of risk occurrence. For example consider the risk of getting into a car collision or the risk of falling down the stairs. The car and the stairs represent the hazards. The likelihood of an accident occurring depends on many conditions: how often you drive or walk down stairs, the other car drivers, driving and walking aids, your balance, etc. There is always some risk that your vehicle will collide into some other car (unless you don't have a car!) or that you might fall down the stairs (unless you never take the stairs!), however, there are also ways to control or mitigate these risks to prevent the hazards from occurring. For example, stairs without a railing will increase the likelihood of a fall. Adding a railing will mitigate the risk that someone would fall. Driving alongside a person without a driver licence potentially increases the risk or likelihood of being in a car accident.


**Figure 1** — Risk can be assessed by the likelihood of a hazard occurring.

Risks in food production are defined by **hazards** in foods, and whether a **hazard** is biological (i.e., microbial), chemical, or physical.

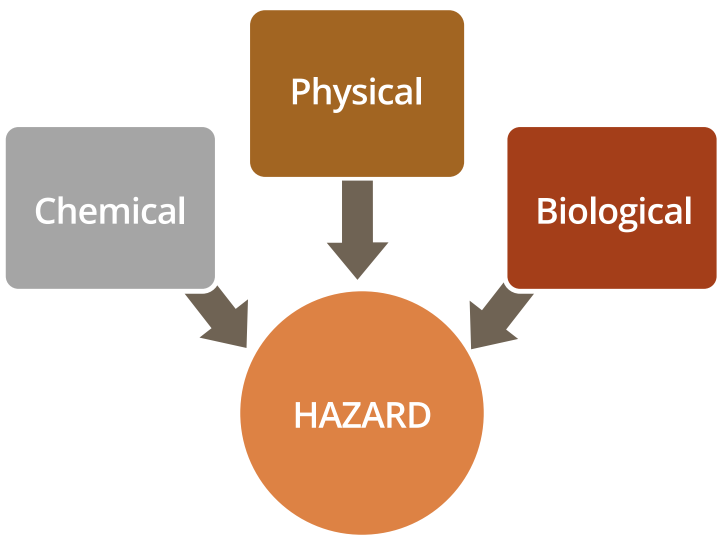



**Figure 2** — Types of hazards in foods

Let's consider the potential microbial risks. To address microbial risk, the following questions should be considered:

- What microorganisms might be present in any given food?
- What microorganisms might begin to grow or be introduced into the environment?
- What would protect the food but not diminish the quality or palatability of the product?

For example, let's consider salmon and the risk differences between wild and farmed salmon. The diet of wild salmon includes other fish, squids, shrimps, insects, and other marine organisms. Due to this varied diet, wild salmon often carry parasites; therefore, this biological hazard must be considered in the food process. One study of wild pink salmon found that over 70% of fillets were infected with parasite worms (Bilska-Zając et al., 2016). Farmed salmon have a diet of heat-treated fish pellets; therefore, parasite risk is not present and this biological hazard is not a concern. This is an important distinction in decisions as to how the fish is processed and consumed. If fish was to be served raw as sushi or sashimi, or was intended to be smoked, wild salmon may carry a parasite risk, but farmed fish should not. The process to manage or mitigate parasite risk in wild salmon might be freezing for parasite control, or it might be cooking (smoking) for parasite control. Both of these processes involve a time and temperature component.

### Activity: Hazards in salmon

Name one chemical and one physical hazard that might occur in wild or farmed salmon.

**Figure 3** — Chinook salmon

Check your answers with the answer key provided at the end of this textbook.

A risk-reduction strategy for salmon would examine the hazard, the process, and the best controls for the process. The hurdles chosen depend on the type of food to be prepared and how that food will be prepared. Cooking, for example, would work as a hurdle to control potential microbial contaminants in chicken. But cooking would not work for salmon intended to be served raw, such as sushi; nor would it work for other foods intended to be served fresh, such as cantaloupe.

## Hazard analysis and critical control points (HAACP)

To formally evaluate the potential for microbial contamination, The National Aeronautics and Space Administration (NASA) devised an approach known as **Hazard Analysis and Critical Control Points** or **HACCP**. When people first went into space, every aspect of the mission was controlled to maximize success. Not surprisingly, this included the foods that the astronauts consumed during their voyage. Food poisoning in a small space capsule needed to be prevented at all costs. Consequently, the focus was on prevention, on identifying potential hazards along the food-production chain, and designing strategies to control the hazards in order to reduce or eliminate them.

Devising a HACCP strategy also requires assessing what combination of controls and hurdles will prevent spoilage and guarantee safety. A HACCP strategy considers every aspect of food production, from the design of buildings (whether on the farm or food preparation site), through the maintenance and inspection of equipment, to the workflow patterns of people as they move through various stages of food harvesting, production, and distribution. HACCP is built upon a solid foundation of good sanitation, cleanliness of equipment, building design, and personnel hygiene, described as prerequisite programs to HACCP. As we learned in a previous lesson, microbial numbers can be controlled when people practice safe food handling; limit opportunities for cross-contamination, and maintain hygiene standards for their premises and themselves. The efficiency of the applied HACCP principles is measured by maintaining microbial numbers below danger levels. Foods must also be assessed for chemical and physical hazards. Ways these can be measured include ensuring chemical contaminants are below acceptable limits and ensuring there are no detectable metal fragments.

Devising effective hurdles can be a challenge because there are many risks that can impact the safety of the food supply. Food manufacturers must consider:

- Each ingredient in the food formulation
- The food process from start to finish
- How the food will be transported and stored
- The optimal shelf life
- Consumer handling before consumption

## Principles of HACCP

The HACCP Risk Assessment plan devised by NASA has seven basic principles, which provide a systematic approach to food safety.

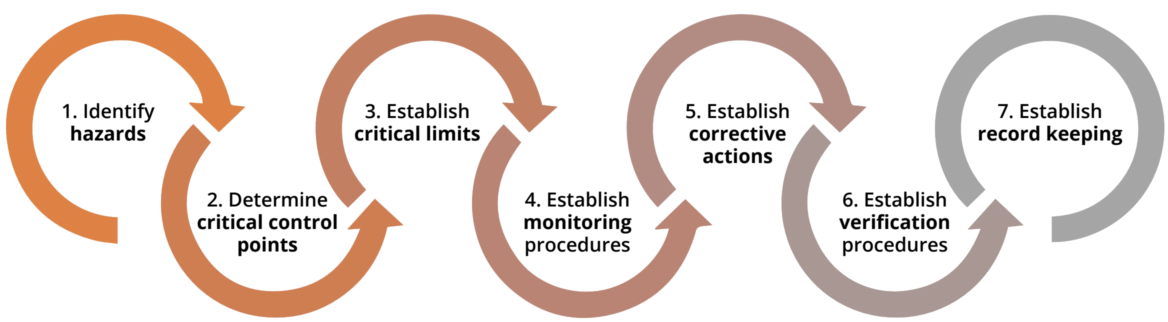



**Figure 4** — The seven principles of HACCP

1. **Identify hazards**
   The first step is to conduct a hazard analysis of ingredients in all the process steps. List ALL ingredients, even water, used to make and prepare the food. Every ingredient is examined in this analysis for hazards. Does the ingredient have any potential for biological, chemical, or physical hazards? For example, biological contaminants (e.g., *Salmonella* or parasites); chemical contaminants (e.g., toxins, pesticides, detergents), and physical contaminants (e.g., paint chips, pieces of equipment, jewelry, or rocks), might need to be considered for smoked fish processing. Once all ingredients are examined, the analysis considers whether potential hazards could be amplified during each process step. The process steps are the "how to" information in the food recipe. At each process step, consider the temperature of the food and the time it stays at that temperature. Is a microbial hazard amplified, reduced, or

unchanged during that process step? Or, could a toxin be created that cannot be eliminated by a cook step, such as heat-stable histamine that may occur in tuna? Water activity and pH information about the food will also be required at one or more of the process steps to examine whether hazards are controlled (or not). Addition of ingredients during the process (i.e., later in the recipe) may also potentially affect microbial loads, change the pH and/or water activity, and increase or decrease potential hazards. Other issues to consider include how equipment may affect food. For example, is there a possibility that slicers could create physical metal hazards if they were to fail, such as during chopping of cabbage for sauerkraut? The best way to identify hazards is to map out the process steps in a food flow-diagram. This can be done alongside the recipe or separately. The food flow-diagram will be used later to identify where control and critical control points exist throughout the process.

2. **Determine critical control points**
   A **critical control point (CCP)** is frequently referred to as the "kill step." This is the step in a process that eliminates or reduces the hazard to an acceptable level; for example, a cook step that reduces *Salmonella* in meat by a 6.5 log reduction. The cook step will have a specific time and temperature that meets this objective. CCPs must be a point in the process that will prevent, eliminate, or reduce the hazard. Not all CCPs are kill steps; some CCPs will prevent the growth of bacteria if the food will not be cooked. Typically, a CCP is also the final point in the process where the particular hazard can be controlled. If a hazard can be controlled later in the process, then this control point is not considered critical. Many HACCP-style approaches will include a decision-tree matrix that addresses the question, "Can the hazard be controlled later in the process?" If you answer "yes" to this question, then this is not the CCP. It is usually best to have as FEW CCPs as possible, because each CCP should be monitored and recorded. Loss of control over this point or step in the process can be expected to result in unacceptable risk to the consumer.

3. **Establish critical limits**
   The **critical limit** is the measurable criteria under which the hazard is controlled by the CCP; for example, the cook step. What temperature is the food cooked to? For most proteins, the temperature is taken using a probe-tip thermometer at the middle or thickest part of the food, and then the food is held at that temperature for a specific length of time to meet the 6.5 log reduction. Time and temperature make only one type of critical limit. There are also chemical limits for the amount of salt, nitrite, or preservative to add to a recipe, or the maximum allowable level of acidity (pH) in a food product before it is sold. Critical limits are often a range of values, and as long as the food falls within those values, it is considered acceptable. For example, nitrite levels in fermented meats are expected to be within 100 ppm to 200 ppm. Values below 100 ppm will fail to control the hazard *C. botulinum*. Values above 200 ppm are a health risk to consumers. Either of those situations is considered unacceptable. Therefore, the critical limit for nitrite is that the recipe must have between 100 ppm to 200 ppm of nitrite.

4. **Establish monitoring procedures**
   The key word in this step is "procedure." It is self-evident that to monitor the temperature, some type of thermometer will be used. The procedure to monitor the temperature, however, should include:
   - How the monitoring is to occur
   - How often the monitoring is to occur
   - Who is responsible to do the monitoring
   - How and where to record the monitoring

For example, keeping perishable foods cold in the refrigerator is a common control. The procedure to conduct this monitoring might be assigned to the kitchen staff, and the task may be to record the temperature seen on the thermometer inside the walk-in cooler twice per day: once when the walk-in cooler is first opened in the morning, and once during the evening shift. The temperature is recorded on a log-sheet that is posted on the walk-in cooler door. A method and frequency for monitoring the temperature of foods in the walk-in cooler, along with who does it and how it is recorded, has been established. The hazards controlled by this monitoring procedure might include, for example, histamine from tuna or spoilage of milk.

5. **Establish corrective actions**
   **Corrective actions** should describe what is done when a critical limit is not achieved. Corrective actions are also referred to as **CCP deviation response**. A **deviation** occurs when there is a failure to meet a critical limit. These procedures describe what happens to food where the CCP has not been met—sometimes the action will specify how to correct the situation so that the CCP can be met, but sometimes the correction action may require destruction of the food if the problem cannot be corrected. Similar to the previous step, corrective action procedures should include:
   - What the corrective action is
   - Who takes the corrective action
   - If it is to be recorded, where and how is this done

   Here is an example of a corrective action: if the reading of the digital thermometer displays a temperature lower than expected for the critical limit, then the food must be cooked longer and the temperature possibly increased.

6. **Establish verification procedures**
   This step ensures that the HACCP plan is working as intended. The procedures may be tests or other objective criteria to assess the process. In food, these are most often microbial tests. A procedure for ensuring the HACCP system is working could include inspection of random products via testing for total aerobic colony counts at particular points in the process. Foods testing with higher bacterial levels than expected would demonstrate that CCPs are failing to control bacterial growth, or that some other process step is allowing bacterial growth to occur. Microbial verification may be required to demonstrate that the food-production lot meets a specific standard; for example, that generic *E. coli* is not present at more than 1000 CFU per gram in the final food product. Verification procedures are generally more rigorous than the monitoring process and are meant to uncover potential issues with the HACCP plan. The verification procedures can be used to ensure that employees responsible for monitoring a CCP are following correct procedures, to find hazards unrecognized in the original HACCP plan, and to ensure critical limits that are in place keep foods safe.

7. **Establish record-keeping procedures**
   The record-keeping step is proof that the HACCP plan is being followed. Record keeping is required in steps 4, 5, and 6 of a HACCP system. **Records** are kept for monitoring, deviations, and verification. This documentation ensures the CCPs are met, tracks steps taken when errors (deviations) in the food process occur, and records the objective data that verifies the food production meets standards. **Records are evidence of the HACCP plan**. They are reviewed by governing bodies to ensure that best practices are being employed by producers, standards of food safety and quality are being met, and appropriate procedures are being enacted when processing deviations are encountered. Records can

also help you troubleshoot failures and trace back the history of a manufacturing process. Records are useful to demonstrate due diligence in the processing of foods, protecting food product from liability, and may be requirements in some jurisdictions (Jouve, 1994).

## Critical control points (CCPs)

It can be challenging to determine where in a process the ***critical*** control points exist. The flow chart in Figure 5 is based on three simple questions to help determine whether the process step is a control point or a critical control point. CCPs are those process steps that eliminate the hazards and where there is no subsequent or later step that can eliminate the hazard.

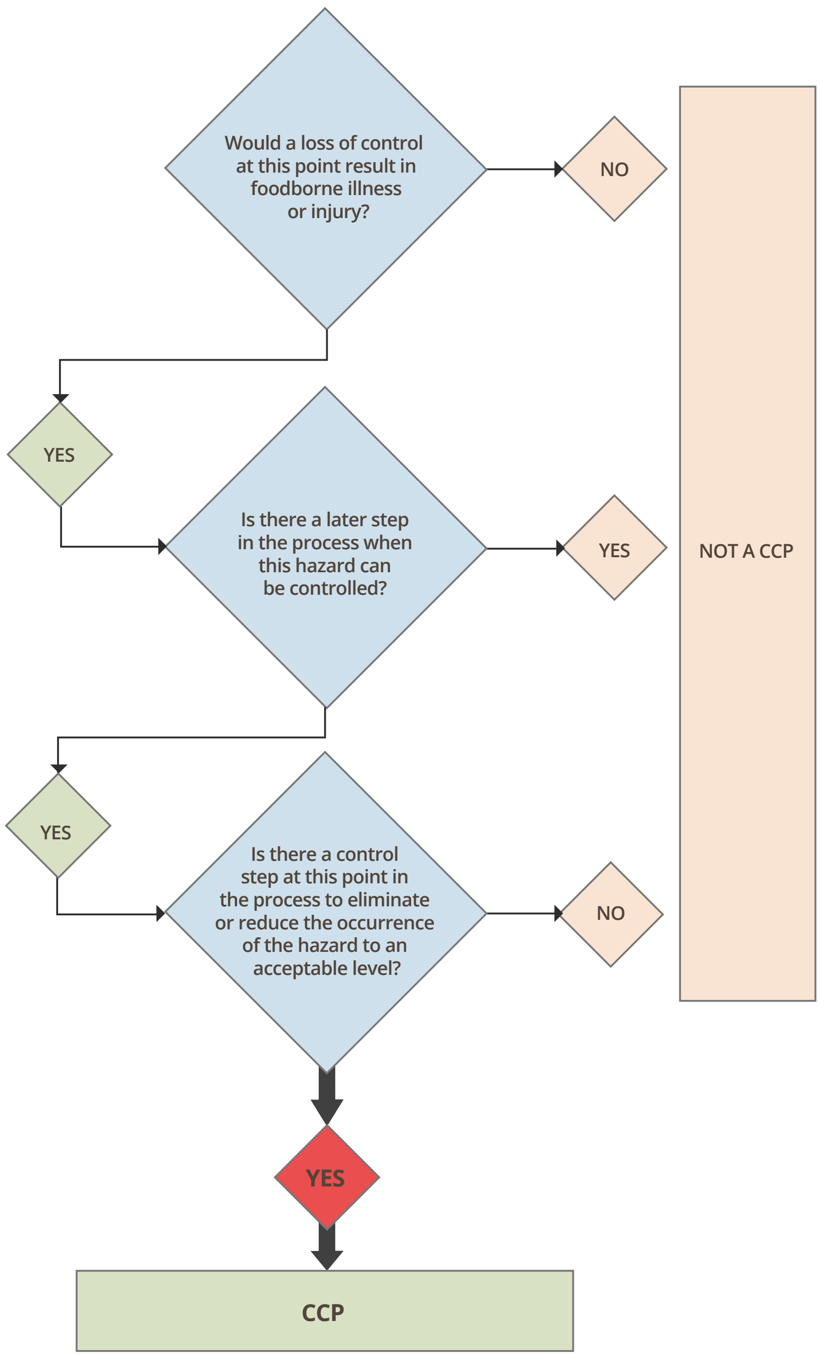



**Figure 5** — Critical-control-point decision tree

Examples of critical control points follow:

| CCP | Example |
|---|---|
| A cook step that provides for a log reduction of pathogens. | • poultry: 7 log reduction for *Salmonella*; e.g., at 60°C, minimum holding time of 16.9 minutes<br>• meat: 6.5 log reduction for *Salmonella*; e.g., at 60°C, minimum holding time of 12 minutes |
| The addition of an ingredient or preservative critical to a recipe. When it is not added, pathogens may grow or, when too much is added, harm may occur. | • nitrite as previously described<br>• pickling recipes for fish have a minimum of 50% acid (e.g. vinegar) |
| Chill and refrigeration procedures (these often occur after a cook step and in some processes when there is no cook step, occur throughout the process). | • raw tuna loin: thawed and maintained at or below 4°C to minimize histamine formation<br>• cooling done within six hours—from 60°C to 20°C in two hours and from 20°C to 4°C in four hours |
| Freezing procedures to kill parasites. | • fish: −35°C for 15 hours or −20°C for seven days<br>• pork: when pork is < 15 cm thick, hold for twenty days at −15°C or ten days at −23°C |
| A temperature and humidity step necessary for fermentation and/or dehydration. | • tempeh: requires between 22°C and 38°C and between 70–85% humidity for *Rhizopus* mould to grow<br>• beef jerky: requires heat to provide log reduction *and* dehydration humidity at a minimum of 90% or higher for at least one hour |

## Control points

**Control points** are still important throughout the process, as described in two different contexts:

- Some control points will already be managed by other programs, such as prerequisite programs, operational controls, environmental controls, and good manufacturing practices (Elliott, Jenner, Johson, Ontario Ministry of Agriculture and Food, & Fulton Food Safety Consultants, 2003).
- Some control points will be part of a food-manufacturing process (i.e., in the recipe).

### Control points managed by other programs

*Note*: None of the following examples are critical control points in a food process.

#### Example 1: Temperature control of foods before a CCP cook step

By limiting the time food is in the danger zone, you also limit the amount of microbial growth. Consider a 7 log reduction of *Salmonella* in raw chicken. If the number of *Salmonella* bacteria is too high in the chicken, a 7 log reduction may still leave behind enough bacteria to cause illness. Before the raw chicken is used in a recipe, the temperature control of the chicken is managed in several of the programs mentioned above, such as:

- Prerequisite program for shipping, receiving, and storage. For example, the food premises will have a written policy for the standards that are expected of food companies and how foods are to be delivered and managed when received at the food premises. These are backed up by operational controls at the food premises.

- Operational control: shipping. For example, companies delivering product to the food premises are cold-chain certified. The food premises will monitor incoming food deliveries to ensure the foods being received are at the correct temperature (i.e., refrigerated or frozen).
- Operational control: receiving and storage. For example, staff will put away orders into refrigerated and frozen storage within two hours of receiving goods at the loading bay.
- Prerequisite program: equipment. For example, the food premises will have a routine scheduled maintenance program for refrigeration and freezing equipment. The food premises will annually certify thermometers used to check temperatures of refrigerators and freezers. These are backed up by operational controls at the food premises.
- Operational control: storage practices. For example, food premises staff will ensure that temperatures of storage areas, refrigerators, and freezers are appropriate. Other operational controls in this section not specific to temperature include food rotation (first in, first out), minimizing contamination from chemicals by segregation, having foods stored off the floor, and others.
- Environmental control: establishment of interior air quality and ventilation. For example, air filters in rooms and in walk-in coolers and freezers will be maintained and changed as required.

### Example 2: Handwashing

When food handlers have hands-on contact with foods during preparation, the possibility of cross-contamination exists. Handwashing is important to overall sanitation and is a control point, but not a CCP. Handwashing policies may be addressed within various contexts as follows:

| Policy type | Specific context |
|---|---|
| Good manufacturing practices | • Injuries and wounds<br>• Personal practices |
| Prerequisite programs | • Illness policies<br>• Training<br>• Sanitation and staff. Personnel sanitation includes food-handling behaviours such as when and how often handwashing should be performed. These are usually covered in a prerequisite program. |

### Example 3: Premises design to limit cross-contamination

This can be defined as an environmental control. By situating entrance and exit pathways for bathroom facilities, lunchroom, and receiving away from areas where foods are prepared and packaged, the possibility of cross-contamination from these sources is controlled.

## Control points within a food-manufacturing process

### Example 1: temperature control of foods before a CCP cook step

Consider the raw poultry example above. A whole chicken left on the counter for half a day allows more time for bacteria to grow before the chicken is cooked (the CCP) than a whole chicken left in the refrigerator. The goal is still the same: to limit pathogen growth before the CCP.

This can be achieved by limiting the time food is spent at ambient temperature (i.e., in the danger zone) before the cook step. When food is taken out of the refrigerator or when it is thawed, times and temperatures are monitored to minimize hazards.

One other example of temperature-control points that may not be critical control points is rehydrating ingredients in water. For example, raw and dried tree nuts (e.g., cashews, almonds, walnuts, hazelnuts) are known reservoirs of *Salmonella*. A recipe that has a cook step such as steaming a Christmas pudding would not consider soaking the nuts as a CCP. It could be good manufacturing practice to soak the nuts under controlled conditions to limit the possibility of pathogen amplification. However, If the recipe had no cook step (CCP) for the nuts, then it would be a necessary to treat this rehydration step as a CCP.

To summarize this section, within a recipe and process there will be several control points that will limit hazards. Control points, however, are not critical control points. Control points do not eliminate the hazard. While there may be limits that should be established and monitored (such as time in the danger zone), these will not require corrective action, verification, or record keeping.

### Flow diagrams and evaluating process steps

Putting a HACCP plan into action requires an understanding of what pathogens might be present in raw food, when pathogens might be introduced into the food-production chain, what can be done to prevent cross-contamination, and whether the process steps in a recipe will amplify the growth of microbes. Step 1 of HACCP is to identify hazards in all ingredients and process steps. The best way to do that is to make a flow diagram. The diagram is a representation of the recipe and the sequence of steps in the production of the food item.

## How to evaluate a food process for control points and CCPs

A food-manufacturing process is more than a recipe. It has the components of a recipe and also incorporates HACCP principles and the concept of constructing a food flow diagram. This stepwise process is depicted in Figure 6. To simplify this concept, in this section we will consider recipes as food-manufacturing processes.

Let's start with a simple recipe: hard boiled eggs. In a typical recipe, you will have a list of ingredients and then instructions on how to make the food. There are two ingredients in this recipe: water and eggs. Eggs are perishable items and are stored in the refrigerator until use. Tap water in this example is presumed to be potable (drinkable). Refrigeration and water are managed by control programs.

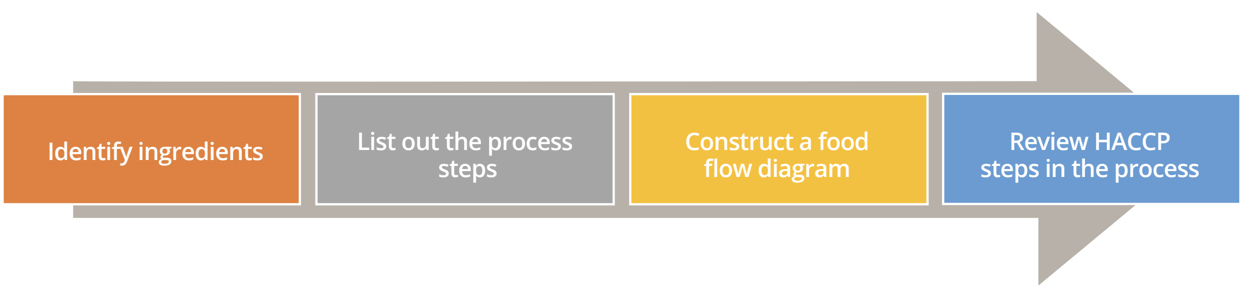



**Figure 6** — Basic approach to evaluating a food process

Once you have a list of ingredients, the next step is to determine the actual process. The process steps are outlined in the instructions for the recipe that follows:

1. Remove the eggs from the refrigerator and let them come to room temperature.
2. Place the eggs in a pot in a single layer.
3. Cover the eggs with tap water.
4. Place the pot with its lid onto high heat and bring it to a boil.
5. Remove the pot from the heat, and let eggs sit in hot water for 12 minutes.
6. Drain the water from the pot and add cold tap water to cool.
7. Refrigerate the eggs.

Draw out the food flow diagram, based on the major process steps.

| Process steps |
|---|
| |
| ↓ |
| |
| ↓ |
| |
| ↓ |
| |
| ↓ |
| |

When "extracted" from the recipe instructions, the process steps can be broken down as follows:

| Process steps |
|---|
| Let eggs come to room temp. |
| ↓ |
| Boil eggs with water. |
| ↓ |
| Let eggs stand in hot water. |
| ↓ |
| Cool eggs with cold water. |
| ↓ |
| Refrigerate eggs. |

### Step 1 of HACCP: Identify the hazards

Consider the hazard(s) associated with eggs. These are based on a history of foodborne illnesses linked to eggs or known to be associated with this ingredient. *Salmonella* enteritidis (SE) bacteria are a common biological hazard associated with chicken eggs that cause *Salmonella* enteritis infections. Another biological hazard associated with eggs is *Campylobacter*. Note that in this step, you've only identified the hazards associated with the one ingredient: eggs. However, you've not identified the hazards during the process steps, when existing microbes might multiply or be introduced through a cross-contamination event.

Unsure of what hazards may be in a particular food or what process steps are of concern? The Reference Database for Hazard Identification (RDHI) created by the CFIA is a good resource to begin your search. For example, enter a search term for eggs

**Reference Database for Hazard Identification (RDHI)**
https://active.inspection.gc.ca/rdhi-bdrid/english/rdhi-bdrid/searece.aspx?i=11

### Step 2 of HACCP: Determine critical control points

Is there a process step where these hazards can be controlled? This is the step where the two previously mentioned hazards are eliminated or reduced to acceptable levels. Is there any other step where this hazard is controlled? If the answer is "no," then this is the CCP. In this food flow diagram, the CCP is the second process step—when eggs are boiled.

| Process steps | CCP? |
|---|---|
| Let eggs come to room temp. | |
| ↓ | |
| **Boil eggs with water.** | **Yes** |
| ↓ | |
| Let eggs stand in hot water. | |
| ↓ | |
| Cool eggs with cold water. | |
| ↓ | |
| Refrigerate eggs. | |

**Figure 7** — Boiled eggs cooling in water

## Step 3 of HAACP: Establish critical limits

The recipe says to boil the eggs, then let them stand in the hot water for twelve minutes. Is the critical limit to monitor the time of twelve minutes after boiling? How do you know if the eggs have reached a safe internal temperature? While a recipe generally outlines the "how" for the process, it may not properly identify a critical limit for it.

Consider the physical parameters for the process step. Once the water is boiled, the internal temperature of the eggs held in the water may increase. What temperature is necessary for *Salmonella* to be controlled? According to the US FDA code, the internal temperature of eggs should be 63°C and held at that temperature for a minimum of fifteen seconds give a 3 log reduction of *Salmonella* (pg. 433). Or eggs may be cooked to a higher temperature of 74°C and held at that temperature for less than one second (or some other equivalent time and temperature) to allow an adequate 7 log reduction of bacteria. The critical limit can be expressed to cook eggs after boiling in the hot water until an acceptable internal temperature is reached for the boiled eggs; i.e., to 63°C for at least fifteen seconds or to 74°C or equivalent. To test the internal temperature of the egg based on the recipe, it will be necessary to insert a probe thermometer through the shell of an egg at the prescribed time to measure and document the process.

**Archived – Annex D: Cooking Time / Temperature Tables**
Canadian Food Inspection Agency
https://www.canada.ca/en/health-canada/services/general-food-safety-tips/safe-internal-cooking-temperatures.html

| Process steps | CCP? | Limits and critical limits |
|---|---|---|
| Let eggs come to room temp. | | |
| ↓ | | |
| Boil eggs with water. | Yes | Critical limit: Cook to internal temperature of 63°C for at least 15 sec. or equivalent |
| ↓ | | |

| | | |
|---|---|---|
| Let eggs stand in hot water. | | |
| ↓ | | |
| Cool eggs with cold water. | | |
| ↓ | | |
| Refrigerate eggs. | | |

Are there other CCPs for this recipe? Cooling and refrigeration of boiled eggs are definitely required, but do they fit the definition of a CCP or are they more accurately described as control points? Control points still require limits and monitoring. Cooling processes are necessary to limit the hazard of bacterial growth leading to the formation of toxins, such as *Staphylococcus* enterotoxin. Bacteria generally do not have an opportunity to grow when temperatures are above 60°C. Cooling should occur from 60°C to 20°C within two hours, and from 20°C to 4°C within four hours. Refrigeration below 4°C controls most microbial growth. For boiled eggs in the shell, the egg is considered low risk for microbial cross-contamination, so there is no other CCP. However, if the shell is cracked, cross-contamination from other sources is a possibility, even if it's remote. Good practice would include additional limits in the HACCP plan to control for these temperature- and time-related issues. Monitoring is required, but these additional activities are not considered CCPs. The only critical limit in this recipe is associated with the CCP and it is shown in red.

| Process steps | CCP? | Limits and critical limits |
|---|---|---|
| Let eggs come to room temp. | | Limit time in danger zone to 2 hr or less |
| ↓ | | |
| Boil eggs with water. | **Yes** | **Cook to internal temperature of 63°C for at least 15 sec. or equivalent** |
| ↓ | | |
| Let eggs stand in hot water. | | Standing water is above 60°C |
| ↓ | | |
| Cool eggs with cold water. | | Cool to at or below 4°C within 6 hours |
| ↓ | | |
| Refrigerate eggs. | | Store at or below 4°C |

### Steps 4 to 6 of HACCP

Steps 4 to 6 of the HACCP plan specify the monitoring, corrective action, and verification procedures needed for the food-production process. To monitor whether the boiled egg achieves the critical limit, a probe-tip thermometer is used to measure the internal temperature of the egg. Not every egg in a batch needs to be checked—a representative egg from the middle of the batch can be checked. If the egg has not achieved an internal temperature of at least 63°C, then the eggs should be held longer in the hot water or re-boiled. A verification procedure for this food-production process might be to measure the temperature of all the eggs.

| Process steps | CCP? | Limits and critical limits | Monitoring | Corrective action | Verification |
|---|---|---|---|---|---|
| Let eggs come to room temp. | | Limit time in danger zone to 2 hr or less | | | |
| ↓ | | | | | |
| **Boil eggs with water.** | **Yes** | **Cook to internal temperature of 63°C for at least 15 sec. or equivalent** | **Measure internal temperature of one boiled egg with a probe-tip thermometer** | **Hold eggs in the hot water longer, and/or return to heat and re-boil** | **Do a temperature check of all eggs** |
| ↓ | | | | | |
| Let eggs stand in hot water. | | Standing water is above 60°C | | | |
| ↓ | | | | | |
| Cool eggs with cold water. | | Cool to at or below 4°C within 6 hours | | | |
| ↓ | | | | | |
| Refrigerate eggs. | | Store at or below 4°C | | | |

This HACCP plan might seem at first glance to be excessive. After all, what can go wrong with boiling eggs? Do you think this recipe would work if you were boiling an egg at the top of Mount Robson, British Columbia's highest mountain in the Rockies? Does water boil at the same temperature in all locations? In fact, the answer to this is "no." It depends on the geographic location, but generally at higher locations, water boils at lower temperatures. With less atmospheric pressure, water takes less energy to boil. With less energy being expended to boil the water, less heat is produced. That is why boiling methods at higher elevations take a longer time. It might mean that the holding time for a hardboiled egg is twenty minutes, not twelve minutes. The only way to verify this is to take the internal temperature of the boiled egg. Food handlers unfamiliar with a recipe need to understand exactly what the process steps are, including what to monitor and how to know if the processing was sufficient to meet the critical limit.

### Calibration of instruments

One final word on this HACCP plan. The thermometer mentioned throughout must also be verified in a prerequisite program. Equipment must be calibrated and verified, usually on an annual basis. This usually involves sending a thermometer to an independent company for a calibration test, and getting a certificate stating the calibration has been performed. The certificate provides the actual true temperature reading of the instrument to a set standard at a set temperature. For example, "this instrument reads at +0.3°C at 20°C in normal atmospheric pressure." Dial-type and some digital thermometers may be calibrated using ice cold and boiling water (see the following video). Walk-in coolers with built-in thermometers should also be calibrated as needed: check the manufacturer's instructions or use a service company. Ensure all offsets (+/- values from 0°C) are recorded on the devices.

**Calibrating a Thermometer**
https://www.youtube.com/watch?v=VpJULQICiGM

**Food Thermometers: Types and Calibration (FOODSAFE Secretariat)**
http://www.foodsafe.ca/docs/Food_Thermometers_Types_and_Calibration.pdf

## Activity: Food flow-chart

1. Design a food flow-chart based on the following recipe for egg-yolk parmesan.
2. Identify the main hazard associated with this recipe.
3. Identify a CCP. If there isn't a CCP, consider how you would amend this recipe process to include a CCP, to control the hazard(s) you've identified.
4. Identify critical limits and monitoring for the CCP.
5. Identify any additional control steps for the process steps in this recipe.

Use the template that follows. This activity is expected to take approximately thirty minutes. The food flow-chart may need to be amended once you consider how to address the CCP for the hazard. It will require some thought and consideration of the process steps.

| Process steps | CCP? | Limits and critical limits | Monitoring |
|---|---|---|---|
| | | | |

**Recipe for dehydrated egg-yolk parmesan**
Ingredients:

- 1 ¾ cup salt (370 grams)
- 1 ½ cup sugar (320 grams)
- 8 eggs
- Water

**Figure 8** — Raw egg yolks on salt and sugar mixture

Instructions:

1. Mix the salt and sugar and spread half into a small 8 × 8 inch pan.
2. Make six wells in the salt/sugar layer for each of the yolks.
3. Crack each egg, carefully remove the yolk, and place it into a well.

4. Cover the yolks with the remaining salt/sugar mixture.
5. Cover the container and let stand for four days.
6. Remove the yolks from the salt mix.
7. Refrigerate the egg yolks until use in a recipe.

Dehydrated egg yolks make a great dairy-free alternative to parmesan cheese!

(Hint: Search the Internet for recipes for cured egg yolk or egg yolk parmesan. There are many different recipe variations. With your knowledge of hazards, microbial growth, and preparing foods safely by limiting microbial hazards and reducing risks in a food recipe, you should be able to pick up some ideas for this recipe.)

This activity was based on questions about egg yolk parmesan safety when Environmental Health Officers found this food in restaurants. The two main issues of concern were how *Salmonella* bacteria present in the egg yolks would be eliminated in the process and the potential growth of *Salmonella* in egg yolks during the dehydration process. The first concern is achieved by a cook step. Investigation into a validated cook step for in shell pasteurized eggs found that a minimum time of 3.5 minutes at 61°C was required for *Salmonella* reduction. The second concern is managed by ensuring the dehydration process is long enough to lower the water activity to below 0.92, below which *Salmonella* bacteria cannot grow. A BCIT EHO student found that a minimum time of 24 hours under refrigerated conditions are required to reduce $a_w$ to <0.92. Based on these facts a food safety fact sheet for this product was created as a resource for EHOs and restaurant operators. To review the fact sheet that includes a food flow diagram, critical control points and best practices, the original student research project and egg pasteurization values please consult the resource following links.

**Fact Sheet: Egg Yolk Parmesan. Guidelines for Safe Preparation. (BC Centre for Disease Control, 2020).**
http://www.bccdc.ca/resource-gallery/Documents/Educational%20Materials/EH/FPS/Food/Egg%20yolk%20parmesan%20fact%20sheet%20BCCDC.pdf

**Evaluating the risk of contracting salmonellosis from egg yolk "parmesan" based on water activity.** Written by Dianna Vuu, 2020. *BCIT Environmental Health Journal*.
https://circuit.bcit.ca/repository/islandora/object/repository%3A969

**Canadian Food Inspection Agency (2019). Preventive controls for eggs and processed egg products.**
https://www.inspection.gc.ca/preventive-controls/eggs-and-processes-egg-products/eng/1524259297433/1524259297745#a5

Check your answers with the answer key provided at the end of this textbook.

There are many process steps that can add or reduce overall risk in a recipe. To evaluate risk, thought must be directed toward the ingredients in the recipe and the individual process steps. In general, risk exists when:

- Any of the process steps occur between 4°C and 60°C (in the danger zone)
- Multiple handling steps and multiple ingredients are present in the process
- Ingredients and preservatives must be measured and added based on weight

You should now have a good foundation for reviewing recipes for potential microbial risks and how processes should control them. This section is not meant to go into great deal on HACCP, as this is covered very well in many other courses and offerings.

### Additional resources for HACCP

If you are a processor and are required to make a HACCP plan for your product, consult the Ministry of Health's online resources for creating a HACCP-based Food Safety Plan.

**Food Safety & Sanitation Plans**
https://www2.gov.bc.ca/gov/content/health/keeping-bc-healthy-safe/food-safety/food-safety-sanitation-plans

To learn more about HACCP and safe food processing, take the ProcessSafe course offered through the FOODSAFE Secretariat.

**FOODSAFE**
http://www.foodsafe.ca/index.html

Other additional resources:

**HACCP Principles & Application Guidelines**
https://www.fda.gov/food/hazard-analysis-critical-control-point-haccp/haccp-principles-application-guidelines#app-e

**MyHACCP**
https://myhaccp.food.gov.uk/help/guidance/introduction-myhaccp

**Process Flow Diagrams** (also found in this lesson)
https://myhaccp.food.gov.uk/help/guidance/process-flow-diagrams

## Evaluating the shelf life of products

Food microbiologists are often asked, "How long is it safe to keep this food in the refrigerator?" Food processors want to know if the vegetable patties or pickled salsa they have made will keep for one week, one month, or one year. Answering this question is not straightforward. While you might be able to guess at the shelf life duration for a particular category of foods, you cannot determine a number unless you know more about how the food was made, how the food was packaged, and the foods' microbial characteristics (intrinsic and extrinsic properties from Lesson B). Fresh-cooked vegetable patties made of beans, vegetables, and spices might keep up to one week when properly refrigerated, or one month if they are pre-cooked and packaged in a reduced-oxygen environment. Often the answer is "it's complicated and it depends."

Furthermore, shelf life does not determine safety. Shelf life is most often used to define the acceptability of the organoleptic and sensory qualities of foods or, rather, its taste, odour, and overall palatability. These are known as the quality attributes of a food. As covered in previous sections, there are many spoilage organisms capable of turning great food into an odorous pile of slimy goop. Evaluating shelf life safety of products requires an examination of the microbiological quality of the food—shelf life testing. One way to do this is to evaluate food at the end of its intended shelf life.

While this is of concern to the manufacturer, consumers also don't want to buy product that has off-odours or flavours. It is more important to understand whether the food could support the growth of pathogens during its shelf life. This type of testing "challenges" the food. A **food challenge study** involves inoculating pathogens into food and then measuring the inactivation and inhibition of the pathogens under a variety of conditions. Types of challenge studies include:

1. Pathogen growth inhibition studies
2. Pathogen inactivation studies
3. Combined growth and inactivation studies

Challenge studies are only required when the intrinsic properties of the food will support the growth of pathogens. Most foods requiring these challenge studies are assessed as perishable; i.e., they are requiring refrigerated temperature control. Differences in storage conditions of the food is one of the conditions that are typically measured in a food challenge study. These will include temperature abuse conditions that may occur with consumer handling. Information about the food is collected that describes product preparation, pH and $a_w$ of the food during processing steps, effects of inhibitors, etc. This is done by measuring pathogen inoculum growth and inhibition, along with changes in indigenous microbial populations affecting the intrinsic properties of the food. Details of how to conduct a challenge study are provided by the National Advisory Committee on Microbiological Criteria for Foods ("Parameters for determining inoculated pack/challenge study protocols," 2010). Conducting a challenge study is, well, challenging! Often a food business will contract a private laboratory and food microbiologist to help them design and carry out food challenge studies.

### Desktop pathogen growth and modelling

There are several publicly available online modelling programs to assist manufacturers, regulators, and the general public with evaluation of food products. Modelling programs are computer simulations of growth and challenge studies. These tools can help assess if there is a perceived risk with food. To use the tools effectively, general values for pH and $a_w$ for the food should be known. Then, a challenge of a target microbe, such as *Salmonella* or a spore forming organism such as *C. perfringens*, can be added and, over time, modelled to evaluate the growth or inhibition. Other tools in the following resource links evaluate seafood safety (FSSP) or meat safety (Process lethality spreadsheet). The spreadsheet tool shows the log reduction of a target pathogen, such as *E. coli* O157 in meat at a specific temperature over time.

Tools include:

**Pathogen Modelling Program (PMP)**
https://pmp.errc.ars.usda.gov/PMPOnline.aspx

**Combase**
https://www.combase.cc/index.php/en/

**Food Spoilage and Safety Predictor (FSSP)**
http://fssp.food.dtu.dk/

**Process lethality spreadsheet**
https://meatpoultryfoundation.org/content/process-lethality-spreadsheet

## Labelling requirements

**Best-before dates**, packed-on date, enjoy-by date, sell-by date, **expiry date**, and use-by date: consumers often get confused by the types of date descriptions on packages. The most important detail from a food microbiologist safety lens is that none of these dates provide a guarantee of product safety.

## Best-before dates

Best-before dates are the most common type of date found on a package. All dates should be written in the YEAR/MM/DD (year, month, day) format. Best-before dates (on some packaging labels written as a "best if used by," "use before" or "enjoy-by" date) refer to flavour and quality (i.e., the durable life of the product). These dates do not guarantee safety, especially if you do not follow the guidelines for storage. The **durable life** of a product refers to the time that an unopened food product will retain its taste, freshness, and nutritional quality if stored as directed.


**Figure 9 —** Best-before date on soup—is the year 2015 or 2021?

All food that should be consumed within ninety days must have a best-before date. The use-by date is the last date recommended for consumption of the product. After this time, the product may lose desirable colour, flavour, and palatability. This does not mean that the product is unsafe to eat, however. Of course, safety depends on how food is stored. All perishable foods must be maintained at or below 4°C to maintain quality. Notice in the label image in Figure 9 above the warning to "refrigerate after opening and use within 7–10 days." Once packaging is opened, the shelf life of a food changes, and you can't tell if a food is safe by how it looks, smells, or tastes. If food is not handled properly, pathogenic microbes may begin to grow before the use-by date, particularly under temperature abuse conditions. Foods with a shelf life of more than ninety days do not have to be labelled with a best-before date.

## Expiry dates

An expiration date is different from a best-before date. Expiry dates are used only on foods that have nutritional and compositional ingredients such as milk formula substitutes, nutrition and meal supplements, liquid diet formulas, and other formulations. These foods must have expiration dates on them and should not be used after the date has passed. Other foods such as yeasts may also have expiration dates on them, after which the manufacturer will not guarantee the activity of the product.

There are many industry food labelling requirements other than dates. Core labelling requirements for foods include:

1. Bilingual labelling
2. Common name of the food
3. Country of origin
4. Date markings and storage instructions
5. Name and principal place of business

6. List of ingredients and allergens
7. Net quantity
8. Nutrition labelling
9. Declarations of sweeteners, food additive, fortification (e.g., added vitamin D)
10. Grades
11. Standards of identity

Resources to help industry with food label requirements can be found on the CFIA website in one of the additional resources.

### Additional resources

**Date labelling on pre-packaged foods**
https://www.inspection.gc.ca/food-safety-for-industry/information-for-consumers/fact-sheets-and-infographics/date-labelling/eng/1332357469487/1332357545633

**Food labelling for industry**
https://www.inspection.gc.ca/food-label-requirements/labelling/industry/eng/1383607266489/1383607344939

**Food labelling requirements checklist**
https://www.inspection.gc.ca/food-label-requirements/labelling/industry/labelling-requirements-checklist/eng/1393275252175/1393275314581

**Canadian Food Labelling Requirements**
http://healthunit.org/wp-content/uploads/CFIA_ACIA-Labelling-Presentation-for-Industry.pdf

## Lesson G Quiz

1. Which statement explains why hazard analysis and critical control points are essential in the food preparation industry?
    a. Critical control points are needed due to the time it takes to prepare the food.
    b. Microbial contamination of foods is always a result of poor temperature control.
    c. Microbial contamination of foods can occur at many different steps during processing.
    d. Critical control points are specific to the type of equipment used by a processing facility.

2. At what point does a HACCP strategy become important in a new food service establishment?
    a. As soon as staff are hired
    b. When the facility is being designed
    c. As soon as the equipment has been installed
    d. When the menus or products have been determined

3. How do you determine the critical control points (CCPs) in a recipe?
    a. CCPs are identified in most published recipes.
    b. Recipe ingredients each have a standard critical control point.
    c. Assess whether the process step is one where microbes can be controlled.

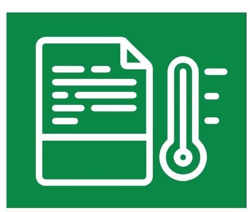
4. Which type of food preparation poses the greatest risk to food safety?
    a. Farm-fresh produce or products
    b. Thawing of ingredients before they are used
    c. Multiple ingredients and several handling steps

5. Which statement does not describe critical control points and critical limits in a food process?
    a. All critical control points in a process have critical limits.
    b. Critical limits are necessary only if there are critical control points in a process.
    c. Steps in a process may include just critical control points, just critical limits, or both.
    d. Critical limits are useful to prevent critical control point failures and prevent product rework

6. Which document is an example of monitoring during food processing?
    a. A calendar of maintenance inspections posted on the door of the walk-in cooler

    b. A record of time and internal temperature kept next to cooling food

    c. A list of the critical control points in a process posted in the work area

7. Which term is used to describe the length of time a packaged food retains its freshness and quality?
   a. Critical limit
   b. Expiry date
   c. Durable life or best-before date
   d. Critical point

8. Which type of temperature control is described in the following example? Staff put orders into refrigerated storage.
   a. Operational control
   b. Prerequisite program
   c. Environmental control

9. Which type of temperature control is described in the following example? Written policies for the expected standards for food delivery are established.
   a. Operational control
   b. Prerequisite program
   c. Environmental control

10. Which type of temperature control is described in the following example? Air ventilators and filters in walk-in coolers are well maintained.
    a. Operational control
    b. Prerequisite program
    c. Environmental control

11. Which type of temperature control is described in the following example? The FIFO principle is applied to food in cold storage.
    a. Operational control
    b. Prerequisite program
    c. Environmental control

12. Which action is an example of monitoring a critical limit?

a. Dedicating a single sink for hand washing

b. Using separate cutting boards for animal proteins and vegetables

c. Checking temperatures during processing

13. Which principle should guide the assessment of risk in food preparation?
    a. All food preparation plans must include multiple critical limits.
    b. Control points are unique to each type of food being processed.
    c. Food microbes can be eradicated from ingredients before food is processed.
    d. Every process step should be evaluated for time, temperature and factors that contribute to microbial growth in food.

14. What is an example of a corrective action?
    a. Modifying a recipe to use safer ingredients
    b. Repairing equipment that has been malfunctioning
    c. Continuing to cook a casserole found to be undercooked
    d. Disciplining a staff member for failing a to wash hands prior to food preparation

15. Examine the food flow chart that follows. Which process step needs to be modified to reduce the risk of microbes growing in the food?

| | |
|---|---|
| Step 1 | Soak dried soybeans overnight in potable water at ambient temperature |
| Step 2 | Drain away water from soybeans |
| Step 3 | Cook soybeans in boiling water for one hour until soft |
| Step 4 | Drain away water from cooked soybeans |
| Step 5 | Press soybeans into clean shaped moulds |
| Step 6 | Cool soybeans in moulds within two hours |
| Step 7 | Vacuum package and freeze soybean cakes |

Check your answers with the answer key provided at the end of this textbook.

# Lesson H: Food Identity, Food Standards, and Certification

## Introduction

As a consumer, you expect the products that you purchase will be as advertised: the jar of honey will contain natural honey, and the peanut butter will contain peanuts. Guidance for what food ingredients may be used to make that honey or peanut butter are referred to as "standards of identity." This module will explain food identity standards and how foods are assessed using attribution plans for microbial and hygiene hazards. Adulterants in the peanut butter, for example, might be accidental or deliberate: a piece of glass, an insect leg, *Salmonella* bacteria, or a protein additive. Food fraud and mislabelling, and the motives behind fraud, will be explained. The final sections in the lesson will review global microbiological standard-setting agencies and the certification standards that industry use to safeguard their products.

### Learning outcomes

Upon completion of this lesson learners will:

- Be able to identify labelling requirements and standards of food identity
- Assess microbiological standards using attribution plans to establish lot acceptance
- Differentiate between acceptable levels of hazards and unacceptable adulteration of foods
- Describe food fraud and differentiate it from food safety and food quality
- Describe international microbial standard-setting organizations
- Describe the purpose for food-manufacture certification bodies

### Terminology

Key terms given in bold

**adulteration**
certification standards for industry
diversion
**extraneous materials**
FAO
food defence
**food fraud**
GMO
halal
kosher
**label claims**
**lot acceptance**
mislabelling
organic
**standard of identity**
substitution
**three-class attribute plans**
**tolerance (allowable and zero tolerance)**
**two-class attribute plans**

## Labelling and standards of identity for foods

Pre-packaged food products sold to consumers at retail are required to have a label with the product name, a nutrition facts table, declared allergen information, and a list of ingredients. There are instructions for industry on what the label should look like and what nutrition and advertising claims a manufacturer may make about a food. The Canadian Food Inspection (CFIA) has many tools for industry about food labelling that will not be reviewed here. If you would like to learn more, consult the CFIA's industry labelling tool at the following link.

**Food labelling for industry**
https://www.inspection.gc.ca/food/requirements-and-guidance/labelling/industry/eng/1383607266489/1383607344939

In this lesson, we are going to review a different aspect of labelling; i.e., the actual name and content of the food. Foods and beverages sold in Canada and elsewhere are required to have labels that identify the name of the product. The product name on foods is regulated and referred to as the **standard of identity**. According to the CFIA, standards of identity currently exist for over 500 foods under the Food and Drugs Regulation (FDR) and the Safe Food for Canadians Regulations (SFCR). Standards of identity for foods will identify the ingredients a food must contain or may contain and the specific manufacturing process requirements.

In Canada, the following groups of foods are regulated for standards of identity (*Standards of identify for food*, 2019):

1. Dairy products
2. Processed egg products
3. Fish
4. Processed fruit or vegetables
5. Honey
6. Maple syrup
7. Meat
8. Icewine

Maple syrup (Figure 1) is defined as a syrup "obtained exclusively by the concentration of maple sap;" honey is defined as a food "derived from the nectar of blossoms" or "from secretions of living plants;" and icewine is defined as a wine "made exclusively from grapes naturally frozen on the vine." These are all examples of foods that must contain a defined ingredient and, for maple syrup and icewine, these standards of identity also specify the acceptable process; i.e., for maple syrup, the "concentration of sap" and for icewine, that grapes must be harvested when "frozen on the vine." The standards for other products, such as meat and dairy are much more detailed. They begin by defining a glossary of terms, and then define specific processes for categories of dairy and meat products.


| Nutrition Facts<br>Valeur nutritive<br>Per 1/4 cup (60 mL)<br>pour 1/4 tasse (60 mL) | |
|---|---|
| Amount<br>Teneur | % Daily Value<br>% valeur quotidienne |
| **Calories / Calories** 210 | |
| **Fat / Lipides** 0 g | 0 % |
| **Sodium / Sodium** 5 mg | 0 % |
| **Carbohydrate / Glucides** 53 g | 18 % |
| Sugars / Sucres 51 g | |
| **Protein / Protéines** 0 g | |
| Calcium / Calcium | 4 % |
| Not a significant source of saturated fat, trans fat, cholesterol, fibre, vitamin A, vitamin C or iron. | |
| Source négligeable de lipides saturés, lipides trans, cholestérol, fibres, vitamine A, vitamine C et fer. | |

**Ingrédients**: Sirop d'érable
**Ingredients**: Maple syrup

**Figure 1** — Bottles of maple syrup and a close-up of the ingredient list for maple syrup

The standards of identity for dairy products states that the definition of milk and whole milk, "as used in the manufacture of dairy products, means the normal lacteal secretion, free from colostrum, obtained for the mammary gland of an animal." Soy and almond milk are very popular beverages, but are they legally allowed to also be called "milk"? According to Canadian regulations described above, and to regulations in the United States, they are not (Pippus, 2019). If you look closely at the packaging of some these items, many do not contain the word "milk;" rather, they are labelled as soy or almond beverages.


**Figure 2** — Fortified almond beverages

Terminology is also problematic when it comes to nut cheeses, as these are dairy-free items. Most of these items are labelled as "vegan" and/or "dairy-free," which are useful terms for marketing these products. Concerning the term "milk," plain-language labelling should use the terminology used by the people, and that common term is "milk," as shown in Figure 3. In the United States, this has become a First Amendment issue, and challenges in court have allowed the use of free speech and continued labelling of products to be what consumers understand them to be (the Food and Drug Administration lost their case) (Sibilla, 2019).


**Figure 3** — Coconut milk

There are other labels on foods in addition to the product names. Manufacturers will add extra information about the food that takes the form of **label claims**. These might include terms such as "organic" or "kosher," or a certification label such as "kosher." Each of these label claims has a specific definition and requirement under Canadian regulations. Labelling and identity standards for foods may be divided into three categories for descriptive purposes: "product names," "production claims" and "inspected and certified."

## Product names

Labelling and identity standards exist for foods that must contain specific ingredients or be manufactured in a specific geographic area. These are often requirements for a food to be identified by a particular name; for example, champagne. Only sparkling wines using grapes grown in the Champagne region of France are permitted to have the word "champagne" on the bottle. A similar sparkling wine made in Canada would not have the label "champagne." Another example is Parmagiano-Reggiano cheese, commonly known as parmesan cheese. Within the countries of the European Union (EU), "parmesan" is legally defined as cheese made from milk (and cows raised in) specific growing areas, such as Parma and Reggio Emilia in Italy. Outside of the EU, the generic term "parmesan cheese" is allowed to be used for cheeses that are processed by the same method. However, the name "Parmagiano-Reggiano" may only be used for cheeses made from milk in those specific growing areas in Italy.

## Production claims

Some labelling and identity standards concern foods that must meet specific growth requirements. One example of this is when foods are grown using organic principles to allow farmers to use the label "organic." Organic agriculture is based on principles of:

- Health for soils, plants, animals and the planet
- Stewardship and awareness of ecological systems
- Using precautionary measures to ensure responsible care
- Embedding fairness into the farming practice and to other farmers

Organic standards are defined by the Government of Canada and Standards Council of Canada in a document that lays out the general principles and management standards for crops and livestock. Organic farming is done without using standard pesticides. Instead, more natural means, such as diatomaceous earth, sulfur, or pyrethrum (Chrysanthemum flower extracts) are used to control pests. Other requirements for organic standards include the prohibition of genetically engineered and nanotech substances, irradiated substances, synthetic compounds, sewage sludge, and many other prohibited materials listed in a separate guidance document called a "permitted substances list." The CFIA is the regulator and certification body for the use of the Canada Organic logo on products sold within Canada and exported outside of Canada. Other examples of production claims on foods include the terms "natural" and "gluten-free." Each of these types of product claims have a specific definition that is regulated by the CFIA.

Learn more about organic standards in Canada from the documents described at the following links.

Government of Canada. Canadian Organic Standards
https://www.inspection.gc.ca/organic-products/standards/eng/1300368619837/1300368673172

Organic production systems. General principles and management standards (March 2021)
https://publications.gc.ca/collections/collection_2020/ongc-cgsb/P29-32-310-2020-eng.pdf

Organic production systems. Permitted Substances Lists (March 2021)
https://publications.gc.ca/collections/collection_2020/ongc-cgsb/P29-32-311-2020-eng.pdf

## Inspected and certified

Other labelling and identity standards relate to foods that are inspected by certified bodies and evaluated to meet a set of criteria. An example of this is "Certified Organic," as described above. Other certifications and label claims, such as "kosher" and "halal," describe foods that have met a religious standard. These foods have some standards in common, such as avoiding pork and shellfish, but also have differences (Figure 4).

| Kosher | Halal |
|---|---|
| **Jewish dietary laws** | **Islamic dietary laws** |
| Consume no pork | |
| Consume no shellfish | Consume no shellfish, with exception of prawns and shrimp |
| May consume alcohol | Consume no alcohol, unless naturally present |
| Meat animals must have cloven hooves and be an ungulate (an animal that chews cud) – other are considered unclean (*Torah*, Deuteronomy 14:3-10) | Dead meat and blood are excluded (*Quran*, Surah 2:172-173) |
| Meat and dairy products must be consumed separately and prepared with separate utensils | Foods in contact with blood or alcohol must be washed |
| Meats must be ritually slaughtered using a blade and inspected (rituals involved reciting of prayers) | |
| Stunning before slaughter not permitted | Stunning before slaughter allowable |
| Inspectors must be trained | Training not required |
| Halal meats are not acceptable under Kosher law | Kosher meats are acceptable under Halal law |
| Not all parts of the animal are permitted to be consumed under kosher law | All parts of the animal may be consumed |

**Figure 4** — Differences between kosher and halal dietary laws

"Kosher" and "halal" are terms that signify compliance related to inspection and certification according to dietary laws. There are many symbols and labels to represent compliance to kosher and halal laws, as shown in Figure 5 respectively. Kosher- and halal-inspected and certified foods are generally accepted to have higher quality and sanitary standards in comparison to non-kosher and non-halal inspected foods. The additional inspections and oversight during preparation of these foods do likely have some impact, although this has not been measured. However, kosher practices of slaughter are not without controversy when animal rights are considered. Some animal rights activists, veterinarians, and industry professionals view the slaughter of animals without stunning or first rendering the animal unconscious to cause needless pain, fear, and suffering, and so would prefer to see such practices changed. These types of ethical issues are complex, particularly when traditional and religious beliefs are involved.

The Canadian Food Inspection Agency recently updated ritual slaughter guideline practices that address practitioner training and animal welfare risks for industry. To learn more about these read the requirements at the following link.

**Canadian Food Inspection Agency. Guidelines for ritual slaughter of food animals without pre-slaughter stunning**
https://www.inspection.gc.ca/food-safety-for-industry/food-specific-requirements-and-guidance/meat-products-and-food-animals/guidelines-for-ritual-slaughter-of-food-animals-wi/eng/1542387114106/1542388400893


**Figure 5**—Certification and kosher symbol card (COR) – top; halal certification symbols – bottom

# Microbiological standards for food

In Canada, the microbiological standards for food set out by Health Canada are used to regulate microbiological levels in foods. The online "Compendium of Analytical Methods" is where you may find a list of Canadian microbiological standards for some foods (but not all; see the following link). The compendium's main purpose is to provide a series of microbiological methods for testing foods. It has five volumes, and describes how to test for water activity, pH, and the presence of insects, foreign materials, bacteria, and viruses in most foods. Volume 1 of the compendium is available on request at the following link and includes an "Interpretive Summary" document that lists the microbiological standards for foods. This document explains the standards for microbiological levels in foods using "criteria for lot acceptance," which are used to establish whether a batch of food (a lot) passes or meets the standard.

**The Compendium of Analytical Methods**
https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods.html

**Health Products and Food Branch (HPFB) Standards and Guidelines for Microbiological Safety of Food: An Interpretive Summary**
https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods/official-methods-microbiological-analysis-foods-compendium-analytical-methods.html

These criteria are based on what are termed "two-class" or "three-class" attribute plans. A mathematical and probability formula is used to determine how many samples from a given lot of food should be collected and, based on the microbiological results of those samples, whether or not the samples pass the criteria. Since it is important to be able to interpret microbiological results from tests, some math is required to explain the interpretative summary information. The variables considered in the tests follow:

$n$ = number of samples

$c$ = maximum number of sample units allowed to fail or have unsatisfactory results

$m$ = the microbiological value above which a sample has unsatisfactory results

$M$ = a second microbiological value above which a sample has unsatisfactory results

In a two-class attribute plan, the variables $n$, $m$, and $c$ are used. A two-class attribute plan is based on microorganisms detected in a sample above the level set by $m$. The lot will fail if multiple samples test positive for microorganisms above the number of samples set by $c$. A two-class attribute plan differs from simple presence/absence test plans in that in a presence/absence plan, any detection of the microorganism is considered unsatisfactory (meaning the lot is defective or fails).

In a three-class attribute plan, the variables $n$, $m$, $M$, and $c$ are used. As in two-class plans, a three-class plan also allows for marginally unacceptable results in a given lot. In a three-class plan, however, if any sample in a lot exceeds $M$, that lot will fail. The microbiological criterion for $M$ is higher than that of $m$, which is usually set at a lower microbiological level—for example, $M$ = 1000 *E. coli* CFU/gram and $m$ = 100 *E. coli* CFU/gram.

Differences in the way a presence/absence system and these two plans are used are shown in Figure 6.

| Presence/absence system | Two-class attribute plan | Three-class attribute plan |
|---|---|---|
| *m* and *n* | *m*, *c*, and *n* | *m*, *M*, *c*, and *n* |
| Decision based on detection of microorganism | Decision based on detection of microorganism at a single defined microbiological criterion | Decision and marginally unacceptable decision based on two microbiological criteria |
| Lot fails when microorganism is detected | Lot fails when the microbiological value of *m* is exceeded by a specified number of samples, *c* | Lot fails when any sample exceeds the microbiological value of *M*, or when the microbiological value of *m* is exceeded by a specified number of samples, *c* |

**Figure 6** — Comparison of three systems for microbiological standards for foods

Which of these three systems is used can affect whether a lot passes a microbiological test. For example, consider how these three systems will work for the following scenario. Five samples were tested for *Listeria* spp. If *Listeria* spp. was detected in any of the samples, the laboratory was instructed to test the sample further to determine if *Listeria monocytogenes* is present and conduct enumeration. The lab results for the five samples were:

- Sample 1. negative
- Sample 2. *Listeria* spp. (*L. innocua* detected)
- Sample 3. *Listeria* spp. (*L. monocytogenes* detected at 250 CFU/gram)
- Sample 4. negative
- Sample 5. *Listeria* spp. (*L. monocytogenes* detected at 850 CFU/gram)

Figure 7 shows how these results were interpreted using each of the three systems.

| Presence/absence system | Two-class attribute plan | Three-class attribute plan |
|---|---|---|
| If *Listeria monocytogenes* (*L.m.*) is detected, lot fails | *n* = 5<br>*c* = 1<br>*m* = 100 *L.m.* CFU/gram | *n* = 5<br>*c* = 3<br>*m* = 100 *L.m.* CFU/gram<br>*M* = 1000 *L.m.* CFU/gram |
| Result: lot fails (*L.m.* detected) | Result: lot fails (two samples of lot were above 100 CFU/gram of *L.m.*) | Result: lot acceptable (up to three samples permitted to have >100 CFU/gram of *L.m.* as long as no single sample is above 1000 CFU/gram of *L.m.*) |

**Figure 7** — Interpretation of results of five food samples tested for *Listeria*

## Activity: Criteria for ice cream

**Task 1.** Look up the criteria for ice cream in the Interpretative Summary (see previous link) and fill in the following table.

| Ice cream | n | m | c | M |
|---|---|---|---|---|
| Aerobic colony count (ACC) | | | | |
| Coliforms | | | | |

Check your answers with the answer key provided at the end of this textbook.

**Task 2.** Three different lots of ice cream were tested and the results obtained are summarized in the following table. Determine which lots failed and explain why, based on the criteria above.

| Lot | ACC results | Coliform results |
|---|---|---|
| Lot 1 | Sample 1. $1.2 \times 10^2$<br>Sample 2. $1.3 \times 10^1$<br>Sample 3. $1.2 \times 10^6$<br>Sample 4. $4.8 \times 10^5$<br>Sample 5. $1.3 \times 10^5$ | Sample 1. < 3 MPN/gram<br>Sample 2. < 3 MPN/gram<br>Sample 3. < 3 MPN/gram<br>Sample 4. < 3 MPN/gram<br>Sample 5. < 3 MPN/gram |
| Lot 2 | Sample 1. $1.2 \times 10^2$<br>Sample 2. $1.3 \times 10^1$<br>Sample 3. $1.2 \times 10^5$<br>Sample 4. $4.8 \times 10^5$<br>Sample 5. $1.3 \times 10^4$ | Sample 1. < 3 MPN/gram<br>Sample 2. < 3 MPN/gram<br>Sample 3. 120 MPN/gram<br>Sample 4. < 3 MPN/gram<br>Sample 5. < 3 MPN/gram |
| Lot 3 | Sample 1. $1.2 \times 10^2$<br>Sample 2. $1.3 \times 10^1$<br>Sample 3. $1.2 \times 10^5$<br>Sample 4. $4.8 \times 10^5$<br>Sample 5. $1.3 \times 10^4$ | Sample 1. < 3 MPN/gram<br>Sample 2. < 3 MPN/gram<br>Sample 3. 1200 MPN/gram<br>Sample 4. < 3 MPN/gram<br>Sample 5. < 3 MPN/gram |

## Presence/absence criteria and allowable tolerances for microbes—*Listeria Monocytogenes*

Presence/absence microbial criteria are applied to foods where the presence of a specific (pathogenic) microbe is undesirable. For example, infant powder formula must not contain microbes such as *Cronobacter*, *Salmonella*, and *Listeria*. This is also referred to as "zero tolerance." In some foods, there can be an allowable tolerance for a microbe, as for *Listeria* in specific food categories in which the food attributes (pH, $a_w$ and intended storage temperature) do not allow *Listeria* to grow. Health Canada defines *Listeria* risk in foods in three separate categories: Category 1 foods, Category 2a foods, and Category 2b foods.

Specific guidance for *Listeria* from Health Canada can be found in the document, *Policy on Listeria monocytogenes in Ready-to-Eat Foods*, available on the Health Canada website.

## Activity: Criteria for *Listeria Monocytogenes* testing

Look up the criteria for *Listeria monocytogenes* testing for Category 1, 2a, and 2b foods, and fill in the following table. (*Hint*: Look at Table 1 in the *Listeria* policy document in the link above).

| Category | Number of samples to test per lot? | Enrichment or direct plating? | Action level | Health risk |
|---|---|---|---|---|
| 1 | | | | |
| 2a | | | | |
| 2b | | | | |

Check your answers with the answer key provided at the end of this textbook.

# Tolerable (acceptable) limits of hazards in foods

Food is not always sterile. It is common and natural for foods to have microbes such as yeasts and bacteria in them and on them. For some types of food manufacturing, these microbes are beneficial and needed, such as for making fermented foods and beverages. However, spoilage microbes will affect food quality. Other issues in food can include presence of worms and other insects, foreign matter (dirt, stones, organic debris), and antibiotic and pesticide residues. For example, flour is generally considered compromised if there are too many weevil insects present. These types of issues are also called **extraneous materials**. But how many is too many?

In this section, we will explore standards that look at the tolerable or acceptable limits of hazards in foods. In the previous section, microbiological guidelines found in the "Compendium of Analytical Methods" described the limits of indicator and pathogen organisms in different food categories.

The compendium also sets limits for extraneous hazards in a separate module called "Guidelines for the General Cleanliness of Foods,".

The compendium also sets limits for extraneous hazards in a separate module called "Guidelines for the General Cleanliness of Foods," available on the Health Canada website.

Once you review these guidelines, you see there are weighted measures and numbers for the amount of pieces of insects (whole or fragments), maggots, mites, moulds, metal fragments, nematodes, and pits or pieces of pits, and rodent hairs. As described previously, the values are given in two- and three-class attribute plans. For example, wheat flour is assessed based on an examination of insect fragments pre- and post-milling (milling refers to the grinding of wheat into flour) and on the number of rodent hairs. Based on the acceptance criteria for ground white flour, out of three samples ($n$ = 3) of 50 grams each, a single sample may have up to ten insect fragments that are greater than 0.2 mm in size, but no sample may have more than twenty insect fragments that are greater than 0.2 mm in size.

### Activity: Acceptance criteria for mushrooms

Describe the sample acceptance criteria for mushrooms.

| Mushrooms | *n* | *c* | *m* | *M* |
|---|---|---|---|---|
| Maggots ≥ 2mm | | | | |
| Mites (dead) | | | | |
| Nematodes | | | | |

Check your answers with the answer key provided at the end of this textbook.

## Food fraud: Adulteration and mislabelling

Food crimes are defined as criminal activity with intent to compromise safety or authenticity of food. There are two types of food crimes: food fraud and food defence. **Food fraud** is motivated by the prospect of economic gain. **Food defence** is motivated by the desire to create harm and terror, but it can still include economic gain. These are both intentional food crimes. Food safety and food quality differ from food fraud and food defence because they are unintentional activities leading to negative consequences.

These differences were discussed by Spink & Moyer (2011) and are summarized in the diagram in Figure 8. Harms arising from food defence issues include public health harms, such as food poisoning. While food fraud does not generally intend to cause harms such as foodborne illness or allergenic reactions, cases are often detected after illness is reported.

| | | |
|---|---|---|
| **Food quality** | **Food fraud*** | Motivation gain: economic |
| **Food safety** | **Food defence** | Harm: public health, economic, or terror |
| Unintentional | Intentional | |
| **Action** | | |
| | | ** includes economically motivated adulteration and food counterfeiting* |

**Figure 8** — Food protection risk matrix (from Spink & Moyer, 2011)

## Methods of food fraud

Section 5(1) of the Food and Drugs Act prohibits "labelling, packaging, treating, processing, selling or advertising food in a manner that is false, misleading or deceptive or is likely to create an erroneous impression."

Food fraud (using food as deception for economic gain) can occur via several methods. Many of the deception methods described in this section are performed together in food fraud cases, such as substitution with mislabelling.

### Adulteration of food

When other ingredients are deliberately added to foods in place of the expected ingredients, this is known as **adulteration**. For example, high fructose corn syrup is fairly inexpensive and has been used in place of or to partly dilute natural honey, maple syrup, and fruit juices such as pomegranate (Everstine, Spink, & Kennedy, 2013). More recently, melamine used as a protein substitute was an adulterant in pet foods and baby infant powder formula. The latter case is featured in an example that follows.

### Substitution

High-value products such as scallops or blue-eye tuna are expensive. By substituting lower-value products, higher profits can be made. Examples of **substitution** include surimi made from inexpensive pollock being substituted for scallops and lower-value tuna being substituted for more expensive blue-eye tuna. These are both examples species substitution. Red snapper is a fish that is commonly found to be mislabelled. Lower-value fish species were found as substitutes for red snapper when species testing surveys of fish at retail markets has been undertaken. Seafood fraud has been found in nearly half of seafood tested in Canada (47%) (Everstine, Spink, & Kennedy, 2013; Oceana, n.d.)

### Diversion

"**Diversion**" occurs when food is diverted to avoid tariffs or other costs such as farm program taxes and to increase profits: this is food fraud. Sales of foods to unintended markets can also cause distribution issues and shortages of foods in some populations. Some countries have over-dumping taxes and duties for undesirable fish species, such as catfish. Catfish mislabelled as grouper has been sold from Vietnam to the United States (Spink & Moyer, 2011; Johnson, 2017).

### Misrepresentation

The mislabelling of a food product or its ingredients, or of the country of origin on packaging are examples of **misrepresentation**. All-natural and organic ingredients are often substituted with synthetic or conventional ingredients. When a package weighs less than its label states (known as "short weighing"), profits are increased. Historically, this was an issue in the liquor industry, where pint sizes and standard measures are of concern. Counterfeit packaging can also be used for lower-quality foods.

## Foods commonly subject to food fraud

In Canada, maple syrup is a food at particularly high risk of food fraud through adulteration. As a high-value product, it has been on lists of the ten most adulterated foods. Food fraud occurs when maple syrup is diluted or its sugar content is substituted with corn syrup (Johnson, 2017). Because pure maple syrup is so expensive, it is a lucrative target for crime. In 2011 and 2012, $18.7 million dollars of maple syrup was siphoned out of containers in a warehouse and sold on the black market (Constable, 2017).

Food fraud was uncovered in the United Kingdom when porcine DNA was found in halal meat products (Fuseini, Wotton, Knowles, & Hadley, 2017). Unscrupulous or ignorant meat processors either added lower-cost porcine meats or processed halal-slaughtered meats in facilities that also processed pork products. In the United States, Canada, and other countries, chloramphenicol-tainted honey from China was sold through other countries to mask the country of origin and avoid antidumping tariffs (Everstine, Spink, & Kennedy, 2013).In Hungary, sixty cases of illness occurred when lead oxide was added to Romanian-sourced paprika that was marketed as Hungarian paprika (Everstine, Spink, & Kennedy, 2013).

Carcinogenic Sudan dyes added to spices to enhance colour have been linked to several food fraud cases (in sauces and spices) (Everstine, Spink, & Kennedy, 2013).

Other foods and food ingredients commonly associated with food fraud include olive oil, fish, honey, milk and dairy products, meat products, grain-based foods, fruit juices, wine and alcoholic beverages, organic foods, spices, coffee, and tea, and highly processed foods.

A collection of food fraud incidents can be found in the online database cited earlier.

**Food fraud risk information database**
https://trello.com/b/aoFO1UEf/food-fraud-risk-information

## Public health issues arising from food fraud

Allergy-related illnesses can occur when substitutions and adulterations with allergen-containing foods are made. For example, highly valued virgin olive oil may be diluted with lesser-value tree nut oils. Ground spices have been found contaminated with the finely ground husks of almonds. This filler is normally undetected unless a sensitive person has an adverse reaction to the allergen. Soy-based vegetable protein has been found as an allergen in meat products.

When extra water is frozen with shrimp or fish to increase the weight, the source water may be contaminated with microbial or chemical hazards. Water sources can also be of concern when foods are diluted, such as in juice concentrates. When food shipments are illegally diverted to avoid authority oversight, poor temperature storage may lead to amplification of microbial hazards.

### Example: Melamine as a protein adulterant in animal food and baby food

Melamine is a compound that is high in nitrogen. In tests for protein that look exclusively at nitrogen levels, melamine added deliberately as an adulterant can give a false positive reading that a food is high in protein. In the mid-2000s, companies in China adulterated various brands of cat and dog treats and foods with this chemical to falsely indicate a high protein level. Several hundred pets died as a result in the United States, Canada, and likely elsewhere where the products were exported. Melamine is excreted through urine and can cause formation of kidney stones and renal injury. Melamine was eventually detected in the pet foods and was also found in other animal feeds. Tragically, this compound was also added to baby foods and infant formula in China, where it was found at levels as high as 2560 mg/kg in powdered infant formula. In China, nearly 300 000 infants became ill after ingesting this formula. Around one third of these children, most under the age of two, were hospitalized; eleven deaths were reported. Although the Chinese government had declared all contaminated product was destroyed, melamine was subsequently detected in powdered infant formulas in Africa. Melamine caused urinary tract stones to form in pets and infants, which can be fatal if not diagnosed and treated (*Melamine*, 2019; Schoder & McCulloch, 2019).

Melamine is also found in dairy products and wheat gluten. Single shipments of fraudulent food can result in large economic gains, estimated at tens of thousands of dollars in illegal profits (Moyer, DeVries & Spink, 2017).

# International standards for foods

International standards for foods are upheld by a number of organizations, which are given in Figure 9. The responsibilities and roles of these organizations are discussed after Figure 9.

| Organization | Acronym | Website |
|---|---|---|
| Food and Agricultural Organization of the United Nations | FAO | http://www.fao.org |
| Codex Alimentarius Commission | CAC | http://www.fao.org/fao-who-codexalimentarius/en/ |
| International Organization for Standardization | ISO | https://www.iso.org/home.html |
| European Food Safety Authority | EFSA | http://www.efsa.europa.eu/ |
| National Advisory Committee on Microbiological Criteria for Foods | NACMSF | https://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/nacmcf/ |

**Figure 9** — Organizations involved in international food standards

## Food and Agricultural Organization of the United Nations

The Food and Agricultural Organization of the United Nations (FAO) promotes food security and fair trade. This organization has over 180 member countries, including Canada. To learn more about what the FAO does, watch the video at the following link.

**10 Achievements of the Food and Agriculture Organization of the United Nations**
https://www.youtube.com/watch?v=wYxMwaTB_AQ

## Codex Alimentarius Commission

The Codex Alimentarius Commission (CAC) was established by the FAO and the World Health Organization (WHO) to protect consumer health and promote fair practices in food trade. This commission is responsible for developing and revising the Codex Alimentarius, the first achievement mentioned in the video above. The Codex is a voluntarily recognized set of food standards of identity, guidelines, codes of practice, and maximum residue limits (of pesticides and veterinary drugs) for foods. The Codex does not take the place of a country's national standard, laws, or regulations. However, it is recognized as a voluntary set of recommendations regarding food quality, food safety, farming, and processing activities.

## International Standards Organization

The International Standards Organization (ISO) publishes standards on a diverse array of topics. These include food technology and detection methods, as well as ceramics, military affairs, and aircraft and information technology. These internationally recognized standards are widely recognized and used by food and manufacturing industries. Food-safety management modules within ISO are used in many private certification bodies. They incorporate ISO modules, such as ISO 22000, into their offerings in order to be more easily recognized and accepted by users. ISO modules are not free and must be purchased. In contrast, government sites publish their laboratory methods online (e.g., the "Compendium of Analytical Methods" on the Health Canada website described earlier in this lesson). Two freely accessible examples from the United States are given at the following links.

**Bacteriological Analytical Manual (BAM)**
https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam

**Microbiology Laboratory Guidebook**
https://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook

Earlier in this lesson, we reviewed food standards and lot acceptance based on microbiological criteria. Some larger members of the food industry will sometimes perform laboratory tests in-house as part of their quality assurance. However, smaller food industry members will likely seek a private laboratory to perform these testing services. It is important that the laboratory service provider is also accredited to perform the test. Laboratories are usually accredited to ISO 17025 standards through the two agencies in Canada at the following links.

**Standards Council of Canada (SCC)**
https://www.scc.ca/

**CALA**
https://cala.ca/

To find an accredited laboratory, an industry seeking a service should search out and choose a laboratory that has been audited and certified to provide this service. This can be done on the SCC site at the following link, through filtering by geographic region and test to find an appropriate laboratory.

**Standards Council of Canada: Accreditation**
https://www.scc.ca/en/search/laboratories/

## European Food Safety Authority

The European Food Safety Authority (EFSA) provides independent scientific advice to food regulators in the European Union. The *EFSA Journal* is a free online journal that publishes this advice.

**EFSA Journal**
https://efsa.onlinelibrary.wiley.com/journal/18314732

Many of the papers EFSA publishes have a title that begins with "Scientific Opinion on." The EFSA is a very useful resource to consult for the latest information on pathogen-food interactions, concerns about pesticides and GMO foods, animal health, food security, and information about outbreaks affecting Europe. For example, a more recent database from the EFSA looks at the combined mixed effects of chemicals on animal feed and human food (see following links).

**When chemicals mix: Assessing the risks to humans, animals and the environment**
https://www.efsa.europa.eu/en/interactive-pages/MixTox

**Chemical mixtures**
https://www.efsa.europa.eu/en/topics/topic/chemical-mixtures

**Chemical mixtures and food safety**
https://youtu.be/0BgpGPzXYy4

## National Advisory Committee on Microbiological Criteria for Foods

The National Advisory Committee on Microbiological Criteria for Foods (NACMSF) is a branch of the Food Safety Inspection Service in the United States that provides scientific advice and recommendations to federal food-safety agencies. Several reports written by this agency have been referenced throughout this lesson. You can read through more reports, such as how *Salmonella* can be controlled in poultry, at the following link.

**NACMSF Reports and Recommendations**
https://www.fsis.usda.gov/wps/portal/fsis/topics/regulations/advisory-committees/nacmcf-reports/nacmcf-reports

# Certification standards for industry

Certification for industry involves compliance to a set of accreditation standards by a recognized body. Industry seeks out certification because many very large retailers and wholesalers demand this as a way to ensure the foods they sell have the highest food safety. If the supplier does not have the required certification, they may not be able to market and sell their goods to clients. Figure 10 lists the bodies that provide certification for industry.

| Name | Acronym | Website |
|---|---|---|
| Global Food Safety Initiative | GFSI | https://mygfsi.com/ |
| International Features Standards | IFS | https://www.ifs-certification.com/index.php/en/ |
| Foundation Food Safety System Certification 22000 | FSSC 22000 | https://www.fssc22000.com/ |
| British Retail Council Global Standard for Food Safety | BRCGS | https://www.foodchainid.com/certification/brc-food-safety-certification |
| Safe Quality Food Institute | SQF | https://www.sqfi.com/ |

**Figure 10** — Organizations involved in food standards for industry

## The Global Food Safety Initiative

The Global Food Safety Initiative (GFSI) is an organization that sets certification standards for industry. It is managed by a volunteer-led industry board of directors. The GFSI aims to improve food safety and business efficiency through a harmonization and "once certified-recognized everywhere" approach. The GFSI has a recognition program to assess whether private certification companies meet their standard, through a process termed "benchmarking." The GFSI bases its food-safety certification standards on recognized international standards, such as the Codex and those of the ISO. The benchmarking process assesses private certification companies for equivalency (more information follows). Once a program meets the benchmark, it is considered equivalent to other certification programs. The goal is to reduce the number of different certifications a single company may have to comply with in order to trade with partners. GFSI standards are made up of three topic areas: food-safety management, HACCP, and good manufacturing practices (GMPs). Recognized certification standards under GFSI are listed on their website at the following link.

**Recognition: A food safety passport**
https://mygfsi.com/how-to-implement/recognition/

## Private certification organizations (IFS, FSSC, BRC, SQF)

The remaining organization listed in Figure 10 are private certification organizations. These include International Features Standards (IFS), Foundation Food Safety System Certification 22000 (FSSC 22000) British Retail Council Global Standard for Food Safety (BRCGS) and Safe Quality Food Institute (SQF), which provide their own variations of food-safety and food-quality programs that are recognized by retailers, brand owners, and food providers (Sansawat & Muliyil, 2011). These programs cover most aspects of the food chain, from agriculture to retail. Many of the programs offer modules that cover specific segments of a certification program, such as management responsibility, internal audits, documentations, purchasing, supplier approval/monitoring, corrective action, and personnel training (Sansawat & Muliyil, 2011).

# Lesson H Quiz

1. What principle underlies the labelling of food products in Canada?
    a. The number of people experiencing intolerances to specific foods is rising.
    b. It is increasingly common that prepared foods contain adulterated ingredients.
    c. The public has a right to know what is in the foods they are purchasing and consuming.
    d. Regulation of the food industry is necessary to combat careless production practices.
2. What is the meaning of intentional adulteration as it applies to food production?
    a. The act of deliberately using an alternate ingredient for economic gain
    b. The unintended act of adding filth to a food such that the food fails sanitary indicator tests
    c. The act of deliberately mislabelling a food product
3. Which two food label terms refer to specific religious standards?
    a. GMO
    b. Pure
    c. Halal
    d. Vegan
    e. Kosher
    f. Organic
4. How are attribute plans used to determine lot acceptance?
    a. They determine the total amount of microbiological activity in the given lot.
    b. They determine the minimum number of lot samples that must show no microbiological activity.
    c. They determine the maximum number of lot samples that may exceed a set microbiological value.
5. Which attribute plan is likely to result in the greatest number of lots being labelled unacceptable?
    a. Two-class attribute plan
    b. Three-class attribute plan
    c. Presence/absence system
6. If a producer alters a food product in a way that lowers food quality, when is it considered food fraud?
    a. When the alteration is done intentionally and leads to economic gain
    b. When the alteration is done unintentionally and leads to economic gain
    c. When the alteration is done intentionally and causes harm to public health
    d. When the alteration is done unintentionally and causes harm to public health
7. What is the principle purpose of certification agents for food processors?
    a. Certification ensures that the food is produced to a specific market standard.
    b. Certification allows processors to market their product with a recognized label.
    c. Certification is a requirement of global retailers and wholesalers to ensure food safety.
    d. Certification opens up markets to allow product sales in other countries.
8. Which aspect of food industry regulation is exemplified in this image?
    a. Certification
    b. Lot acceptance
    c. Best before date
    d. Standard of identity

9. Which of the following descriptions applies to the Food and Agricultural Organization of the United Nations?
    a. Promotes food security and fair trade
    b. Provides up-to-date information about pesticide use and GMO foods
    c. Sets standards for food technology, aircraft and information technology

10. Which of the following descriptions applies to the European Food Safety Authority?
    a. Promotes food security and fair trade
    b. Provides up-to-date information about pesticide use and GMO foods
    c. Sets standards for food technology, aircraft and information technology

11. Which of the following descriptions applies to the International Standards Organization?
    a. Promotes food security and fair trade
    b. Provides up-to-date information about pesticide use and GMO foods
    c. Sets standards for food technology, aircraft and information technology

Check your answers with the answer key provided at the end of this textbook.

# Appendix A: Techniques for Counting and Characterizing Bacteria

## Preparing food samples for counting and characterizing

To prepare a food sample for bacteria counting or characterization techniques, a quantity of food is first measured out and mixed with a dilute nutrient broth to form a 10 % solution (for e.g., 25 g of food and 225 mL of broth). It is then mixed in a piece of equipment called a stomacher (Figure 1), which has two metal paddles that mix the food inside a sterile plastic bag. From this solution (called the food homogenate) (bacteria counts and investigations into the type(s) of bacteria growing can be performed. Blenders are sometimes used for this step, but they can be too rough.

**Figure 1** — Food sample after mixing in a stomacher

## Counting bacteria in food samples

There are many times when a food microbiologist needs to know the number of bacteria in a sample. For example, bacteria counts are needed to determine D-values or to run tests for food quality or food safety. Bacterial counts can be carried out on bacteria grown on agar plates or in liquid nutrient broth. In this section, we will look at a number of concepts and techniques important to the process of determining the number of bacteria in a sample namely: serial dilutions, colony forming units (CFUs), most probable number (MPN), and streaking.

## Serial dilutions

The first step to determine how many bacteria are present in a food sample, water sample, or a culture of bacteria growing in liquid media is to prepare a series of dilutions. Serial dilution is usually a set of three tenfold dilutions (Figure 2). Some methods require a dilution series of five or more. A measured volume of each serial dilution is then spread on an agar plate and allowed to grow so that the number of bacteria can be determined (Figure 3).

**Figure 2** — Serial dilution of food sample and inoculation into nutrient broth

**Figure 3** — Plating of food samples and serial dilutions onto nutrient agar to establish standard plate count

Suppose you wanted to prepare a serial dilution from a food sample, for e.g., a salad processed in a stomacher; referred to as the sample homogenate. The original homogenate is the first tenfold dilution in the series, as it is prepared using one part salad sample and nine parts water. For the next dilution in the series, 1 mL of the original homogenate is added to 9 mL of water or diluent buffer. This dilution is a 100-fold dilution of the original food sample. Then, 1 mL of this dilution is added to another 9 mL of water or buffer. This dilution is a 1000-fold dilution of the original food sample. It follows that if you observed forty-two bacterial colonies on the plate from the 1000-fold dilution, that number would be equivalent to 42,000 bacteria from the original sample.

Although they are long, the following videos provide an excellent overview of serial dilution and spread plate techniques.

**Serial Dilution for the Standard Plate Count**
https://www.youtube.com/watch?v=FdzKgotzjC4

**The Spread Plate Technique for Standard Plate Count**
https://www.youtube.com/watch?v=FwW_2ii1Zoo

## Colony forming units

The term, colony forming unit or CFU is used to describe a single bacterial cell, or clump of bacterial cells stuck together. It is assumed that each individual bacteria will form a round spot on the plate. This is the term that is often used in laboratory report forms to describe how many bacteria are present in a food or water sample. Figure 4 shows individual bacteria or colony forming units on plates prepared from two dilutions in the series shown in Figure 3.

**Figure 4** — Counts from the nutrient agar serial dilutions (10 × dilution left; 100 × dilution, right)

In Figure 4, there are approximately twenty-two bacterial colonies on the right-hand plate: some of these are just tiny points and some are quite large. This plate was made from a 1:9 dilution (1 mL homogenate + 9 mL broth) of the already diluted food homogenate (25 g of food + 225 mL of broth) shown in Figure 1. So, the dilution in this case is by a factor of 100. To make the spread plate, 0.1 mL was used. The bacterial count is:

Total plate count = $(22 \times 100)/0.1 = 22\,000$ CFU/mL or 4.3 log10 CFU/mL

For more information on calculating the number of bacteria in a sample, and on the nutrient agar plates, watch the two videos that follow.

**Colony Forming Units cfu/mL Calculation**
https://www.youtube.com/watch?v=jdnOjoOO6qY

**Colony Forming Units**
https://www.youtube.com/watch?v=HDsy--ya2sY

## Most probable number (MPN)

Bacteria in liquid samples may be counted by a method called most probable number or MPN. This method is based on a statistical calculation (Poisson) of the number of dilutions in replicate. For example, three dilutions might be used (e.g., 10 ×, 100 ×, 1000 ×), and three replicates (copies) of each dilution are used for the sample (as shown in Figure 2). In food and water samples, this method will determine the count of bacteria without the need for plating the samples onto semi-solid agar. The dilutions are instead made using a indicator for the presence of bacteria, such as letheen broth (Figure 5). A positive reaction for letheen broth is the production of gas in the tube, caused by bacterial growth (see the left-hand tube). No gas is produced when no bacterial growth occurs, as seen in the right-hand tube in Figure 5.

**Figure 5** — Growth of *E. coli* in letheen broth: positive reaction (left tube), negative reaction (right tube)

Suppose you wanted to use this method to find out how many *E. coli* are present in the food sample. First, you would prepare three replicates of each of three dilutions of 0.1, 0.01, and 0.001 (10 ×, 100 ×, 1000 ×). Then, you would determine the number of tubes positive for *E. coli* so you would be able to estimate the bacterial count.

Assume you found a count of 3, 1, 0. This means that for the 0.1 dilution, all three tubes were positive; for the 0.01 dilution, one tube was positive; and for the 0.001 dilution, no tubes (zero) were positive. By this method, the total *E. coli* count would be 43 MPN per gram. You would obtain this number by consulting the tables shown in the following resource link.

For more information on how to calculate most probable numbers and to access the tables mentioned, go to this link from the United States Food and Drug Administration Bacteriological Analytical Manual (BAM) in Appendix 2.

**BAM Appendix 2: Most Probable Number from Serial Dilutions**
https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm

## Streaking techniques

Bacteria can be plated out onto agar using several techniques.

To count bacteria, techniques that evenly distribute bacteria over a plate are best and these are referred to as spread plate methods. Sterilized bent glass rods or sterile disposable miniaturized plastic hockey sticks can be used to manually spread the bacteria. Automated spiral plating machines can also perform this task.

To isolate bacteria, another type of streaking technique is used. In this method, a loop of wire on a handle is first heated in a flame to sterilize it, then touched to a sample and run along the surface of an agar plate. When done correctly, isolated colonies will grow on the plate that can be more easily counted.

View the following videos to see examples of the different types of streaking techniques for isolating and counting (even growth) bacteria.

**Streak Plate Method**
https://www.youtube.com/watch?v=_1KP9zOtjXk

**Culture Plate Streaking Technique**
https://www.youtube.com/watch?v=PgOotP5e2VM

**The Spread Plate Technique**
https://www.youtube.com/watch?v=1gs4JQ4B1TU

## Using environmental conditions to characterize bacteria

Different species and/or different variants (or strains) of bacteria often need different environmental conditions, including different food sources, in order to grow. Microbiologists may try to grow bacteria on various different types of agar or nutrient broth to identify or characterize them.

For example, the strain of *E. coli* bacteria responsible for hamburger disease, *E. coli* O157:H7, is the only *E. coli* strain that is unable to consume the sugar sorbitol. Figure 6 shows the growth of *E. coli* O157:H7 on two different types of nutrient agars that have a dye (neutral red) that is also added to the plate. When bacteria digest the sugars in the agar, acid is formed that raises the pH and activates the dye to show a red colour. The plate on the right contains a type of agar called Sorbitol-MacConkey (S-MAC), which contains only sorbitol as a sugar source. *E. coli* O157:H7 bacteria growing on this plate have a pale, translucent colour because they cannot digest the sugar sorbitol. Other strains of *E. coli* would appear dark pink in colour on this type of agar similar in colour on the left plate. On the left-hand plate normal MacConkey agar (MAC) contains lactose as the primary sugar source. Here, the bacterial colonies are stained pink because of because all types of E.coli ferment lactose, this sugar supports the growth of both normal, non-disease-causing strains of E.coli and the hamburger-disease-causing strain *E. coli* O157:H7.

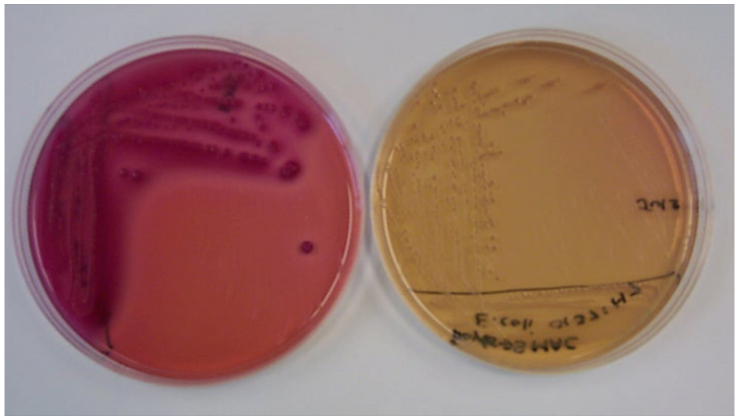


**Figure 6** — *E. coli* O157:H7 bacteria growing on MAC agar (left) and S-MAC agar (right)

Figure 7 shows various types of agar that can be used to determine if a case of food poisoning has occurred. Samples of the food suspected of causing illness are plated directly onto these agar, or mixed into special nutrient broth to determine if a bacteria species or variant known to cause food poisoning is present.

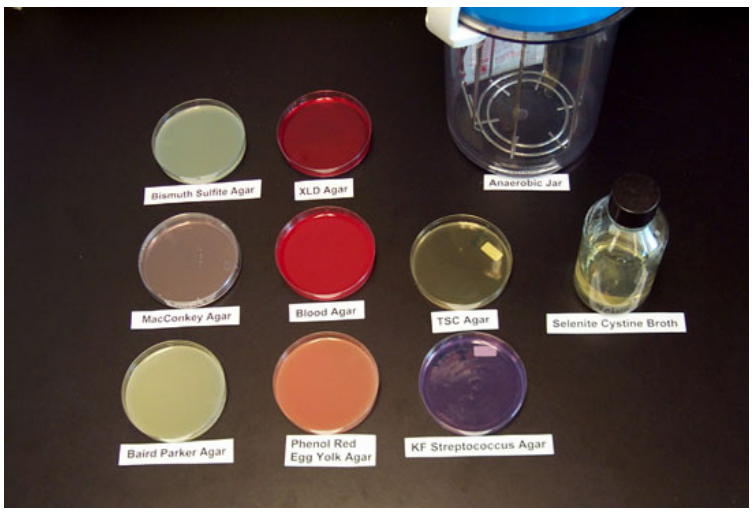



**Figure 7** — Types of nutrient agar used in a food poisoning investigation

To learn more about metabolic function, the structure and function of proteins, enzymes, and different types of cellular respiration in microbes, view the following video:

**Bacterial Metabolism**
https://www.youtube.com/watch?v=GLwsj13AACs

# Appendix B: Statistics on foodborne illness

For more statistics on foodborne illness, visit these resources:

**Chronic Disease Dashboard**
http://www.bccdc.ca/health-professionals/data-reports/chronic-disease-dashboard

**Chronic Disease Data and Indicators**
https://www.canada.ca/en/public-health/services/chronic-diseases/chronic-disease-facts-figures.html

**Integrated Surveillance of Foodborne Pathogens**
http://www.bccdc.ca/health-info/diseases-conditions/salmonella-infection/integrated-surveillance-of-foodborne-pathogens

**Notifiable diseases online**
https://dsol-smed.phac-aspc.gc.ca/notifiable/

**Reportable Diseases Data Dashboard**
http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard

**Surveillance of food-borne illness in Canada**
https://www.canada.ca/en/public-health/services/food-borne-illness-canada/surveillance-food-borne-illness-canada.html

**Yearly food-borne illness estimates for Canada**
http://healthycanadians.gc.ca/eating-nutrition/risks-recalls-rappels-risques/surveillance/illness-estimates-estimations-maladies/yearly-annuel-eng.php#ov

# Appendix C: Common pathogens

Bacteria:

- *Campylobacter jejuni*
- *Clostridium botulinum*
- *Clostridium perfringens*
- *E. coli* O157:H7
- *Listeria monocytogenes*
- *Salmonella* spp.
- *Shigella* spp.
- *Staphylococcus aureus*
- *Vibrio* spp.
- *Yersinia enterocolitica*

Viruses:

- *Hepatitis A*
- Norovirus

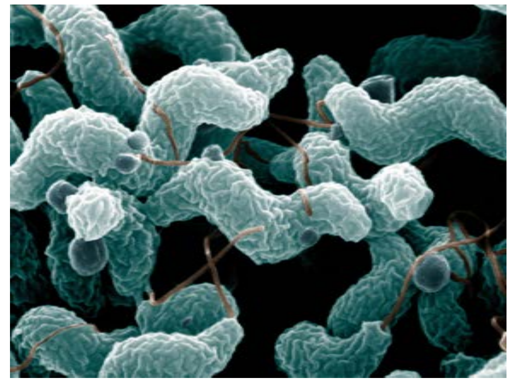
Parasites:

- *Cryptosporidium*
- *Cyclospora*
- *Toxoplasma gondii*

## *Campylobacter*

**Description**

*Campylobacter* are spiral-shaped bacteria that can cause disease in humans and animals. Most human illness is caused by one species, *Campylobacter jejuni*, but human illness can also be caused by other species. *Campylobacter* is often found in raw poultry. In some cases, it has been spread through contaminated drinking water. The bacteria infect the intestinal tract and sometimes the blood. Campylobacteriosis is the most frequently reported food-related illness in Canada. (www.phac-aspc.gc.ca)



*Campylobacter* bacteria

**Sources**

*Campylobacter* live in the intestines of many animals, including chickens, cows, pigs, sheep, dogs, cats, and humans. Fecal material from infected humans or animals can get into our mouths by:

- Consuming contaminated food or drink, particularly undercooked poultry, beef, pork, and lamb, as well as shellfish and untreated drinking water
- Contact with feces of domestic or wild animals, including pets and farm animals
- Contact with feces of infected humans that is not followed by proper handwashing

**Symptoms**

Mild to severe diarrhea, bloody diarrhea, nausea, stomach pain, vomiting, fever, muscle pain, headache

A person can be infected and have no symptoms.

**Incubation**

Two to five days after eating contaminated food

**Duration**

Two to ten days

**Prevention**

- Treat all raw poultry and meat as if it is contaminated.
- Keep raw meat well-wrapped and on lower refrigerator shelves, to avoid blood dripping down.
- Avoid direct contact between raw meats and other uncooked foods.
- Use a meat thermometer to ensure poultry and meats are cooked to 74° C or higher.
- Do not eat raw eggs. Do not drink unpasteurized milk or juices.
- Drink treated drinking water and wash all fruits and vegetables prior to consumption.
- Wash your hands before eating, before handling food, immediately after handling raw poultry or meat and before touching anything else, after using the toilet or changing diapers, and after touching animals.

**Outbreaks**

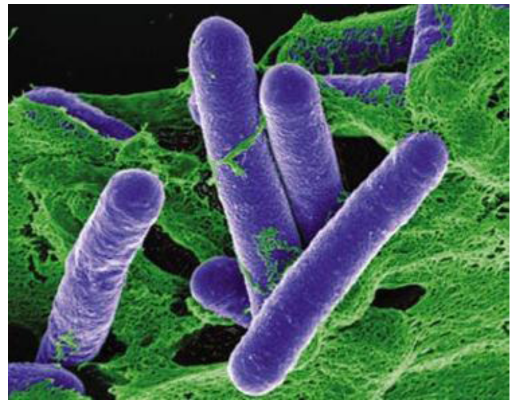
- *Campylobacteriosis* is the most frequently reported food-related illness in Canada. Many of these illnesses are sporadic cases, but some are part of outbreaks. Outbreaks of *campylobacteriosis* are uncommon in Canada but do occur.
- The Government of Canada estimates that approximately 145 000 illnesses and 565 hospitalizations occur due to *Campylobacter* annually. (www.healthycanadians.gc.ca)
- In 2018 there were 1,687 *campylobacteriosis* cases in BC (33.8 cases per 100,000 population). Rates in BC are much higher in this province in comparison to the rest of the country. (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

## *Clostridium Botulinum*

**Description**

The spores of the bacteria that causes botulism—*Clostridium botulinum (C. botulinum)*—are widespread in nature, commonly being found in soil and dust. These bacterial spores cannot grow or produce the toxins that cause illness in aerobic conditions, but can do so under anaerobic conditions (e.g., improperly canned foods).

Botulism is a rare, but serious, paralytic illness caused by a nerve poison produced by *C. botulinum*. Foodborne botulism is caused by eating foods that contain the botulism toxin. Botulism may also be contracted from an infected wound. All forms of botulism can cause paralysis and be fatal. (www.phac-aspc.gc.ca/)



*Clostridium botulinum* bacteria

**Sources**

Botulism is rare in Canada. *C. botulinum* spores can be found in soil and dust, and so can cause problems when foods are stored or prepared improperly.

The most common way of getting botulism is by eating or drinking contaminated foods and beverages, such as:

- Improperly prepared low-acid, home-canned foods (such as asparagus, beets, green beans, mushrooms, peppers)
- Improperly smoked fish
- Vacuum-packed and tightly wrapped food
- Improperly prepared raw marine mammal meat (such as whale, walrus, seal)
- Non-refrigerated storage of low-acid fruit juices (such as carrot juice)
- Baked potatoes stored in aluminum foil
- Honey (linked to infant botulism)

(www.phac-aspc.gc.ca/)

**Symptoms**

Nausea, vomiting, diarrhea (early), constipation (late), fatigue, weakness and dizziness, blurred or double vision, dry mouth, difficulty speaking and swallowing; descending paralysis of the arms, legs, trunk, and breathing muscles (starts in the arms and moves down)

If not diagnosed and treated, botulism can cause death from respiratory failure within three to ten days.

**Incubation**

Twelve to seventy-two hours after eating contaminated food (in infants, three to thirty days)

**Duration**

Between one week to one year for recovery

**Prevention**

Foods contaminated with *C. botulinum* toxin might look, smell, and taste normal. *C. botulinum* spores are not necessarily destroyed by cooking, so preventing the toxin from forming is key.

- When canning or bottling low-acid foods at home, use up-to-date recipes and equipment, and follow all instructions carefully.
- Foods stored in oil should be keep them in the refrigerator and used within ten days.
- Do not give honey (even pasteurized honey) to children under one year old.
- Never eat food from cans that are dented, bulging, or leaking.
- Aluminum-foil-wrapped potatoes or other vegetables should be eaten after baking or refrigerated immediately.
- Keep all low-acid juices (such as carrot juice) and other products labelled "keep refrigerated" in the fridge. (www.phac-aspc.gc.ca/)

**Outbreaks**

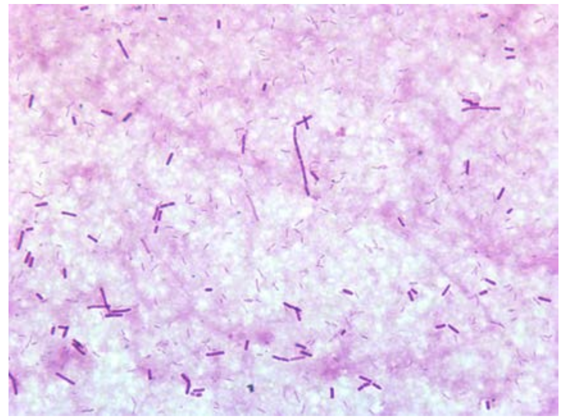
- The largest botulism outbreak in the U.S. in nearly 40 years occurred in 2015. The cause of the outbreak was potato salad prepared with improperly home-canned potatoes. The salad was served during a community potluck meal. Twenty-nine people became ill and one person died. (www.cdc.gov)
- There were three cases of botulism in BC in 2018. (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

# *Clostridium perfringens*

Description

*Clostridium perfringens* (*C. perfringens*) is a bacterium commonly found in the intestines of humans and animals, and in the environment in soil, sewage, or dust. It is anaerobic spore-former, like *C. botulinum*.

A person can become infected with *C. perfringens* by eating contaminated food that was temperature abused. Once in the intestines, the bacteria produce toxins that cause human illness. (www.phac-aspc.gc.ca/)



*Clostridium perfringens* bacteria

**Sources**

Food becomes contaminated with *C. perfringens* from the environment, as these bacteria can be found in soil, sewage, or dust. Contaminated food typically must have large numbers of bacteria present to cause illness.

At temperatures above 10°C and up to 60°C, the bacteria grow and multiply. Therefore, low-oxygen foods that are improperly stored, cooled, or reheated are more likely to cause illness (e.g., soups, stews, marinades). When bacteria reach the intestines, they produce toxins that cause a person to become sick. (www.phac-aspc.gc.ca/)

**Symptoms**

Diarrhea, abdominal cramps, nausea, loss of appetite, weight loss, muscle aches, fatigue

*Clostridium perfringens* does not generally cause vomiting or fever.

**Incubation**

Eight to sixteen hours after eating contaminated food

**Duration**

One day or less

**Prevention**

Foods contaminated with *Clostridium perfringens* may look and smell normal. To reduce the risk of illness, follow these safety tips:

- Thoroughly cook and reheat all food to temperatures of 74°C or higher.
- Keep cold foods below 4°C and hot foods at or above 60°C.
- Refrigerate food after cooking and cool within six hours to below 4°C.
- Divide leftovers into smaller amounts without covering to allow for faster cooling in the refrigerator.
- Freeze or consume leftovers within four days of cooking. (www.phac-aspc.gc.ca/)

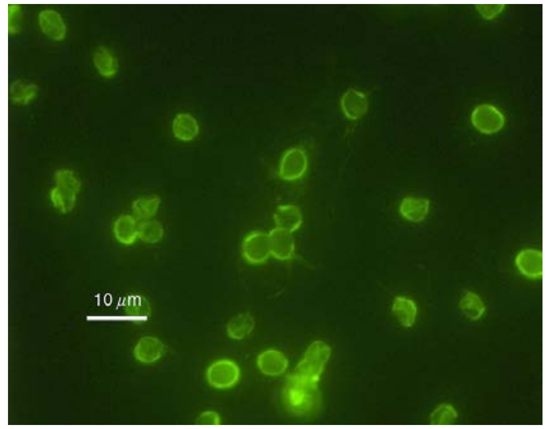
**Outbreaks**

- *Clostridium perfringens* outbreaks are common in Canada. Outbreaks occur most frequently in household settings and have occurred at community events and institutions, including hospitals, cafeterias, catering firms, and long-term-care facilities.
- In 2012, a *Clostridium perfringens* outbreak at a community church supper on Prince Edward Island was caused by improperly reheated roast beef. The reheating temperature was not sufficient to destroy toxin-producing bacteria in the large volume of meat on the trays. 209 people became ill. (pubs.ciphi.ca)

# *Cryptosporidium*

**Description**

*Cryptosporidium parvum* is a single-celled parasite (protozoan) that has caused some significant water-borne outbreaks of gastroenteritis. Infection by *Cryptosporidium parvum* is not a serious health threat for people with a healthy immune system. Supportive rehydration and electrolyte therapy are used in severe cases of diarrhea. Nitazoxanide is approved for use in children with severe infection but this medication has not been effective in immunocompromised patients. Rifamycins, mixed drug therapies, and antiretroviral therapy in cases with HIV have been helpful. Even with treatment cryptosporidiosis can cause prolonged illness in people who are immunocompromised. [cdc.gov and www.ncbi.nlm.nih.gov/pmc/articles/PMC4640180/]




*Cryptosporidium* oocysts

**Sources**

Cryptosporidiosis is commonly a water-borne disease. *Cryptosporidium parvum* persists for long periods in cold water in the environment, as the protozoa can form hardy spores (called oocysts) that are able to survive when conditions are not optimal for growth. Outbreaks of cryptosporidiosis are usually due to contamination of a water system (www.bccdc.ca).

The most common ways of getting cryptosporidiosis are by:

- Consuming contaminated food or drinking water
- Contact with the feces of infected humans (e.g. changing diapers) that is not followed by proper handwashing
- Contact with the feces of domestic or wild animals, including pets and farm animals, which is not followed by proper handwashing

**Symptoms**

Frequent, watery diarrhea; stomach cramps, nausea, vomiting, mild fever and dehydration

**Incubation**

An average of seven days (range two to ten days) after exposure to the parasite

**Duration**

One to two weeks in people who are otherwise healthy

**Prevention**

- If your local drinking water provider has issued a boil-water notice for your community water system, take the advice seriously.
- Do not drink untreated surface water from a spring, stream, river, lake, pond, or shallow well. Assume it is contaminated with animal feces. Boil or filter water from these sources.
- Do not drink unpasteurized milk or juices.
- Wash your hands before eating, before handling food, after using the toilet or changing a diaper, and after touching animals.
- *Cryptosporidium parvum* is resistant to chlorine. Treating water with chlorine will NOT remove the parasite. (www.bccdc.ca)

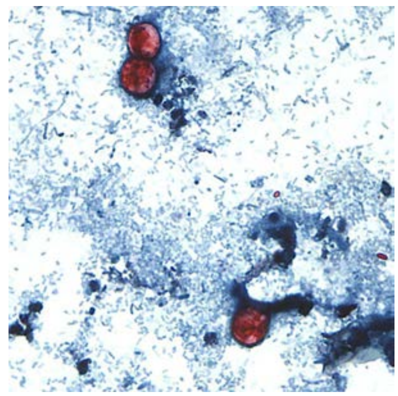
**Outbreaks**

- Outbreaks of illness linked to *Cryptosporidium parvum* in drinking water have been reported in several Canadian provinces. Their spread in swimming pools has also been reported. In 2016, 112 cases of *Cryptosporidium* were reported in BC. Cryptosporidiosis illnesses decreased in 2018 to 60 cases (1.2 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

## *Cyclospora cayetanensis*

**Description**

*Cyclospora cayetanensis* is a parasite that causes gastrointestinal illness. Most infections are linked to travel to other countries, but locally acquired infections have increased in recent years. Outbreaks of locally acquired disease tend to occur in the late spring to early summer and have been linked to the consumption of contaminated, imported fresh produce, especially leafy green vegetables, fresh herbs, raspberries, and blackberries. *(*www.bccdc.ca)



*Cyclospora cayetanensis* oocyts

**Sources**

*Cyclospora* are spread through consumption of food or water contaminated by human or animal feces. The parasite is not spread person to person. It is not infectious when passed in the stools of an infected person. Instead, it must remain in the environment, outside the host, for several days to become infectious.

Contaminated fruits and vegetables are the main source of *Cyclospora* infections. Produce may become contaminated when it is watered with or washed in water containing the parasite, or when handled by infected workers. *(*www.bccdc.ca*)*

**Symptoms**

Frequent, watery, often explosive diarrhea; abdominal cramps, nausea, vomiting, loss of appetite, weight loss, fatigue, fever (occasionally)

**Incubation**

Approximately one week after exposure to the parasite

**Duration**

From a few days to several weeks

**Prevention**

- Wash fruits and vegetables as thoroughly as possible before eating them. However, note that washing produce will not completely remove the possibility of infection. When travelling to developing countries, avoid any fruits and vegetables that cannot be peeled or cooked.
- Do not drink untreated surface water from a spring, stream, river, lake, pond, or shallow well. When travelling to a developing country, make sure to drink only bottled water from a reputable supplier. (www.bccdc.ca)

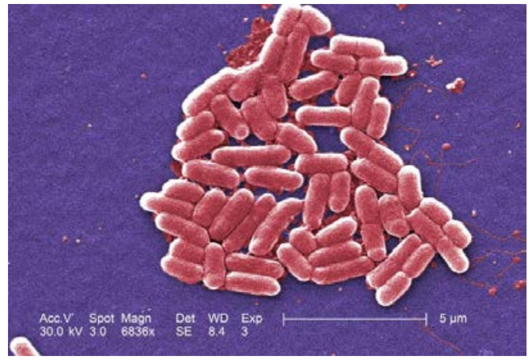
**Outbreaks**

- In 2015, a total of ninety-seven cases of *Cyclospora* infection were reported in British Columbia, Alberta, Ontario, and Quebec. Two people were hospitalized. No deaths were reported. The source of the outbreak was not identified. (www.phac-aspc.gc.ca/)
- In 2018 there were 41 cases of cyclosporiasis in BC (0.8 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

## *Shigatoxigenic Escherichia coli*

**Description**

*Escherichia coli*, commonly referred to as *E. coli*, are bacteria commonly found in the intestines of humans and animals. Most strains of *E. coli* are harmless; however, some strains, such as *E. coli* O157:H7 can make people sick by releasing toxins. These *E. coli* strains are described as shigatoxigenic and toxins released by these bacteria can cause severe stomach cramps, diarrhea, and vomiting. Serious complications of an *E. coli* O157:H7 infection can include kidney failure. (www.phac-aspc.gc.ca/)




*E. coli* bacteria

**Sources**

Animals and people can be carriers of *E. coli* without showing signs of illness. They can then spread the bacteria to foods, surfaces, or other people.

Food can become contaminated with *E. coli* during:

- Butchering, if feces on the carcass gets into the meat
- Handling of food by an *E. coli* infected person
- Improper handling of raw food, leading to cross-contamination of other foods

Two of the most common ways to come into contact with *E. coli* are by improperly handling raw ground meat and by eating ground meat that is undercooked.

Exposure to *E. coli* can occur by eating or drinking:

- Raw and undercooked meat, especially ground or needle tenderized beef
- Contaminated raw fruits and vegetables, including sprouts
- Untreated water
- Unpasteurized (raw) milk and (raw) milk products, including raw milk cheese
- Unpasteurized apple juice or cider

*E. coli* infection can also occur through contact with the feces of infected people, or the feces of cattle or other farm animals (including at petting zoos and fairs). (www.healthycanadians.gc.ca)

**Symptoms**

Severe stomach cramps, diarrhea (often watery and may become bloody), vomiting, slight fever

Five to ten percent of those who get sick from *E. coli* O157:H7 overall and about fifteen percent of young children and the elderly develop hemolytic uremic syndrome (HUS), which can be fatal. Symptoms of HUS vary. Some people have seizures or strokes, and some need blood transfusions and kidney dialysis. Others live with long-term side effects, such as permanent kidney damage. (http://www.phac-aspc.gc.ca/)

**Incubation**

Usually three to four days after ingestion, but may occur from one to ten days after eating contaminated food

**Duration**

Five to ten days

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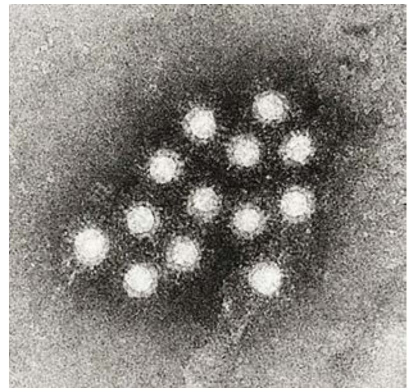
| **Prevention**<br>• Wash hands thoroughly with soap and warm water before and after handling all types of food products.<br>• Cook food to a safe internal temperature that has been checked using a digital thermometer.<br>• Keep raw food away from other foods while shopping, storing, repackaging, cooking, and serving foods.<br>• Use warm, soapy water to clean knives, cutting boards, utensils, your hands, and any surfaces that have come in contact with food, especially meat, poultry, and fish.<br>• Wash your hands before eating; before handling food; immediately after handling raw poultry or meat and before touching anything else; after using the toilet or changing diapers, and after touching animals. |
| **Outbreaks**<br>• In a 2006 outbreak in the U.S. 199 people were infected with *E. coli* from contaminated, uncooked spinach. 102 people were hospitalized; thirty-one suffered kidney failure, and three people died. (www.cdc.gov)<br>• An outbreak of twenty-nine cases of *E. coli* involving four Canadian provinces was reported in 2015. Individuals became sick between July and September. The majority of cases were male, with an average age of twenty-three years. Seven people were hospitalized. The source of the outbreak was not identified. (www.phac-aspc.gc.ca)<br>• In 2018 there were 193 cases of shigatoxigenic *E. coli* in BC (3.9 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard) |

## Hepatitis A

**Description**
Hepatitis is a general term referring to inflammation of the liver. Although there are many forms of hepatitis, it is most commonly caused by the viruses *Hepatitis A, B, C, D,* and/or *E*. (www.bccdc.ca)



Hepatitis virus particles

**Sources**
The hepatitis A virus (HAV) is found in the bowel movements of an infected person. Even if a person does not feel ill, they are still able to spread HAV to others. HAV infection can occur from:

- Eating food that has been touched by contaminated hands
- Using contaminated illicit drugs
- Eating raw or undercooked shellfish, such as crabs, clams, oysters, or mussels, that have been exposed to contaminated sewage
- Eating contaminated fruits or vegetables
- Drinking contaminated water or ice

In developing countries where HAV infection is common and there are poor sanitary conditions or poor personal hygiene, travellers are more likely to come into contact with contaminated food and water. (www.bccdc.ca)

**Symptoms**
Fever, jaundice, joint pain, nausea, vomiting, loss of appetite, stomach pain, dark urine, clay-coloured stool, soreness in the upper-right stomach area, fatigue

Symptoms can be so mild that people may not be aware they have been infected with HAV.

**Incubation**
Fifteen to fifty days

**Duration**
One to two weeks to several months

**Prevention**

- Always wash your hands with soap and water after using the bathroom, before preparing meals, and before eating.
- Get vaccinated, particularly if travelling to areas of high Hepatitis A virus activity.
- When travelling abroad avoid peeled fruit and raw vegetables, salads, dairy products with unpasteurized milk, and raw or undercooked meat, fish, and shellfish, and any food sold by street vendors.
- Swim only in chlorinated pools.
- Do not share food, drinks, or cigarettes. (www.bccdc.ca)

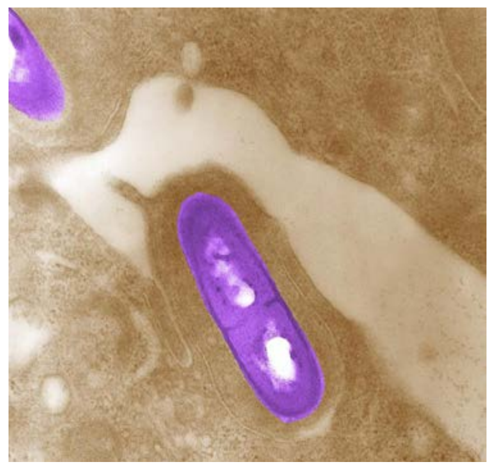
**Outbreaks**

- In 2013, a U.S. nationwide outbreak of HAV linked to frozen berries sickened 118 people in eight states. Forty-seven people were hospitalized. (www.cdc.gov)
- In 2003, an outbreak of Hepatitis A was traced to a restaurant in Pennsylvania. The outbreak was associated with the consumption of raw or undercooked green onions. More than 650 cases of HAV infection were confirmed. The victims included at least thirteen employees of the restaurant, and numerous residents of six other states. Four people died. More than 9 000 people received immune globulin shots as protection against the Hepatitis A virus. (www.about-hepatitis.com/)
- In 2018 there were 25 cases of HAV infection in BC (0.5 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

## *Listeria monocytogenes*

**Description**

*Listeria monocytogenes* (commonly called *Listeria*) is a bacterial species found in food and the environment. *Listeria* can cause a rare, but serious disease, called listeriosis. Pregnant women, the elderly, and individuals with weakened immune systems are at higher risk than others. In serious cases, listeriosis can lead to brain infection and death. (www.phac-aspc.gc.ca/)



*Listeria monocytogenes* bacteria

**Sources**

*Listeria* is found in soil, vegetation, water, sewage, some types of livestock feed, and in the feces of humans and animals. Animals and humans can carry the bacteria without knowing it. Plants and vegetables can become contaminated with *Listeria* from soil, water, and manure-based fertilizers.

Unlike most bacteria, *Listeria* can survive and sometimes grow on foods stored in the refrigerator. *Listeria*-contaminated foods look, smell, and taste normal.

Infection with the bacteria can occur through eating or drinking contaminated food or beverages. Listeriosis can also result from cross-contamination during food preparation in the kitchen or commercial plant where the food was processed.

About five percent of healthy adults are carriers of *Listeria* and have no symptoms. (www.phac-aspc.gc.ca/)

**Symptoms**

Vomiting, nausea, cramps, muscle aches, diarrhea, severe headache, constipation, persistent fever

When the infection spreads to the nervous system, symptoms include headache, stiff neck, confusion, and loss of balance.

**Incubation**

The mild form of listeriosis usually begins about three days after eating heavily contaminated food. There is a more serious form that has a generally much longer incubation period—up to seventy days after exposure.

**Duration**

Varies depending on severity of illness

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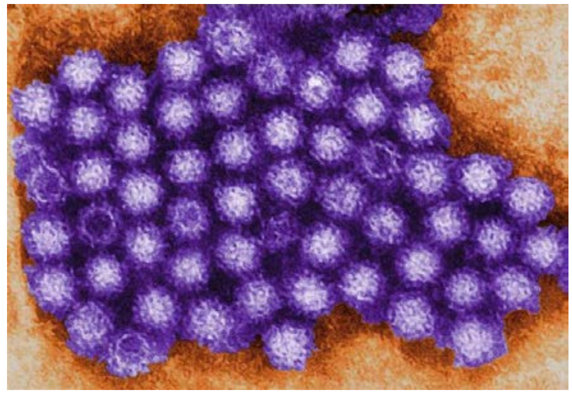
| **Prevention**<br>• After handling foods, especially raw foods such as meat and fish, thoroughly clean and sanitize all surfaces used for food preparation with a kitchen sanitizer (following the directions on the container) or a bleach solution (add 5 mL household bleach to 750 mL of water), and then rinse with water.<br>• To avoid cross-contamination, clean all knives, cutting boards, and utensils used for raw food before using them again.<br>• Thoroughly clean fruits and vegetables before eating them.<br>• Some foods are more likely to carry *Listeria*, including raw or contaminated milk, soft cheeses, and ready-to-eat meats, such as hot dogs, pâté, smoked fish, and deli meats. Individuals at high risk, such as pregnant women, the elderly, and those with weakened immune systems, should avoid these foods to reduce their risk of developing listeriosis. (www.phac-aspc.gc.ca/) |
| **Outbreaks**<br>• In 2008, a listeriosis outbreak in Canada caused fifty-six illnesses in seven provinces; twenty-one people died. The source of the outbreak was two production lines at a Maple Leaf Foods packaging plant in Toronto.<br>• An outbreak of *Listeria monocytogenes* between May 2015 and February 2016 caused fourteen infections in five Canadian provinces. All cases were hospitalized. The outbreak was linked to packaged salad products produced from a U.S. processing facility. (www.phac-aspc.gc.ca)<br>• In recent years, an average of 132 cases of listeriosis were reported annually in Canada. (www.phac-aspc.gc.ca/). In 2018 there were 9 cases of listeriosis in BC (0.2 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard) |

# Norovirus

**Description**

*Norovirus* are a genus of viruses in the *Calicivirus* family. Noroviruses cause gastroenteritis (inflammation of the stomach and small intestine) commonly called "winter vomiting disease" or, incorrectly, "stomach flu." Norovirus infection is a common illness and should not be confused with influenza (the "flu"). These viruses cause 1 000 000 cases of foodborne illness per year in Canada. Outbreaks are more noticeable in closed communities such as long-term and acute care facilities than in the general community. (www.bccdc.ca)



Norovirus particles

**Sources**

- The main source of norovirus infection is stool and vomit from infected people passed through the fecal-oral route, including spread from person to person on unwashed hands.
- The viruses can be spread by food, water, or ice that has been handled by a sick person.
- Vomiting may spread the viruses in droplets.
- The viruses can survive on surfaces, such as countertops or sink taps, for a long time.
- Norovirus can also contaminate shellfish, water, and has been linked to outbreaks of frozen berries. (www.bccdc.ca)

**Symptoms**

Sudden onset of nausea, cramping, chills, fever, vomiting and/or diarrhea (usually)

**Incubation**

Twelve to forty-eight hours after infection

**Duration**

Twenty-four to seventy-two hours

**Prevention**

There is neither a vaccine for Norovirus, nor a treatment that can cure the infection. People can get infected with norovirus multiple times, as there are many different strains and any immunity will be only to the strain(s) a person has been infected with previously.

- Handwashing is critical to reducing person-to-person spread of norovirus. Handwashing should be under warm running water, using soap. Hands should be rubbed together for thirty seconds, and care taken to clean the fingertips and under the nails, using a nail brush if necessary.
- Ill people should avoid going to work for at least forty-eight hours after symptoms have ended, especially food handlers or caregivers. Food for other people should never be prepared by people who are ill.
- Carefully wash fruits and vegetables with clean water.
- Avoid eating raw shellfish, including oysters. Cook these foods thoroughly before eating. (www.bccdc.ca)

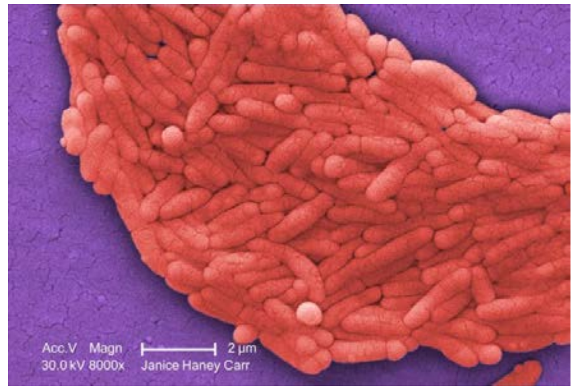
**Outbreaks**

- The largest norovirus outbreak in Canadian history was linked to raw oyster consumption that occurred in 2016–2017, from B.C.-sourced oysters. Over 400 illnesses occurred across Canada, with more occurring in the U.S. Human-source fecal contamination of the oyster shellfish farms and specific environmental conditions led to the oysters becoming contaminated and the closure of twelve shellfish farms. (www.bccdc.ca )
- Hundreds of outbreaks of norovirus are reported to the National Enteric Surveillance Program at the Public Health Agency of Canada (PHAC) each year. Only the common cold occurs more often. Outbreaks occur more frequently during the fall and winter months. Many outbreaks go unreported. (http://www.phac-aspc.gc.ca/)

# *Salmonella* spp.

**Description**

*Salmonella* spp. are rod-shaped, Gram-negative bacteria found naturally in the intestines of animals, reptiles, and birds. These bacteria are usually transmitted to people when they eat foods or beverages contaminated with animal feces. Contaminated foods can come from animal sources, such as poultry, beef, milk, or eggs, and from fruits and vegetables. Salmonellosis is the second most frequently reported food-related illness in Canada. (www.phac-aspc.gc.ca)




*Salmonella* bacteria

**Sources**

*Salmonella* spp. can occur in or on:

- Raw or undercooked meat (especially poultry)
- Raw or undercooked eggs
- Homemade salad dressings, hollandaise sauce, mayonnaise, ice cream, cookie dough, tiramisu, and frostings
- Raw fruits and vegetables (especially sprouts and cantaloupes) and their juices
- Unpasteurized dairy products, such as raw milk and raw milk cheeses
- Raw or undercooked fish and shrimp
- Pet food and treats

Infection by *Salmonella* spp. can also result from contact with infected animals, reptiles, amphibians, rodents, birds, livestock, dogs, and cats. These may carry the bacteria even when they are healthy.

**Symptoms**

Diarrhea, fever, chills, headache, nausea, stomach cramps, vomiting

Symptoms can develop into serious illness with long-lasting effects. Symptoms can be more severe in pregnant women, people with weakened immune systems, young children, and the elderly.

**Incubation**

Twelve to seventy-two hours after eating contaminated food

**Duration**

Four to seven days

**Prevention**

Foods contaminated with *Salmonella* spp. look, smell, and taste normal. To prevent illness, follow these safety tips:

- Wash hands thoroughly with soap and warm water before and after handling all types of food products.
- Cook food to a safe internal temperature that has been checked using a digital thermometer.
- Fully cook eggs and egg-based foods to ensure they are safe to eat.
- Keep raw food away from other food while shopping, storing, repackaging, cooking, and serving foods.
- Use warm soapy water to clean knives, cutting boards, utensils, your hands, and any surfaces that have come in contact with food, especially meat, poultry, and fish.
- Do not cook food for other people when infected with *Salmonella* spp.

**Outbreaks**

- In 2015 in the U.S., imported cucumbers contaminated with *Salmonella* spp. caused 907 people to become ill; 204 were hospitalized and four died. (www.cdc.gov)
- The Public Health Agency of Canada collaborated with federal and provincial public health partners to investigate an outbreak of *Salmonella infantis* in nine provinces. Individuals became sick between March 2015 and January 2016. Twenty-one people were hospitalized. No deaths were reported. Investigations conducted by local, provincial, and federal officials indicated that exposure to fresh, raw chicken was the likely source of the outbreak. (www.phac-aspc.gc.ca)
- The Government of Canada estimates that approximately 87,000 illnesses, 925 hospitalizations, and seventeen deaths occur due to non-typhoidal *Salmonella* spp. annually. (www.healthycanadians.gc.ca)
- The World Health Organization estimates that tens of millions of cases of non-typhoidal salmonellosis occur worldwide every year, and the disease results in more than 100 000 deaths. (www.who.int/mediacentre/factsheets/en/)
- In 2018, there were 979 cases of non-typhoidal salmonellosis in BC (19.6 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

# *Shigella* spp.

**Description**

*Shigella* spp. are Gram-negative rod-shaped pathogenic bacteria. The bacteria are passed person to person via food as a result of poor hygiene, especially poor handwashing. Only humans (and other primates) carry these bacteria and is thought to be one of the oldest human-specific pathogens.

https://www.sciencedirect.com/topics/immunology-and-microbiology/shigella

*Shigella* bacteria

**Sources**

Infections of *Shigella* spp. result from the bacteria passing from stools or soiled fingers of one person to the mouth of another person, when sanitation and hygiene are poor.

Transmission may occur after being exposed to:

- Infected stool or to objects contaminated with stool, or
- Water or food such as salads, milk and other dairy products, raw oysters, ground beef, and poultry. (www.bccdc.ca/)

**Symptoms**

Diarrhea, sometimes with blood and/or mucus; fever, nausea, cramps, vomiting; toxins (or "poisons") in the blood (toxemia); an urge to void, with no passage of stool or urine (called "tenesmus")

**Incubation**

One to two days after eating contaminated food

**Duration**

Five to seven days

**Prevention**

- Carefully wash hands with soap before eating and after using the washroom or changing a diaper.
- Avoid swallowing water from ponds, lakes, or untreated swimming pools.
- When travelling internationally, follow food and water precautions strictly and wash hands with soap frequently.

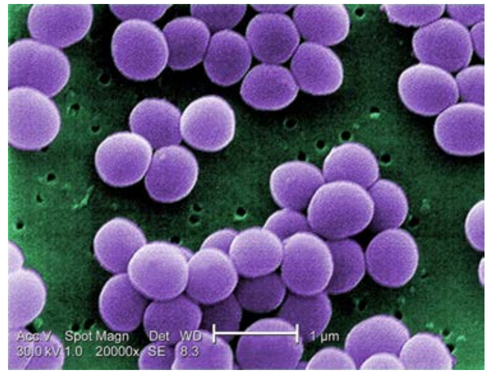
**Outbreaks**

- In 2015, highly contagious *Shigella* bacteria caused an outbreak in the San Francisco area that sickened nearly 200 people. Many of those affected were hospitalized, with several people being admitted to intensive care. The source of the outbreak was a seafood restaurant. (https://www.reuters.com/article/us-sanfrancisco-outbreak-idUSKCN0SK2KW20151026)
- In 2018 there were 122 cases of shigellosis infections in BC (2.4 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

# *Staphylococcus aureus*

**Description**

*Staphylococcus aureus* (*S. aureus*) are Gram-positive, catalase-positive cocci belonging to the *Staphylococcaceae* family. These bacteria produce a toxin (enterotoxin) that causes acute food poisoning and are among the most common causes of gastroenteritis worldwide. Consuming foodx contaminated with enterotoxin produced by these bacteria result in illness. (www.bccdc.ca/)




*Staphylococcus aureus* bacteria

**Sources**

Illness occurs following ingestion of a food contaminated with *S. aureus* or its toxins. Preformed toxins in foods occur when foods are stored for prolonged periods at incorrect temperatures (intoxication), otherwise there must be enough time to allow growth of this pathogen and the production of enterotoxin in the human gut (infection).

Staphylococcal bacteria contaminate ready-to-eat food via cross-contamination from:

- Food handlers who carry *Staphylococcus aureus*, or
- Raw food

Approximately 20% of the population carry *S. aureus* in their nasal cavity all the time, 60% of the population carry it intermittently, and 20% never carry it.

(www.bccdc.ca/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC172932/)

**Symptoms**

Vomiting, nausea; diarrhea, usually watery, but sometimes with blood; cramps; may include mild fever, weakness, dizziness, and chills

**Incubation**

One to ten hours after eating contaminated food

**Duration**

One to two days

**Prevention**

Control measures should be applied to avoid contaminating food with *S. aureus* and also to prevent growth and the formation of enterotoxin in the food.

To avoid contaminating food with *S. aureus*, follow these safety tips to handle and prepare food safely:

- Ensure raw foods of animal origin are obtained following good hygienic practices.
- Ensure food handlers use appropriate protective clothing (e.g. gloves) and thoroughly wash hands.
- Ensure food handlers with skin lesions have them properly covered prior to handling food. If this is not possible, they should not work while handling food until the lesions have healed.
- Avoid cross-contamination by keeping work surfaces clean and ensuring separation between areas where raw and cooked foods are handled.

To prevent growth of *S. aureus* and the formation of enterotoxin, follow these practices:

- Ensure food is maintained either above 60°C or below 4°C.
- Cool cooked foods that will not be immediately consumed to below 4°C within six hours.
- When reheating food, ensure that the temperature reaches at least 74°C. (www.bccdc.ca/)

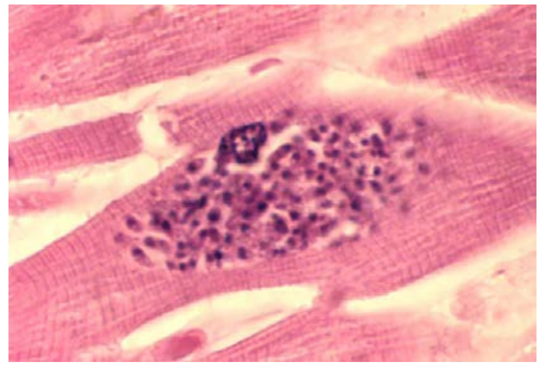
**Outbreaks**

- In 2012, an outbreak of staphylococcal illness sickened twenty-two people at a lunch work-party at a military base in the U.S. The cause of the outbreak was determined to have been poor food-handling practices, and improper reheating and inadequate refrigeration of foods (www.cdc.gov).

## *Toxoplasma gondii*

**Description**

*Toxoplasma gondii* is a parasitic protozoan that causes the disease toxoplasmosis. The parasite infects wild and domestic animals, most commonly cats and other animals such as sheep, goats, cattle, pigs, poultry and other birds. Toxoplasmosis is common disease but seldom recognized, because most infected people do not become sick. It can cause significant illness for the fetus during pregnancy such as mental disability and blindness. Estimates suggest that one-third of Canadians have been infected. (www.ccohs.ca/)



*Toxoplasma* parasites in tissue

**Sources**

All animals and birds can be infected with the *Toxoplasma gondii*. The parasite enters the muscles of a bird or animal when it eats raw meat or drinks the milk of an infected animal. Cats can also spread the parasite in their feces. Humans can become infected with Toxoplasma gondii when changing a cat litter box or working in an area contaminated with cat feces.

Common ways for people to get toxoplasmosis include:

- Eating raw or undercooked meats,
- Drinking unpasteurized milk,
- Cleaning cat litter boxes,
- Working in gardens or playing in sandboxes that contain cat feces, and
- Drinking water contaminated with *Toxoplasma gondii*. (www.bccdc.ca)

**Symptoms**

None in most infected people; fever, sore throat, sore muscles, fatigue; swollen glands in the neck, armpits, or groin that are usually not sore; temporary blurred vision or loss of vision in some cases

**Incubation**

Five to twenty-three days

**Duration**

Two to four weeks

**Prevention**

- Order or cook your meat well-done. Do not eat raw or undercooked meat.
- Do not eat raw eggs. Do not drink unpasteurized milk from any animal.
- Wash your hands, utensils, and cutting boards after handling raw meat to prevent contamination of other foods.
- Avoid cleaning cat litter boxes if you are pregnant or trying to become pregnant. Wear gloves when cleaning the cat litter box, and then wash your hands.
- Place a secure lid on your sandbox to prevent cats from using it as a litter box.
- Wear gloves when gardening, and then wash your hands. (www.bccdc.ca)

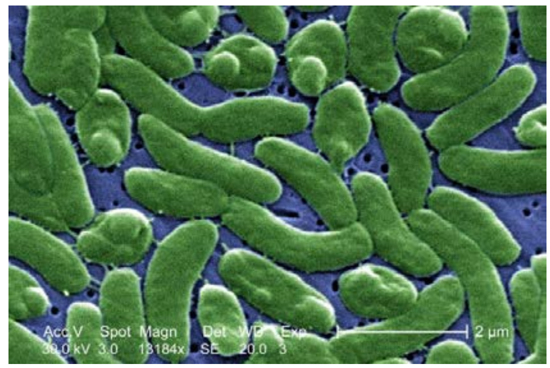
**Outbreaks**

- In 1995, more than one hundred people were confirmed to have been infected with *Toxoplasma gondii* in Greater Victoria, B.C. It was estimated that more than 3 000 people had been exposed to the pathogen. The source of the outbreak was determined to have been the local water supply.
- There were no cases of toxoplasmosis in BC in 2018 and two cases reported in 2017. (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

# *Vibrio* spp.

**Description**

*Vibrio* spp. are toxin-producing bacteria found naturally in water, fish, and shellfish. They cause infection when foods or beverages are contaminated by the bacteria, and from exposure to contaminated water through contact. Several *Vibrio spp*. can cause illness, including salt-loving species that mainly live in marine environments, such as *V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus*. Some freshwater species, such as *V. cholerae*, also can cause illness. (www.healthycanadians.gc.ca/)




*Vibrio* bacteria

**Sources**

*V. parahaemolyticus* grows naturally in coastal waters and can grow to large numbers when the ocean is warm. Bivalve molluscs (i.e. oysters, clams, cockles, scallops, and mussels) are filter feeders that eat by straining plankton and bacteria from seawater. These shellfish species can have high numbers of *V. parahaemolyticus* either when they are harvested or if they are not kept cold after harvesting. Illness can occur when shellfish with high numbers of *V. parahaemolyticus* is eaten undercooked or raw. The illness is not spread from person to person. (www.bccdc.ca)

**Symptoms**

Diarrhea, abdominal cramps, nausea, vomiting fever, headache, bloody stools

**Incubation**

Four to ninety-six hours after eating contaminated food

**Duration**

Two to seven days

**Prevention**

- Drink water from a safe (treated or boiled) water supply.
- Buy shellfish from reputable suppliers. Cook shellfish thoroughly before eating, especially oysters. Do not eat raw shellfish.
- Always keep raw and cooked shellfish separate.
- Avoid taking antacids prior to eating oysters or other seafood, as reduced stomach acid may favour the survival and growth of *Vibrio* spp.
- Avoid exposing open wounds or broken skin to warm salt or brackish water, or to raw shellfish. Wear protective clothing (such as gloves) when handling raw shellfish.
- When travelling to developing countries, drink water from a safe (treated or boiled) source. Eat only cooked hot food. Eat only fruit that can be peeled.
- Always wash hands for thirty seconds with soap after using the bathroom.
- Wash hands well with soap before handling any food. Be sure to wash hands, cutting boards, counters, knives, and other utensils after preparing raw foods. (www.healthycanadians.gc.ca/)

**Outbreaks**

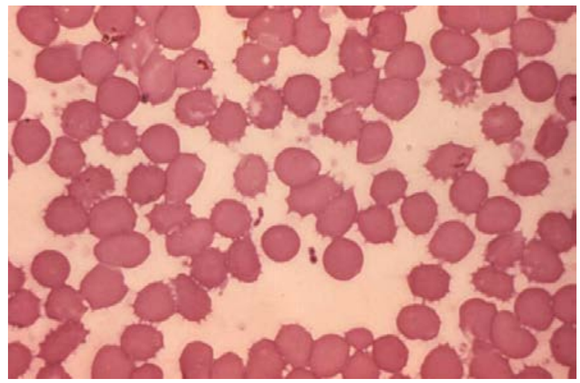
- In a 2015 outbreak of *Vibrio parahaemolyticus*, a total of eighty-two cases were reported in British Columbia, Alberta, Saskatchewan, and Ontario. One person was hospitalized. No deaths were reported. Individuals who became sick all reported consumption of raw or undercooked shellfish, primarily oysters. (www.phac-aspc.gc.ca)
- In 2018 there were 64 cases of *Vibrio* infection reported in BC (1.3 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard)

## *Yersinia enterocolitica*

**Description**

*Yersinia enterocolitica* is a pleomorphic, Gram-negative bacillus that belongs to the family *Enterobacteriaceae*.

In several countries, *Y enterocolitica* has eclipsed Shigella species and approaches Salmonella and Campylobacter species as the predominant cause of acute bacterial gastroenteritis. (emedicine.medscape.com/)



*Yersinia* bacteria

**Sources**

*Yersinia* are spread via the fecal-oral route. Fecal material from infected humans or animals can get into the mouth by:

- Consumption of contaminated food or drink
- Contact with the feces of infected humans that is not followed by proper handwashing
- Contact with the feces of domestic or wild animals that is not followed by proper handwashing

Common sources of infection include:

- Undercooked pork and other meats
- Unpasteurized (raw) milk
- Other contaminated foods
- Water (www.bccdc.ca)

**Symptoms**

Abdominal pain, diarrhea, fever, bloody diarrhea

**Incubation**

One to two days after eating contaminated food

**Duration**

One to two weeks

| |
|---|
| **Prevention**<br>Be aware of the risks associated with the food products you buy and know how to prepare your food safely. Pork is most commonly associated with yersiniosis, but treat all raw meat as if it is contaminated, handling it accordingly. Handle and prepare food safely, including the following:<br>• Wash hands thoroughly with soap and warm water before and after handling all types of food products.<br>• Keep raw food away from other food while shopping, storing, repackaging, cooking, and serving foods.<br>• Thaw meat in the refrigerator, microwave, or under cold running water, not at room temperature. Refrigerate foods promptly.<br>• Do not eat raw or undercooked meats.<br>• Avoid direct contact between raw meats and other uncooked foods.<br>• Wash and sanitize items that do not go into the dishwasher.<br>• If infected, do not cook food for other people. (www.bccdc.ca) |
| **Outbreaks**<br>• From 1999 through 2008, 770 infections per year, on average, were reported to the B.C. Centre for Disease Control. (www.bccdc.ca)<br>• In 2011 an outbreak of *Yersinia enterocolitica* in Norway was associated with ready-to-eat salad mix. Twenty-one people became ill. (wwwnc.cdc.gov)<br>• In 2018 there were 758 cases of yersiniosis reported in BC (15.2 cases per 100,000 population). (http://www.bccdc.ca/health-professionals/data-reports/reportable-diseases-data-dashboard) |

# Appendix D: Sample cleaning schedule and sanitation plans

In this supplemental material to lesson E, we will discuss how poor sanitation, poor personal hygiene, pests, or cross-contamination events lead to foodborne illness. Examples along the modern food production chain will illustrate how to limit cross-contamination.

## Cleaning and sanitizing

Every wondered what an actual cleaning schedule might look like?

The following cleaning calendar itemizes tasks on a weekly schedule. Slower days of the week, Monday and Tuesday, are when many tasks are assigned.

| | MON | TUES | WED | THURS | FRI | SAT | SUN |
|---|---|---|---|---|---|---|---|
| Drawers | X | | | | | | |
| Shelves | | | | X | | | X |
| Cooler walls | | | X | | | | |
| Dessert fridge | | | X | | | | |
| Soup warmer | | | | | | | X |
| Concession fridge | X | | | | | | |
| Broiler | | | X | | | | |
| Burners | X | X | | | | | |
| Grease trays | X | X | | | | | |
| Rotate woodgrill | | | | X | | | |
| Hood vents | | X | | | | | |
| Mop bucket | X | | | | | | |
| Floor mats | | | | | | | X |

**Figure 1** — Cleaning schedule for restaurant

The next photo is the sanitation plan for the same restaurant. This is the document that outlines when the activity is performed — either in the morning, afternoon, weekly or monthly. It also states who does it by position: cooks, dishwashers, or janitors. Some of the instructions for how to clean are also given here, or referred to in a separate document. This kind of plan is very valuable for staff who at a glance can easily see what they are responsible to clean, when and how often.

| Item or area | When | Who | Instructions |
|---|---|---|---|
| Countertops | as needed | cooks | clean using hot water and Advance pot & pan degreaser mixed 20:1, rinse. spray sanitizer mixed 7ml bleach to 1L water, leave on 2 minutes, & wipe dry |
| Passbar | daily cleaning | cooks | clean & sanitize as above |
| Drawer fronts | daily cleaning | cooks | clean & sanitize as above |
| Cooler floor | daily cleaning | cooks | sweep, mop with hot bleach water |
| Microwaves | as needed | cooks | clean inside and out & sanitize as above |
| Globe mixers | after each use | cooks | clean & sanitize as above |
| Steamer | daily cleaning | cooks | clean & sanitize as above |
| Printers | daily cleaning | cooks | wipe with clean damp cloth |
| Bain marie | daily cleaning | cooks | clean top, drain & wipe inside well clean |
| Drawers | MON close | cooks | clean & sanitize as above |
| Shelves | SUN & THURS | cooks | clean & sanitize as above |
| Walls | as needed | cooks | clean & sanitize as above |
| Cooler walls | 1st WED | cooks | remove racks, clean walls, doors and ceiling |
| Dessert fridge | WED am shift | cooks | remove all food items, put racks through dishwasher, clean & sanitize all surfaces |
| Soup warmer | Sun. 10 am | dishwasher | unplug, clean & sanitize as above |
| Steam kettle | after use | dishwasher | clean & sanitize as above |
| Bread warmer | daily 10 am | dishwasher | clean & sanitize all exterior surfaces as above |
| Concess fridge | MON 10 am | cooks | remove all trays, clean & sanitize all interior surfaces |
| Broiler | 1st WED | cooks | disassemble and degrease all surfaces |
| Stove burners | MON & TUES am | cooks | burn out and degrease |
| Grease trays | MON & TUES am | cooks | change foil |
| Slicer | after each use | cooks | take apart, put through machine, clean and sanitize as above |
| Cutting boards | MON & FRI 8 am | dishwasher | soak in bleach solution 1 hr. |
| Mop bucket | MON am | dishwasher | degrease |
| Floors | daily | janitor | see tricom |
| Walls | MON pm | janitor | see tricom |
| Stovetops | daily pm | janitor | see tricom |
| Ovens | daily pm | janitor | see tricom |
| Hoods & walls | daily pm | janitor | see tricom |
| Flat top grill | daily pm | janitor | see tricom |
| Ceiling | monthly | janitor | see tricom |

**Figure 2** — Sanitation plan for restaurant

# Appendix E: Journal of food protection abstracts

**Series of article abstracts published in the Journal of Food Protection on food worker outbreaks from 1927 to 2006.**

Greig, J. D., E. C. Todd, C. A. Bartleson and B. S. Michaels (2007). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 1. Description of the problem, methods, and agents involved." *Journal of Food Protection 70*(7): 1752-1761. (Greig, Todd, Bartleson, & Michaels, 2007)

> Food workers in many settings have been responsible for foodborne disease outbreaks for decades, and there is no indication that this is diminishing. The Committee on Control of Foodborne Illnesses of the International Association for Food Protection was tasked with collecting and evaluating any data on worker-associated outbreaks. A total of 816 reports with 80,682 cases were collected from events that occurred from 1927 until the first quarter of 2006. Most of the outbreaks reviewed were from the United States, Canada, Europe, and Australia, with relatively few from other parts of the world, indicating the skewed set of data because of availability in the literature or personal contact. Outbreaks were caused by 14 agents: norovirus or probable norovirus (338), Salmonella enterica (151), hepatitis A virus (84), Staphylococcus aureus (53), *Shigella* spp. (33), Streptococcus Lancefield groups A and G (17), and parasites Cyclospora, Giardia, and Cryptosporidium (23). Streptococcal, staphylococcal, and typhoid outbreaks seem to be diminishing over time; hepatitis A virus remains static, whereas norovirus and maybe nontyphoidal Salmonella are increasing. Multiple foods and multi-ingredient foods were identified most frequently with outbreaks, perhaps because of more frequent hand contact during preparation and serving.

Todd, E. C., J. D. Greig, C. A. Bartleson and B. S. Michaels (2007). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 2. Description of outbreaks by size, severity and settings." *Journal of Food Protection 70*(8): 1975-1993. (Todd, Greig, Bartleson, & Michaels, 2007a)

> This article is the second in a series of several by members of the Committee on the Control of Foodborne Illness of the International Association of Food Protection, and it continues the analysis of 816 outbreaks where food workers were implicated in the spread of foodborne disease. In this article, we discuss case morbidity and mortality and the settings where the 816 outbreaks occurred. Some of the outbreaks were very large; 11 involved more than 1,000 persons, 4 with more than 3,000 ill. The larger outbreaks tended to be extended over several days with a continuing source of infections, such as at festivals, resorts, and community events, or the contaminated product had been shipped to a large number of customers, e.g., icing on cakes or exported raspberries. There were five outbreaks with more than 100 persons hospitalized, with rates ranging from 9.9 to 100%. However, overall, the hospitalization rate was low (1.4%), and deaths were rare (0.11% of the 80,682 cases). Many of the deaths were associated with high-risk persons (i.e., those who had underlying diseases, malnutrition, or both, as in a refugee camp, or young children), but a few occurred with apparently healthy adults. An analysis of the settings for the food worker–related events showed that most of the outbreaks came from food service facilities (376 outbreaks [46.1%]), followed by catered events (126 outbreaks [15.4%]), the home (83 outbreaks [10.2%]), schools and day care centers (49 [6.0%]), and health care institutions (43 outbreaks [5.3%]). However, many cases resulted from relatively few outbreaks ( 30 each) associated with community events (9,726), processing plants (8,580), mobile/temporary service (5,367), and camps/ armed forces (5,117). The single most frequently reported setting was restaurants, with 324 outbreaks and 16,938 cases. Improper hygienic practices in homes, on

picnics, or at community events accounted for 89 of the 816 outbreaks. There were 18 outbreaks associated with commercial travel in air flights, trains, and cruise ships over several decades, although only the last seems to be a major concern today. Sixteen outbreaks occurred where food, primarily produce, was harvested and shipped from one country to another. Sometimes the presence of an infected worker preparing food was only one of several factors contributing to the outbreak.

Todd, E. C., J. D. Greig, C. A. Bartleson and B. S. Michaels (2007). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 3. Factors contributing to outbreaks and description of outbreak categories." *Journal of Food Protection 70*(9): 2199-2217. (Todd, Greig, Bartleson, & Michaels, 2007b)

In this article, the third in a series of several reviewing the role of food workers in 816 foodborne outbreaks, factors contributing to outbreaks and descriptions of different categories of worker involvement are discussed. All the outbreaks had worker involvement of some kind, and the majority of food workers were infected. The most frequently reported factor associated with the involvement of the infected worker was bare hand contact with the food followed by failure to properly wash hands, inadequate cleaning of processing or preparation equipment or utensils, cross-contamination of ready-to-eat foods by contaminated raw ingredients, and (for bacterial pathogens) temperature abuse. Many of the workers were asymptomatic shedders or had infected family members and/or used improper hygienic practices. Outbreaks were sorted into categories based on how many workers were implicated, the origin of the infective agent (outbreak setting or off site), the degree of certainty that the worker(s) were the cause or were victims, whether or not the workers denied illness, the ability of the agent to grow in the food, whether only the workers and not the patrons were ill, and whether patrons were more responsible for their illnesses than were the workers. The most frequent scenarios were (i) a single worker causing an outbreak by directly infecting patrons; (ii) an infected worker fecally contaminating foods that were then temperature abused, leading to an outbreak; and (iii) multiple workers linked to an outbreak but with no clear initiating source. Multi-ingredient foods with limited descriptions were most frequently implicated and usually were served in restaurants or hotels, at schools, and at catered events. Identified contaminated ready-to-eat foods included produce, baked goods, beverages, and meat and poultry items. In some situations, it was not clear whether some of the workers were the cause or the victims of the outbreak. However, in other situations there may have been an underestimation of the role of the worker. For instance, workers sometimes denied infection or illness for a variety of reasons, but subsequent investigation provided evidence of infection.

Todd, E. C., J. D. Greig, C. A. Bartleson and B. S. Michaels (2008). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 4. Infective doses and pathogen carriage." *Journal of Food Protection 71*(11): 2339-2373. (Todd, Greig, Bartleson, & Michaels, 2008a)

In this article, the fourth in a series reviewing the role of food workers in foodborne outbreaks, background information on the presence of enteric pathogens in the community, the numbers of organisms required to initiate an infection, and the length of carriage are presented. Although workers have been implicated in outbreaks, they were not always aware of their infections, either because they were in the prodromic phase before symptoms began or because they were asymptomatic carriers. Pathogens of fecal, nose or throat, and skin origin are most likely to be transmitted by the hands, highlighting the need for effective hand hygiene and other barriers to pathogen contamination, such as no bare hand contact with ready-to-eat food. The pathogens most likely to be transmitted by food workers are norovirus, hepatitis A virus, *Salmonella*, *Shigella*, and Staphylococcus aureus. However, other pathogens have been implicated in worker-associated outbreaks or have the potential to be implicated. In this study, the likelihood of pathogen involvement in foodborne outbreaks where infected workers have been implicated was examined, based on infectious dose, carriage rate in the community, duration of illness, and length of pathogen excretion. Infectious dose estimates are based on volunteer studies (mostly early experiments) or data from outbreaks. Although there is considerable uncertainty associated with these data, some pathogens appear to be able to infect at doses as low as 1 to 100 units, including viruses, parasites, and some bacteria. Lengthy postsymptomatic shedding periods and excretion by asymptomatic individuals of many enteric pathogens is an important issue for the hygienic management of food workers.

Todd, E. C., J. D. Greig, C. A. Bartleson and B. S. Michaels (2008). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 5. Sources of contamination and pathogen excretion from infected persons." *Journal of Food Protection 71*(12): 2582-2595. (Todd, Greig, Bartleson, & Michaels, 2008b)

In this article, the fifth in a series reviewing the role of food workers in foodborne outbreaks, background information on the routes of infection for food workers is considered. Contamination most frequently occurs via the fecal-oral route, when pathogens are present in the feces of ill, convalescent, or otherwise colonized persons. It is difficult for managers of food operations to identify food workers who may be excreting pathogens, even when these workers report their illnesses, because workers can shed pathogens during the prodrome phase of illness or can be long-term excretors or asymptomatic carriers. Some convalescing individuals excreted Salmonella for 102 days. Exclusion policies based on stool testing have been evaluated but currently are not considered effective for reducing the risk of enteric disease. A worker may exhibit obvious signs of illness, such as vomiting, but even if the ill worker immediately leaves the work environment, residual vomitus can contaminate food, contact surfaces, and fellow workers unless the clean-up process is meticulous. Skin infections and nasopharyngeal or oropharyngeal staphylococcal or streptococcal secretions also have been linked frequently to worker-associated outbreaks. Dermatitis, rashes, and painful hand lesions may cause workers to reduce or avoid handwashing. Regardless of the origin of the contamination, pathogens are most likely to be transmitted through the hands touching a variety of surfaces, highlighting the need for effective hand hygiene and the use of barriers throughout the work shift.

Todd, E. C., J. D. Greig, C. A. Bartleson and B. S. Michaels (2008). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment." *Journal of Food Protection 72*(1): 202-219. (Todd, Greig, Bartleson, & Michaels, 2008c)

This article, the sixth in a series reviewing the role of food workers in foodborne outbreaks, describes the source and means of pathogen transfer. The transmission and survival of enteric pathogens in the food processing and preparation environment through human and raw food sources is reviewed, with the main objective of providing information critical to the reduction of illness due to foodborne outbreaks. Pathogens in the food preparation area can originate from infected food workers, raw foods, or other environmental sources. These pathogens can then spread within food preparation or processing facilities through sometimes complex pathways and may infect one or more workers or the consumer of foods processed or prepared by these infected workers. The most frequent means of worker contamination is the fecal-oral route, and study results have indicated that toilet paper may not stop transmission of pathogens to hands. However, contact with raw foods of animal origin, worker aerosols (from sneezes), vomitus, and exposed hand lesions also have been associated with outbreaks. Transfer of pathogens has been documented through contaminated fabrics and carpets, rings, currency, skin surfaces, dust, and aerosols and though person-to-person transmission. Results of experiments on pathogen survival have indicated that transmission depends on the species, the inoculum delivery route, the contact surface type, the duration and temperature of exposure, and the relative humidity. Generally, viruses and encysted parasites are more resistant than enteric bacteria to adverse environmental conditions, but all pathogens can survive long enough for transfer from a contaminated worker to food, food contact surfaces, or fellow workers.

Todd, E. C., B. S. Michaels, J. D. Greig, D. Smith, J. Holah and C. A. Bartleson (2010). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 7. Barriers to reduce contamination of food by workers." *Journal of Food Protection 73*(8): 1552-1565. (Todd, Michaels, Greig, Smith, Holah, et al., 2010)

Contamination of food and individuals by food workers has been identified as an important contributing factor during foodborne illness investigations. Physical and chemical barriers to prevent microbial contamination of food are hurdles that block or reduce the transfer of pathogens to the food surface from the hands of a food worker, from other foods, or from the environment. In food service operations, direct contact of food by hands should be prevented by the use of barriers, especially when gloves are not worn. Although these barriers have been used for decades in food processing and food service operations, their effectiveness is sometimes questioned or their use may be ignored. Physical barriers include properly engineered building walls and doors to minimize the flow of outside particles and pests to food storage and food preparation areas; food shields to prevent aerosol contamination of displayed food by customers and workers; work clothing designated strictly for work (clothing worn outdoors can carry undesirable microorganisms, including pathogens from infected family members, into the work environment); and utensils such as spoons, tongs, and deli papers to prevent direct contact between hands and the food being prepared or served. Money and ready-to-eat foods should be handled as two separate operations, preferably by two workers. Chemical barriers include sanitizing solutions used to remove microorganisms (including pathogens) from objects or materials used during food production and preparation and to launder uniforms, work clothes, and soiled linens. However, laundering as normally practiced may not effectively eliminate viral pathogens.

Todd, E. C., B. S. Michaels, J. D. Greig, D. Smith and C. A. Bartleson (2010). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 8. Gloves as barriers to prevent contamination of food by workers." *Journal of Food Protection 73*(9): 1762-1773. (Todd, Michaels, Greig, Smith, & Bartleson, 2010)

The role played by food workers and other individuals in the contamination of food has been identified as an important contributing factor leading to foodborne outbreaks. To prevent direct bare hand contact with food and food surfaces, many jurisdictions have made glove use compulsory for food production and preparation. When properly used, gloves can substantially reduce opportunities for food contamination. However, gloves have limitations and may become a source of contamination if they are punctured or improperly used. Experiments conducted in clinical and dental settings have revealed pinhole leaks in gloves. Although such loss of glove integrity can lead to contamination of foods and surfaces, in the food industry improper use of gloves is more likely than leakage to lead to food contamination and outbreaks. Wearing jewelry (e.g., rings) and artificial nails is discouraged because these items can puncture gloves and allow accumulation of microbial populations under them. Occlusion of the skin during long-term glove use in food operations creates the warm, moist conditions necessary for microbial proliferation and can increase pathogen transfer onto foods through leaks or exposed skin or during glove removal. The most important issue is that glove use can create a false sense of security, resulting in more high-risk behaviors that can lead to cross-contamination when employees are not adequately trained.

Todd, E. C., B. S. Michaels, D. Smith, J. D. Greig and C. A. Bartleson (2010). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 9. Washing and drying of hands to reduce microbial contamination." *Journal Of Food Protection 73*(10): 1937-1955. (Todd, Michaels, Smith, Greig, & Bartleson, 2010)

> During various daily activities at home and work, hands quickly become contaminated. Some activities increase the risk of finger contamination by pathogens more than others, such as the use of toilet paper to clean up following a diarrheal episode, changing the diaper of a sick infant, blowing a nose, or touching raw food materials. Many foodborne outbreak investigation reports have identified the hands of food workers as the source of pathogens in the implicated food. The most convenient and efficient way of removing pathogens from hands is through handwashing. Important components of Handwashing are potable water for rinsing and soaps to loosen microbes from the skin. Handwashing should occur after any activity that soils hands and certainly before preparing, serving, or eating food. Antimicrobial soaps are marginally more effective than plain soaps, but constant use results in a buildup of the antimicrobial compound on the skin. The time taken to wash hands and the degree of friction generated during lathering are more important than water temperature for removing soil and microorganisms. However, excessive washing and scrubbing can cause skin damage and infections. Drying hands with a towel removes pathogens first by friction during rubbing with the drying material and then by wicking away the moisture into that material. Paper rather than cloth towels should be encouraged, although single-use cloth towels are present in the washrooms of higher class hotels and restaurants. Warm air dryers remove moisture and any surface microorganisms loosened by washing from hands by evaporation while the hands are rubbed together vigorously; however, these dryers take too long for efficient use. The newer dryers with high-speed air blades can achieve dryness in 10 to 15 s without hand rubbing.

Todd, E. C., B. S. Michaels, J. Holah, D. Smith, J. D. Greig and C. A. Bartleson (2010). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 10. Alcohol-based antiseptics for hand disinfection and a comparison of their effectiveness with soaps." *Journal of Food Protection 73*(11): 2128-2140. (Todd, Michaels, Holah, et al., 2010)

> Alcohol compounds are increasingly used as a substitute for Handwashing in health care environments and some public places because these compounds are easy to use and do not require water or hand drying materials. However, the effectiveness of these compounds depends on how much soil (bioburden) is present on the hands. Workers in health care environments and other public places must wash their hands before using antiseptics and/or wearing gloves. However, alcohol-based antiseptics, also called rubs and sanitizers, can be very effective for rapidly destroying some pathogens by the action of the aqueous alcohol solution without the need for water or drying with towels. Alcohol-based compounds seem to be the most effective treatment against gram-negative bacteria on lightly soiled hands, but antimicrobial soaps are as good or better when hands are more heavily contaminated. Instant sanitizers have no residual effect, unlike some antimicrobial soaps that retain antimicrobial activity after the hygienic action has been completed, e.g., after Handwashing. Many alcohol-based hand rubs have antimicrobial agents added to them, but each formulation must be evaluated against the target pathogens in the environment of concern before being considered for use. Wipes also are widely used for quick cleanups of hands, other body parts, and surfaces. These wipes often contain alcohol and/or antimicrobial compounds and are used for personal hygiene where water is limited. However, antiseptics and wipes are not panaceas for every situation and are less effective in the presence of more than a light soil load and against most enteric viruses.

Todd, E. C., J. D. Greig, B. S. Michaels, C. A. Bartleson, D. Smith and J. Holah (2010). "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 11. Use of antiseptics and sanitizers in community settings and issues of hand hygiene compliance in health care and food industries." *Journal Of Food Protection 73* (12): 2306-2320. (Todd, Greig, et al., 2010)

> Handwashing with soap is a practice that has long been recognized as a major barrier to the spread of disease in food production, preparation, and service and in health care settings, including hospitals, child care centers, and elder care facilities. Many of these settings present multiple opportunities for spread of pathogens within at-risk populations, and extra vigilance must be applied. Unfortunately, hand hygiene is not always carried out effectively, and both enteric and respiratory diseases are easily spread in these environments. Where water is limited or frequent hand hygiene is required on a daily basis, such as for many patients in hospitals and astronauts in space travel, instant sanitizers or sanitary wipes are thought to be an effective way of preventing contamination and spread of organisms among coworkers and others. Most concerns regarding compliance are associated with the health care field, but the food industry also must be considered. Specific reasons for not washing hands at appropriate times are laziness, time pressure, inadequate facilities and supplies, lack of accountability, and lack of involvement by companies, managers, and workers in supporting proper Handwashing. To facilitate improvements in hand hygiene, measurement of compliant and noncompliant actions is necessary before implementing any procedural changes. Training alone is not sufficient for long-lasting improvement. Multiactivity strategies also must include modification of the organization culture to encourage safe hygienic practices, motivation of employees willing to use peer pressure on noncompliant coworkers, a reward and/or penalty system, and an operational design that facilitates regular hand hygiene.

# Appendix F: Food production chain

At each step in the food production chain microbes may cross from one substrate to another; and at every step humans may unknowingly come into contact with these microbes. Even if a person in contact with a pathogenic microbe does not get sick themselves, they may pass on the pathogens to more vulnerable populations such as children, the elderly or people with compromised immune systems less able to fight off an illness. Food poisoning affects millions of people a year and if you work in the food industry you must not ignore this risk, even if you cannot see the pathogens because they are so small.

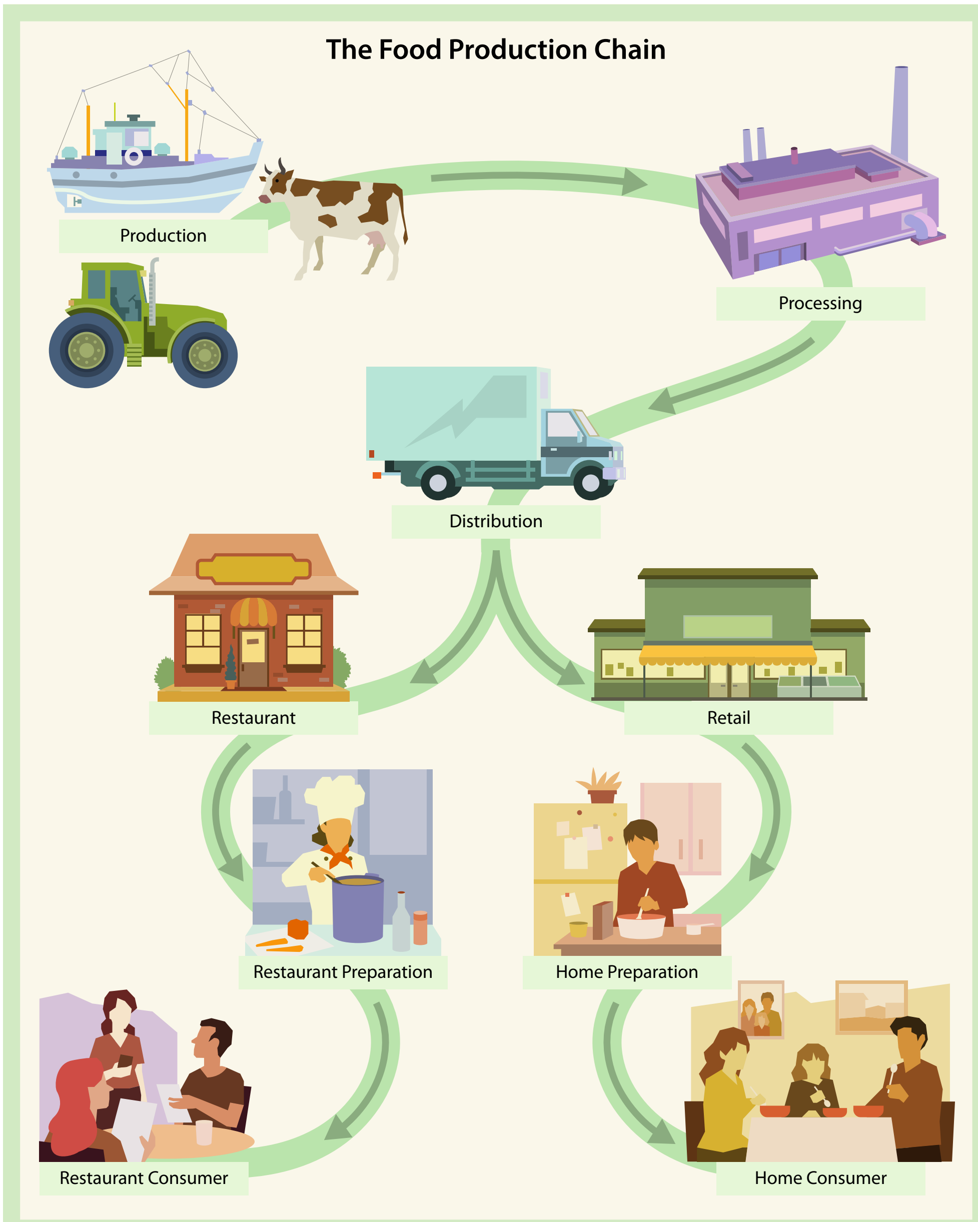



**Figure 1** — The food production chain

**Figure 2** — Rounds of cheese inside a processing plant

In this section we will take a closer look at cross contamination in six key locations:

- Stop 1: outdoor farms and
- Stop 2: indoor animal farm production,
- Stop 3: slaughterhouses and meat processing plants,
- Stop 4: fruits and vegetables processing plants,
- Stop 5: transportation to the kitchen and arrival at the kitchen,
- Stop 6: preparation in the kitchen.

## Food production: From field to fork

### Stop 1: The outdoor farm

At the farm, imagine where microbes might be if you see:

- Cattle grazing off in the distance
- An orchard
- A field of crops
- A farm house where people live with their pets
- A barn where equipment, supplies, animal feed, fertilizer and crops are stored
- A dairy barn where cows are milked and the owners pasteurize milk and make butter and cheese
- Structures for food storage, packing, processing or transportation
- Chicken coops, pigsties or livestock facilities

Now imagine how those microbes might be transported: by a bird, a rodent, a cow, a dog, a human, by water or air.

**Figure 3** — A farmer preparing the field for a crop

Typical activities on small farms include feeding chickens and collecting eggs, milking cows, tending crops, grooming horses, and daily interactions with other animals, such as sheep, pigs, farm cats and dogs.

**Figure 4** — In what ways might these two animals transport microbes?

Equipment used in the fields and stored in the barn are a potential transfer point of microbes from the field to the barn and the barn to the field. Plants (leaves, fruits and vegetables) can be intermediaries between contaminated soil or water. Humans and animals may become infected through direct (consumption) or indirect contact. Humans and animals can consume microbes in drinking water or direct contact with other animals. People can get fecal matter on their hands when milking cows or spreading manure. The most common way for people to get fecal matter on their hands is after using the toilet and failing to properly wash their hands. However, once infected, microbes multiply in the host animal or human with potential to cross-contaminate other areas or foods on the farm.

Studies in BC have shown that fresh produce requiring a lot of handling, such as fresh picked cherries, are often contaminated by human food pickers. *E. coli* bacteria was not detected in any of the cherries on orchard trees, but it was recovered from pickers' and sorters' hands and in birds at the cherry farms. Pickers' hands, even when they are wearing gloves, can transfer bacteria from one fruit to another during picking activities. (Bach & Delaquis, 2009)

As seen from the photos in Figure 5, mangoes and papayas are tree fruits and cantaloupes are vine fruits that are grown on top of the soil. Contamination from the environment may be through bird feces and insects (mangoes, papayas) or soil (cantaloupes). Contamination of the outer surface during picking by workers is also of concern. All of these fruits are peeled or sliced before consumption, yet these fruits are still linked to outbreaks of food poisoning. When slicing into fruits any contamination on the outer rind surface can contaminate the knife. The knife can then transfer harmful microbes into the flesh of the fruit, where microbes may be able to multiply. If the pH of the fruit is not acidic enough, i.e., not below a pH of 4.6, then growth of microbes is more likely.

Mangoes

Mangoes growing in a tree

Canteloupes growing in a field

Papayas growing on a tree

**Figure 5** — Outbreaks associated with fruits (mangoes, papayas and cantaloupes)

Take a look at the pH range for common fruits in the chart. What other fruits besides papaya and cantaloupe are higher risk? Any fruits with pH above 4.6 offer a more hospitable environment for microbial growth.

| Fruit | pH range |
|---|---|
| Cantaloupe | 6.2 - 6.5 |
| Watermelon | 5.8 - 6.0 |
| Papaya | 4.5 - 6.0 |
| Banana | 4.5 - 5.2 |
| Pear | 3.4 - 4.7 |
| Cherry | 3.2 - 4.7 |
| Plum | 2.8 - 4.6 |
| Mango | 3.3 - 3.7 |
| Apple | 3.3 - 4.1 |
| Pineapple | 3.2 - 4.0 |
| Peach | 3.1 - 4.2 |
| Grape | 3.0 - 4.5 |
| Blackberry | 3.0 - 4.2 |
| Orange | 3.0 - 4.0 |
| Guava | 3.0 - 3.2 |
| Grapefruit | 2.0 - 3.4 |
| Passion fruit | 2.6 - 3.3 |
| Cranberry | 2.5 - 2.7 |
| Lemon | 2.2 - 2.4 |

**Figure 6** — Typical pH ranges for fruits

**Examples of *Salmonella* outbreaks**

*The U.S. Center for Disease Control reports eight outbreaks of Salmonella spp linked to* mangoes *between 1998 and 2017 (*www.cdc.gov *at the NORS dashboard – national outbreak reporting system)*. In the US in 2012, in one outbreak 127 illnesses (cases) were reported from 15 different states; in Canada over 20 people became ill as a result of *Salmonella* contamination from mangoes(Centers for Disease Control and Prevention). Nearly one million mangoes traced to a grower near the coast of the Sea of Cortez in Mexico were recalled.

A series of outbreaks in 2017 were linked to Maradol style papayas. Multiple strains of *Salmonella* were found *S. Urbana*, *S. Newport*, *S. Infantis* and *S. Anatum*. A total of 31 illnesses and one death were reported in these outbreaks. Although the same type of papayas (Maradol) were linked to the illnesses, there were three different outbreak clusters. Each cluster was traced back to different farms in Mexico. One farms' papayas were contaminated with *S.* Urbana, another with *S.* Anatum and the third farms' papayas were contaminated with *S. Newport* and *S. Infantis*. (Centers for Disease Control and Prevention, 2017a, 2017b, 2017c). The reasons why that years papayas were prone to *Salmonella* contamination in 2017 is unclear.

**Example of *Listeria* outbreak**

A very serious outbreak of listeriosis was linked to cantaloupes in 2011 that persisted into 2012. A total of 147 illnesses, 143 hospitalizations, and 33 deaths occurred in 28 different US states (Centers for Disease Control and Prevention, 2012a). An investigation of the farm where the cantaloupes were grown found old and rusty equipment in use that was previously used for potato farming. *Listeria* was found in water, on the floor of the packing plant and 2cts, rodents, birds, wildlife, and domestic animals also shed and pick up microbes wherever they go. Most people who walk through a muddy field do not take shoes off before entering a barn or delivering their crops to market. But on those muddy boots, there is an opportunity for cross-contamination from *E. coli*, *Campylobacter*, *Salmonella* and *Listeria* that might be present in the soil. And this is only the first step on our journey to the dinner plate.

## Stop 2: The indoor farm

In indoor farms where animals live in close contact cross contamination becomes more likely in areas where animal feces accumulate. Fecal material may become airborne, stick to the floors, walls, ceiling and be transported on the feet of workers and other animals passing through the area.

Poultry farms will be used in this example. Both *Campylobacter* and *Salmonella* reside in the gut of chickens.

**Figure 7** — Poultry in an enclosed space

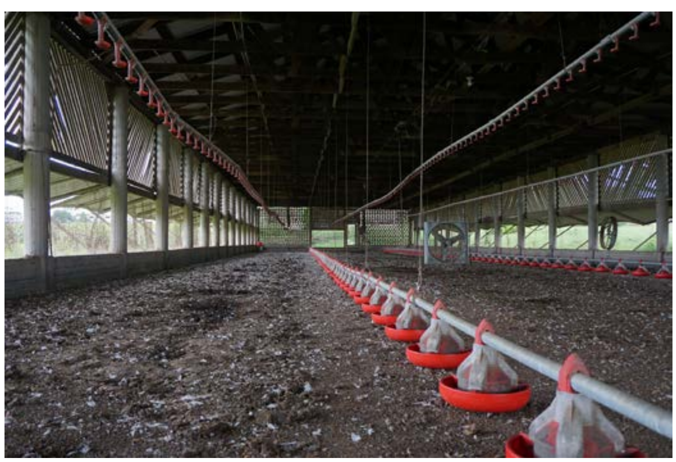


**Figure 8** — Empty poultry barn with feeding stations

In a poultry farm where birds and bird cages are next to each other, bacteria in the feces of hens may move from one bird to another. If an uninfected hen acquires bacteria this way it is known as horizontal transmission ( → ). When the hen lays an egg, the bacteria may be present on the surface of the layer eggs. However, one of these bacteria, *Salmonella*, can also be passed from the hen to the egg when the egg is formed inside the hen. This type of transmission is known as vertical transmission ( ↓ ). Most shell eggs become contaminated this way, from the layer hen to the egg. This means that the *Salmonella* may be present inside the egg shell in the yolk and egg white (albumen). In Canada, risk estimates of layer eggs predict 1.7 million eggs will be contaminated with *Salmonella* Enteritidis annually from a production of 6.3 billion eggs, resulting in an estimated 120 illnesses annually *(DeWinter, Ross, Couture, & Farber, 2011)*. This is actually a very low contamination rate, based on prevalence of contamination variation over a year, between 3 and 4 eggs would be contaminated out of every 10 million. As the eggs age and particularly under poor storage and handling conditions the potential for *Salmonella* present in an egg to multiply increases (DeWinter et al., 2011).

To control *Salmonella* in layer hens and in broiler hens, processors must control:

- Pests, particularly rodents and insect intrusion into the barn
- Water, surface water contaminated by wild birds could be a transmission source for *Salmonella* and other pathogens (avian influenza)
- Feed sources, ensuring feed is free of pathogens such as *Salmonella*,
- Bedding, ensuring sawdust or other materials are clean and not re-used between flocks,
- Transport cages and transport trucks, to ensure these are clean and sanitized between flocks,
- Positive flocks, by testing of "poultry fluff" or feathers. When a flock tests positive for *Salmonella*, the barn may be de-populated (all birds killed and disposed of), the barn completely cleaned and sanitized, before restocking with new birds.

Potential human pathogens from other domestic animals such as cattle will also include *E. coli, Clostridium perfringens* and *Listeria*. These bacteria reside in the intestines of healthy animals (*E. coli*), or can survive in the soil or in manure (*Listeria*) for up to a year.

## Stop 3: Slaughterhouses and meat processing plants

From the farm or ranch, animals are transported to slaughter houses and meat processing plants. This is a stressful time for animals.

**Figure 9** — Beef carcasses hanging in a processing plant

During transport fecal material is often released and will spread from contact between animals and contaminate surfaces of the vehicle and pens. In areas where animals are slaughtered microbes from intestines can result in gross contamination of the carcass, and can be transferred to knives and equipment.

Poultry slaughter involves passage of the birds into different water tanks to remove feathers, remove surface contamination and blood and to clean the cavity of birds. This water quickly becomes contaminated allowing microbe movement from bird to bird via liquid transmission. Thus, if one bird is infected with *Salmonella* all the birds have potential to become infected. Although chemicals are added to the wash water to kill bacteria, organic loading is high, so disinfectants may not be entirely effective and not all bacteria are killed.

Beef slaughter is a multi-step process. These large animals are stunned, bled, and have their hides removed. Beef must have their bungs (anal openings) tied before the intestines are removed, otherwise fecal material may be deposited elsewhere on the carcass. To remove surface contamination from the meat carcass spray washers are used, rather than water tanks. Similar to poultry the spray water will have chemicals added to kill bacteria. However, the spraying process can transmit fecal material from one carcass to another when outer surface contamination is physically sprayed off one carcass and then this contaminated liquid lands on another carcass. Preventing fecal material transfer at the beginning if the process is more effective. Tools, working tables, equipment as well as clothes, counter tops, floors, walls, ceilings and human hands may all be cross contaminated by fecal sources during slaughter.

There is also the potential for contamination to occur during grinding and cutting of meat when the surface of the meat remains contaminated. Pathogenic *E. coli* in ground beef meat is known as "hamburger disease" and was first recognized as an issue in the 1980's. While whole meat cuts are generally regarded as not having bacteria in the interior of the meat muscle, there are exceptions. Beef packaged into large Cryovac plastic containers can allow bacteria to spread through the blood to all surfaces of the meat in the package. Tenderizing of meat using needles or pins can also push *E. coli* bacteria into the interior of the meat.

**Additional resources:**

**Canadian Food Inspection Agency Beef Processing and Inspection**
http://www.inspection.gc.ca/food/information-for-consumers/food-safety-system/beef-processing-and-inspection/eng/1374555766340/1374821164166

**Manitoba Agriculture. Food Safety Program, for Processors and Distributors Fact Sheet #7 Cross-contamination**
https://www.gov.mb.ca/agriculture/food-safety/at-the-food-processor/food-safety-program/pubs/fs_7.pdf

## Processing plant and *E. coli* outbreak

During a routine audit of imported meat from Canada, the United States Department of Agriculture Food Safety Inspection Service (FSIS) identified E. coli in meat supplied by XL Foods (a large slaughterhouse). The Canadian Food Inspection Agency (CFIA) confirmed these findings the following day. *E. coli* O157:H7 was present in beef from a meat packing plant in Alberta supplied by XL Foods. More positive tests of *E. coli* were reported in meat originating from XL Foods, resulting in recalls, and 10 days from the FSIS finding, the first illness was reported, using modern genetic techniques that matched *E. coli* strains in ill people to the same strains found in beef from the plant.

CFIA issued multiple health hazard alerts to the public and distributors, grocery store chains and food service establishments over the following weeks – in total 21 separate health hazard alerts were issued during the complicated trackback. For the first time, in addition to ground beef (hamburger) whole cut steaks were also implicated in illnesses. Steaks purchased from an Edmonton's Costco were linked to four illnesses and subsequently found to have *E. coli* contamination matching to the illnesses. Investigation at the Costco found they employed the practice of needle puncturing for tenderizing of whole steaks prior to packaging. This was the first illness report in Canada of whole steaks acquiring *E. coli* through this practice and eventually led to a new Health Canada recommendation of cooking needle punctured meat to an internal temperature of at least 63°C and to flip or turn over meats (e.g., steaks or other beef cuts) at least twice during the cooking period.

Raw beef meats (not poultry, pork or other meats) that have been needle punctured or mechanically tenderized are required to have these labelling instructions on the outside of the packages as it is very difficult for consumers to know from appearance alone if raw beef meats have been mechanically tenderized.

The source of contamination was traced to a plant in Brooks, Alberta, "Establishment 38" of XL Foods Inc. Two sealed trailers of contaminated meat products were escorted from the plant by CFIA inspectors in Canada's largest beef recall. A total of 45 million pounds of meat was destroyed.

What went wrong? Federal inspectors are always on-site at large meat processing plants. They examine carcasses for disease and evidence of poor quality sanitation. Inspectors and staff are also tasked with doing carcass swabs to detect the presence of coliforms and *E. coli*. This was a very large plant that was open 24 hours a day, had two 12 hour shifts operating and processed 300 cattle per hour. The line speed was set at such a high rate carcasses passed inspectors on average every 12 seconds, allowing very little time to inspect each carcass for signs of disease or fecal contamination. Although meat carcass swabbing was done, CFIA inspectors did not look at the result trends and failed to see increasing counts in the production. It is likely that anal bung ties were not performed properly during evisceration allowing feces to spread onto the meat. Another failure was noted in the carcass cleaning areas where spray nozzles were plugged and did not undergo proper maintenance or servicing.

In the aftermath of the XL foods *E. coli* O157:H7 outbreak the Government of Canada appointed an independent panel to conduct a review of the events and circumstances that led to the recall. The report makes for interesting reading.

**Archived - Independent Review of XL Foods Inc. Beef Recall 2012**
[http://www.inspection.gc.ca/food/information-for-consumers/food-safety-investigations/independent-review/eng/1370367689068/1370367776627](http://www.inspection.gc.ca/food/information-for-consumers/food-safety-investigations/independent-review/eng/1370367689068/1370367776627)

The first recommendation of the Independent Review of XL Foods Inc. Beef Recall 2012 is:

A strong food safety culture must be developed within the processing plant, and adopted by both plant and Canadian Food Inspection Agency (CFIA, or Agency) staff – at all levels.

The report identified that to build a strong culture, food safety must be the top priority and a shared value across all levels of the organization. It further noted that all staff contributing to the building of a strong food safety culture should be recognized for their efforts. Every staff member should be empowered to report to their supervisor any concerns they might have regarding food safety.

It was also noted that employee training on food safety principles as well as on-going refresher courses on hygiene, plant sanitation, and related areas should be integrated into plant operations.

### Further information

Health Canada and Canadian Food Inspection Agency labelling requirements:

**Guidance on Mandatory Labelling for Mechanically Tenderized Beef**
https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidance-mandatory-labelling-mechanically-tenderized-beef.html

**Labelling requirements for meat and poultry products: Mechanically tenderized beef**
https://www.inspection.gc.ca/food-label-requirements/labelling/industry/meat-and-poultry-products/eng/1393979114983/1393979162475?chap=17

**One year later: Beef industry bounces back after massive XL Foods recall (CTV News, 2013)**
https://www.ctvnews.ca/business/one-year-later-beef-industry-bounces-back-after-massive-xl-foods-recall-1.1445299

Listen to this presentation from BCCDC to UBC Population and Public Health Students recorded Oct 12, 2012. The presentation provides details on what went wrong during the slaughter process.

**Xtremely Large Foods recall and E.coli O157:H7 outbreak in Canada, 2012**
https://www.youtube.com/watch?v=eb_mEuI2kKk&feature=youtu.be

## Stop 4: Vegetable and fruit processing

From the field to plate vegetables and fruits must also be carefully handled and monitored to limit cross-contamination.

We've reviewed on-farm scenarios where vegetables and fruits might acquire microbial pathogens. The next stop is the processing and packaging plant. Pathogenic microbes might be found in many places in the processing facility including floors and drains, on surfaces where produce is sorted and graded, in packing equipment as well as from human carriers.

Watch the following video of Chef Gilbert Noussitou's visit to Sysco Victoria to find out how salad greens are processed from delivery of salad greens to packaging for distribution to local food establishments. Pay particular attention to how and what information is collected and recorded to ensure that all products can be traced back from the food establishment to the field in which it was produced.

**L 1 H Sysco Produce Department**
Access from: https://www.youtube.com/watch?v=_f858r_afiU

**Additional resources:**

**Canada GAP**
https://www.canadagap.ca

**Health Canada: Produce Safety**
https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/safe-food-handling-tips/produce-safety.html

**USDA (2008) FDA Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables**
https://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/produceplantproducts/ucm064458.htm

**USDA (2017) Guide to Minimize Microbial Food Safety Hazards Fact Sheet**
https://www.fda.gov/food/produce-plant-products-guidance-documents-regulatory-information/guide-minimize-microbial-food-safety-hazards-fact-sheet

**California Commodity Specific Food Safety Guidelines for the production and harvest of lettuce and leafy greens (April 2019)**
https://lgma.ca.gov/wp-content/uploads/2019/04/190419-Metrics-with-Water-Updates.pdf

## Stop 5: Transportation and arrival of foods at the kitchen

What is wrong in the photo of the watermelons being delivered in the yellow car? You need not be a food safety expert to recognize some potential microbial issues.

**Figure 10** — Melons being transported to a kitchen

**Possible answers:**

- The fruits are not protected from contamination inside the vehicle, the vehicle is not a sanitary container, microbes in the car can be transferred to the fruits.
- The fruits are not refrigerated, microbial spoilage can occur more quickly.
- Two types of fruits are in the vehicle, striped and solid colored melons. Because the melons are mixed, contamination from one type of melon may be transferred to another melon.
- There is no evidence of labels on the melons and they may be sourced from more than one farm.

At the kitchen, the delivery truck arrives. People begin to unload and bring the different foods into the back of the restaurant. Microbes that began in the water or soil on the farms or the processing plant or anywhere else along the may now have entered your food preparation site. Think of everything you cannot see: the microbes in the truck, on the crates and other containers, on the clothes and hands of the delivery driver. Kitchen and delivery personnel who load and unload food are also potential sources of cross contamination.

As the food is brought inside, if it is put onto the floor temporarily, there is potential that microbes on the floor will cross-contaminate the packaging crate. These microbes may then be transferred to another surface, perhaps a sorting table or the refrigerator. Eventually these microbes may be transferred to the food.

> ***Did you know?***
> The famed five second rule for dropping food on the floor is unfortunately a myth. As soon as one surface touches another cross-contamination can occur.
>
> "Even if something spends a mere millisecond on the floor, it attracts bacteria. How dirty it gets depends on the food's moisture, surface geometry and floor condition—***not time***."*

If the packaging crate is already contaminated, then putting it on the floor can potentially transfer microbes from the crate to the floor. Perhaps the microbes will transfer to a bit of food on the floor, or to the bottom of your shoes and then to your hands. Until the cycle of cross contamination is broken, through cleaning and sanitizing of the crate, packaging table, the floor, use of sanitizer boot dips for footwear or simple handwashing, the risk of cross-contamination remains.

During a typical delivery your supplier may deliver a whole host of foodstuffs from various environments. Whole chickens, various cuts and quality of beef, fish, shellfish, different kinds of vegetables and fruit, dairy products, eggs, potatoes, ingredients to make bread and the desserts and everything else that is necessary to make all those delicious offerings on today's menu.

To reduce the risk of cross-contamination, follow these four steps:

1. **Clean**: Wash your hands and any surfaces often.
2. **Separate**: For example don't use the same knives, cutting boards and other surfaces for preparing raw protein foods (meat, poultry, seafood, etc.) as you do raw vegetables and fruits.
3. **Chill**: Keep cold things cold, stay out of the danger zone
4. **Cook**: Make sure that food is properly cooked, use a thermometer to check the internal temperature of protein foods.

Remember, any contact provides an opportunity for microbes to move and cross-contaminate. What tempting substrates does the kitchen offer?

* **Mythbusters Database 5 Second Rule With Food on Floor**
http://www.discovery.com/tv-shows/mythbusters/mythbusters-database/5-second-rule-with-food/

## Stop 6: Preparing foods

Limiting cross-contamination within the kitchen can be challenging in a busy dynamic environment. Common areas where cross-contamination occurs and issues are described.

- Cutting boards are notorious for their role in cross-contamination. NEVER cut vegetables or fruit on a cutting board used to cut raw meat, poultry, or fish. Food establishments often have separate cutting boards for different food groups.
- Temperature is one of the ways to control microbial growth, but if well-cooked hamburger is placed back onto the plate that held the raw meat, the cooked meat is at risk of picking up *E. coli* or other bacteria from the raw meat juices left behind on the plate.
- Marinating sauce used for raw meats and fish should never be applied onto cooked foods. Instead, boil the marinating sauce for at least one minute before using it as a baste or sauce for the cooked foods. An even better method is to prepare extra marinade that is only used for basting cooked foods.
- The same utensils used to transfer raw meat onto the grill should not be then used to take the cooked meat off the grill. During this action, microbes may be transferring from the raw to the cooked meats through the use of tongs or spatulas.

**Washing meat or poultry**

When preparing foods, be aware of the risks of rinsing raw foods in the sink. Water can spread microbes over large areas.

In the video food safety specialist Jasmina Egeler and chef Gilbert Noussitou use glo germ to demonstrate the perils of washing a chicken in the kitchen sink and the implications for cross-contamination.

**Washing Chicken**
https://www.youtube.com/watch?v=1KjRQzmxNNw

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# Answer Key

## Lesson A: Food Microbiology—An Introduction

### Activity: Classifying bacteria

1. *Escherichia coli* 0157:H7
2. *Clostridium botulinum*
3. *Campylobacter*

### Lesson A Quiz

1. d. microscope
2. b. Each person carries a unique combination of living bacteria, viruses and fungi.
3. b. Spore-forming bacteria
4. b. Can be beneficial, neutral, or harmful to humans
5. b. Yeast bread
6. d. Pathogenic
7. a. Virus
8. Functions of microbes and survival of microbes
9. Habitats of microbes and survival of microbes
10. Survival of microbes
11. c. Whether it requires oxygen or not
12. c. Some microbes transform foods in a beneficial way, others may cause food spoilage or illness.
13. c. 6 groups: bacteria, virus, parasites, prions, algae and fungi
14. d. By pH (acidity)

## Lesson B: Microbial Growth

### Activity: Testing for proper cleaning and sanitation

1. a. ATP test
   c. sugar test
   d. Total plate count test
2. a. ATP test
   b. protein test

## Activity: Understanding Laboratory Reports

### Part A

1. c. Water activity, $a_w$
   e. Acidity of foods, pH

2. a. Total aerobic plate count
   d. *E. coli*;
   f. Yeasts and moulds
   g. Total coliforms

3. h. *Staphylococcus aureus*
   I. *Salmonella* spp.
   j. *Listeria* spp.

### Part B

1. d. Yes, because the combination of pH and water activity will not prevent microbial growth in this food.

2. a. Yes, because the acidity is not low enough.

3. d. No, the < 10 means there were not any *E. coli* detected .

4. b. The second batch has detectable levels of *E. coli* and *Staphylococcus aureus*, along with higher pH and water activity..

5. a. Add citric acid or lemon juice to bring down the pH.

## Lesson B Quiz

1. c. Processing factors

2. b. Potentially hazardous foods

3. c. Indicator tests

4. a. Intrinsic factors—pH of the food

5. b. Extrinsic factors—relative humidity

6. c. Food processing

7. d. Other microbes in the food

8. c. The acidity levels that allow pathogen growth are found in most foods we eat.

9. a. An intrinsic factor of a foods ability to accept or transfer electrons

10. a. Temperature

11. c. Atmosphere

12. b. Time

13. a. Temperature

14. d. Relative humidity

15. b. The log phase

16. Will grow in refrigerators and on frozen foods

17. b. Most human pathogens are of this class

18. c. Tolerate the highest temperatures of any known organism

19. a. Indicators show that pathogens may be present in a sample.

20. b. So all meat process controls can be developed to ensure opportunities for pathogen growth are limited and microbial counts are reduced by temperature and time.

21. b. No

22. d. Any number at or below 124

# Lesson C: Fermentation and Spoilage

## Activity: Fermentation problems with kombucha tea

Dizziness and headache; alcohol

Acidosis; acetic acid

## Activity: Extrinsic and intrinsic characteristics in microbial growth

Factors extrinsic to the food are essentially the environmental surroundings of the food. They include:

- The temperature of the food during preparation and storage. Most microbes prefer warm conditions. If temperature is not managed and controlled, microbes can grow.
- The time available for microbes present in food to grow, from the time of manufacture to the time of consumption. This includes shelf life considerations for the food as well.
- The atmosphere for the food during preparation and storage. This is a very important concept—food packaging that changes the normal atmospheric conditions of oxygen, nitrogen, and carbon dioxide is called "reduced oxygen packaging" (covered in detail in Lesson F). As we learned in Lesson A, some bacteria survive best in normal aerobic conditions, while others, such as C. botulinum, prefer an anaerobic environment.
- Relative humidity. This is the level of moisture around the food—is it dry or wet?

Factors intrinsic refer to aspects of the composition of the food itself. They include:

- Nutrients are available to microbes, including sugars, carbohydrates, proteins, and others
- Acidity/alkalinity or pH of the food
- The amount of water available in the food for microbes (water activity or $a_w$)
- Whether the food contains natural antimicrobial chemicals or features that limit growth
- How much natural oxygen is in the food (redox potential)

### Lesson C Quiz

1. c. A process by which microbes produce energy by converting sugars into simpler compounds
2. c. A portion of a batch of food is used to create a starter for a new batch of food.
3. Yogurt—c. Lactic acid fermentation
   Beer—a. Yeast fermentation
   Kombucha—b. Mixed fermentations
   Sauerkraut—c. Lactic acid fermentation
4. b. Probiotics provide nutrients to the bacterial microbes that are essential to gut health.
5. Slimy cucumbers—c. Microbial spoilage
   Freezer burnt turkey—a. Chemical spoilage
   Overripe bananas—b. Enzymatic spoilage
6. Acidity—a. intrinsic
   Temperature—b. extrinsic
   Mould—b. extrinsic
   Moisture content—a. intrinsic
7. a. Reduce oxygen
8. a. Fungal organisms
9. a. After death, the cells of an organism can no longer control enzyme activity.
10. b. The biproducts created by microbial growth in foods

## Lesson D: Foodborne Illness

### Lesson D Quiz

1. a. They harm or cause disease in a host organism.
2. c. The people most susceptible to foodborne illnesses
3. b. Illness results from eating food in which illness causing pathogens are present. The pathogens multiply in tissues in the body.
4. a. Illness results from eating food which requires bacterial growth during its preparation.

   d. Illness results from eating food with pre-formed toxins from bacteria, fungus, algae or other microbes. The toxins are present in the food before it is ingested.
5. d. *Listeria monocytogenes*
6. *Clostridium botulinum*
7. b. *Salmonella*
8. b. They may be more susceptible due to age or pre-existing condition.
9. c. It is a chronic condition which may be the result of a foodborne infection.

10. a. Food intolerance—It occurs when the body is unable to absorb food nutrients; Food allergy—It occurs when the body reacts to nutrients it has absorbed.

11. a. Food service workers are the most common source of pathogens in prepared food.

12. b. Produce, meat and poultry, fish and shellfish

13. Contamination
    1; (f.) Incubation period
    2; (c.) Prodromal (Symptoms begin)
    3; (e.) Acute peak illness
    4; (a.) Decline (Symptoms end)
    5; (d.) Recovery
    6; (b.) Chronic illness (New symptoms occur)

14. c. How often do you wash your hands?

# Lesson E: Sanitation, Personal Hygiene, Pests and Cross-Contamination

## Activity: Why is this lesson important?

You should have answered "yes" to every item on this list.

## Activity: Creating transmission pathways

**Direct:** Crickets are contaminated with Salmonella and are dipped in melted chocolate.

**Direct:** Crickets are contaminated with Salmonella and are ground up into a protein bar.

**Indirect surface-to-surface:** Crickets contaminated with Salmonella crawl on tomatoes. The tomatoes become contaminated with Salmonella.

**Indirect liquid:** Irrigation water is contaminated by Salmonella (by crickets or by some other agent, such as birds). The tomatoes absorb the bacteria through the irrigation water and become contaminated.

**Indirect liquid:** Crickets are contaminated by Salmonella and the bacteria transfer into the melted chocolate. The remainder of the liquid chocolate is used to coat strawberries that become contaminated with Salmonella.

**Indirect airborne:** Dried feces from a nearby farm are blown by a strong wind into the field and contaminate the surface of tomatoes.

## Activity: Bleach dilution calculator

42 mL

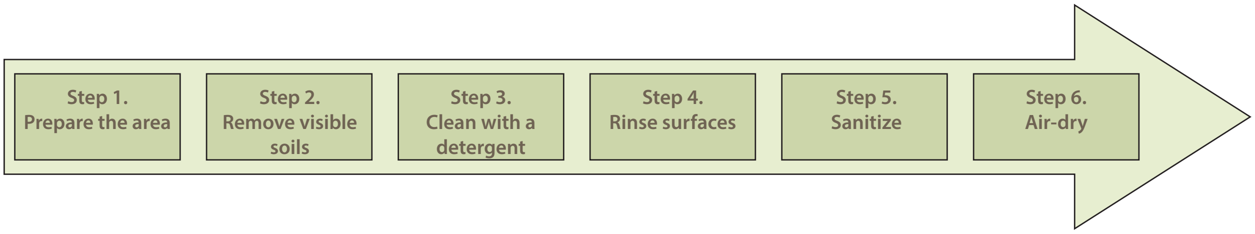
## Lesson E Quiz

1. a. Cleaning is important to remove debris before sanitizing occurs.

2. b. Chemical contamination
   c. Biological contamination
   a. Physical contamination

3. c. effective at reducing pathogens

4.




5. a. Harmful bacteria form biofilms that are more resistant to sanitization.

6. a. and d. Ensure that handwashing is done properly and frequently to prevent contamination of food; Ensure that food-grade gloves are worn by workers and changed as often as necessary to prevent contamination of food

7. c. Limit access by using window screens and keeping doors closed

8. a. Block entry, enclose garbage and implement a pest control monitoring

9. b. Can be best prevented by limiting access to food and water.

10. b. So that appropriate actions are taken by supervisors and employees when communicable illness occurs in the work site

11. b. A worker preparing ground meat shares a prep surface with another worker cutting cucumber for the salad bar.

12. c. Food handlers can reduce the chances of foodborne illness being caused by microbial growth.

13. c. Training cleaning staff

14. d. *Staphylococcus aureus* and a. Norovirus

15. e. *Bacillus cereus* and f. *Clostridium botulinum*;

16. b. Cryptosporidium spp. and c. *Salmonella* spp.

# Lesson F: Microbial Barriers for Food Preservation

## Activity: Intrinsic microbial barriers: pH and $a_w$

1. The statement is false. While most pathogenic microbes will not grow when pH is 4.6 or less, or if $a_w$ is 0.85 or less, there are many spoilage microbes and even a few pathogenic microbes that can survive and grow under these conditions. For example, moulds and yeasts can grow under conditions where $a_w$ is 0.61 to 0.62.

2. Pathogenic bacteria able to grow at $a_w$ less than 0.85:
   - *Staphylococcus aureus*

Pathogenic bacteria able to grow at pH less than 4.6:

- *Bacillus cereus*
- *Listeria moncytogenes*
- *Salmonella*
- *Staphylococcus aureus* (growth)
- *Staphylococcus aureus* (toxin formation)
- *Yesinia enterocolitica*

3. The pH is 5.3 or lower at the end of the fermentation and the $a_w$ is 0.90 or lower in the end product.

## Activity: Vacuum packaging fill in the blank

Name this bacteria: Clostridium botulinum

Many foodborne pathogens are facultative anaerobes, and can survive in reduced oxygen packaging environments. Name at least three other bacteria of concern mentioned in the food code:

- *Salmonella*
- *Listeria*
- *E. coli*

## Activity: Fill in the blank

The answer includes 12 "9's," 10 after the decimal point. 99.9999999999

# Activity: Hurdle technologies and lasagna

## Part 1: Hurdle identification during food processing

| **Choose hurdle for each ingredient** | **Lasagna noodles** | **Tomato sauce** | **Spices and flour** | **Italian sausage** | **Eggs and cream** | **Spinach** | **Parmesan, ricotta and mozzarella cheese** |
|---|---|---|---|---|---|---|---|
| Acidic pH | | ✔ | | ✔ | | | ✔ |
| Canning | | ✔ | | | | | |
| Curing | | | | ✔ | | | |
| Drying or dehydration | ✔ | | ✔ | ✔ | | | |
| Fermentation/ microbes competing | | | | ✔ | | | ✔ |
| Irradiation | | | ✔ | | | | |
| Low water activity ($a_w$) | ✔ | | ✔ | ✔ | | | ✔ |
| Pasteurization, cooking or heating | | ✔ | | | ✔ | ✔ | ✔ |
| Preservatives | | ✔ | | ✔ | | | |
| Reduced oxygen packaging | | ✔ | | ✔ | | | ✔ |
| Refrigeration or Freezing | | | | ✔ | ✔ | ✔ | |

## Lasagna noodles

Chosen hurdles could be drying or dehydration, or "lower water activity ($a_w$).

**Drying or dehydration.** These lasagna noodles have been dried to remove moisture and are shelfstable, the water activity is very low, and they are not a PHF or ingredient.

**$a_w$.** The noodles have a very low $a_w$, of 0.44 to 0.56. These conditions are not conducive to the growth bacteria, fungi, or moulds.

## Tomato sauce

Chosen hurdles could be canning; pH; pasteurization, cooking or heating, preservatives; refrigeration or freezing, reduced oxygen packaging (ROP).

**Canning**

Canning is a hurdle that extends the shelf life of the food and improves safety. Canning involves high temperature and pressure. *Clostridium botulinum* spores and the bacterium can only be eliminated when temperature is sustained above 100°C for a specific time, which requires the use of a pressure canner. Commercial canning requires a 12D or 12 log reduction.

**pH**

Raw tomatoes have a slightly acidic pH of around 4.6, but there are a number of microbes that can grow at this pH range that could cause illness or spoilage, such as acid tolerant *Salmonella*, yeast and mould.

**Preservatives**

The first step to canning tomato sauce is adding some lemon juice or citric acid to lower pH. If the tomato sauce includes other vegetables or herbs, such as basil, its pH may be higher, and so it may be necessary to add more citric acid to lower the pH.

**Pasteurization, cooking or heating**

Before the tomato sauce is put into cans and the canning process begins, tomatoes and spices in the sauce are boiled for ten minutes or longer. Then the sauce is placed into cans or jars and sealed.

**Reduced oxygen packaging**

By packaging foods into a jar or a can, this prevents microbial access to oxygen, and this packaging is referred to as **reduced oxygen packaging**.

**Refrigeration**

There is a reason you are told to refrigerate items after opening a can. By opening a can or jar of tomato sauce or any other canned product, you are providing oxygen to any microbes present or entry to any microbes in your kitchen environment. Thus, it is important to look for another hurdle; in this case, refrigeration.

## Spices and flour

Chosen hurdles could be: drying or dehydration, irradiation,or lower $a_w$.

**Drying or dehydration**

Spices used in this recipe are dried and dehydrated, not fresh. Flour is made from milled grain and also dried and dehydrated. Dehydration may not be done at high enough temperatures to eliminate pathogens, but they should not grow further.

**irradiation**

Spices and flour are permitted to be irradiated in Canada. This process is an effective hurdle that eliminates pathogens in these products. Ionizing radiation energy is used to disrupt the internal DNA of microbes, but is not enough to damage nutritional quality of the foods.

**Lower $a_w$**

Spices and flour have low water activities of < 0.7 and lower. Microbes will not grow at these moisture levels, but pathogens can sometimes survive for extended periods. Although spices and flour have a low $a_w$, they do not receive any thermal treatment and therefore have the potential to carry microbes that can cause foodborne illnesses, particularly *Salmonella* and shigatoxigenic *E.coli*. That is why these ingredients are considered raw.

## Italian sausage

Chosen hurdles could be curing, drying, preservatives, low pH, low water activity, fermentation, refrigeration, or ROP. Depending on how the Italian sausage may have been processed (it was described as spicy, dry and ready-to-eat), it may have contained preservatives, been dried, cured, and/or fermented. These hurdles inhibit bacteria and lower water activity and pH, which work together to keep many bacteria in either the lag or stationary phase of growth. Another hurdle, not shown, is time- and temperature-dependent (degree hours calculation to control *Staphylococcus* aureus toxin formation).

**Curing**

The curing step for sausages involves the addition of the preservatives nitrite and sometimes nitrate (for slow fermentations), and is a specific requirement. Elsewhere in the course, curing is explained as addition of salts or sugars, but for fermented sausage, curing includes nitrites. Most rapid sausage fermentation also uses the addition of sugars (lactose, glucose or GDL-gluco delta-lactone, a fermented rice sugar) to feed the starter culture, LAB, so they can being growing quickly.

**Drying or dehydration**

Sausages are hung after fermentation for days to months. The longer the sausage dries and moisture is lost, the harder it is for microbes to grow.

**Fermentation/microbes competing**

The fermentation process used to make the sausage uses added LAB as a culture starter that will lower pH, and compete with spoilage and pathogenic microbes.

**Lower water activity, $a_w$**

While fresh meat has an $a_w$ of 0.99, drying may reduce the $a_w$ to between 0.85 to 0.91 or even lower. *Campylobacter* requires a minimum $a_w$ of 0.987 for growth, so the final $a_w$ achieved will control growth. *E. coli* can grow at an $a_w$ of 0.95. Until $a_w$ is lowered, however, preservatives will retard growth. Acidic pH also works in combination to keep these pathogens under control.

**pH**

The fermentation process lowers the pH of fresh meat from around 6.0 to between 5.0 and 5.3 for dry sausage, and from pH 4.6 to pH 5.2 for semi-dry sausage. *E. coli* can grow when pH is as low as 4.0 to 4.9, so pH by itself is not always protective in sausages. Lower water activity is also required. Campylobacter prefers an environment with pH between 6.5 and 7.5, and will not grow when pH is below 4.9. However, pH tolerance is temperature-dependent, as the environmental temperature will impact how well the microorganism adapts to the hurdle of a particular pH.

**Preservatives**
Nitrites and nitrates are preservatives required when preparing fermented sausages, and these chemical compounds will limit growth of pathogens. Ethanol is produced during the fermentation process, and also has antimicrobial properties. Salt also has an inhibitory effect on some microbial growth. Smoked sausages will also receive some inhibitory effect from the smoke, although not considered an added preservative.

**Refrigeration or freezing**
Most sausages are either frozen or refrigerated, and are usually packaged. Microbes will not grow when frozen, or grow very slowly under refrigeration, making this an effective additional hurdle.

**Reduced oxygen packaging**
Sausage packaging is usually vacuum packed. If refrigerated, packaging will likely be in some kind of modified atmosphere packaging (puffed up plastic packaging). Both types are reduced oxygen packaging, which limits spoilage organisms' ability to grow without oxygen.

## Eggs and cream

Chosen hurdles could be pasteurization, cooking or heating, and refrigeration or freezing.

**Pasteurization, cooking or heating**
Cream is made from milk with a higher fat content and is pasteurized before sale. The principle hurdle for most dairy products is pasteurization, the goal of which is to reduce the presence of potential pathogens to tolerable levels. Milk pasteurization involves heating to 72°C for 15 to 20 seconds and then quickly cooling it. Higher temperatures can be used, requiring less time. Pasteurization does not typically remove all microbes; spore formers will survive. Most eggs are not sold pasteurized, but they can be pasteurized in the shell in lower temperature water baths for longer times. Effective pasteurization for eggs is 60°C or lower for 45 minutes or longer. At these temperatures the egg white will not turn white, but pathogens in the shell eggs are eliminated.

**Refrigeration or freezing**
Cream and eggs are sold refrigerated and this will extend the shelf life of these products. Once a package of cream (or milk) is opened, spoilage microbes may enter. In Canada, eggs are pre-washed to remove outer soil, but this damages the shell. Refrigeration is required for eggs in Canada, to slow and inhibit the growth of Salmonella that may be inside the shell or still on the outside.

## Spinach

Chosen hurdles could be pasteurization, cooking or heating, and refrigeration or freezing.

**Pasteurization, cooking or heating**
Fresh spinach is highly perishable, lasting a very short time after harvest. To destroy microbes it is common to wash and blanch the spinach. Blanching is a process where vegetables are immersed in boiling water, then rapidly cooled.

**Refrigeration or freezing**
Fresh spinach is highly perishable, lasting a very short time after harvest. To preserve spinach, it is common to refrigerate it to keep it fresh longer, or to freeze into blocks. Microbes cannot grow at frozen temperatures.

## Cheese (ricotta, parmesan or mozzarella)

Chosen hurdles could be fermentation/microbes competing, low $a_w$; pasteurization, cooking or heating, pH, refrigeration or freezing, or ROP.

**Fermentation/microbes competing**
Fermentative lactic acid bacteria (LAB) from a commercially purchased starter culture are used to make all of the cheeses in our lasagna. Rapid growth of LAB will reduce pH and prevent growth of other spoilage and harmful microbes.

**Low $a_w$**
Our lasagna contains ricotta, dry parmesan, and mozzarella cheese. Milk has a high $a_w$ of 0.97 or higher. There are differences in the final water activity of each type of cheese, but generally the $a_w$ is lower than milk, though not protective enough to allow the cheese to go unrefrigerated (another hurdle). Parmesan is aged (not dried) for a long time, so it has a very low water activity of less than 0.8 ranging to 0.9 depending on aging. If aged long enough it is normal to see parmesan stored at room temperature, however, after opening it is best to refrigerate.

**Pasteurization, cooking or heating**
The principle hurdle for most dairy products is pasteurization, the goal of which is to reduce the presence of potential pathogens to tolerable levels. Milk pasteurization involves heating to 72°C for fifteen to twenty seconds and then quickly cooling it. Higher temperatures can be used, requiring less time.

**pH**
Our lasagna contains ricotta, dry parmesan, and mozzarella cheese. Milk normally has a pH of 6.5 to 6.7. The process of making these cheeses will lower the pH but there are differences in the final pH of each type of cheese, as each process is slightly different. Ricotta cheese has a pH of 5.9 to 6.0. As this cheese has a high moisture content and relatively high pH, ricotta provides an appealing environment for many pathogenic bacteria, including Listeria monocytogenes, and so has a fairly short shelf life. Ricotta should be refrigerated as an additional hurdle. Mozzarella cheese has a lower pH of 5.5 or less and parmesan has a slightly lower pH ranging from a pH of 5.0 to 5.4. The $a_w$ of the cheeses also differ: mozzarella is typically refrigerated, while refrigeration for parmesan may not always be necessary, when water activity is lower.

**Refrigeration or freezing**
Parmesan may be stored under refrigeration or at room temperature. Mozzarella is a semi-soft cheese and so should be refrigerated. Depending on its type and how fresh it is, the pH of mozzarella may be around 5.2 to 5.5. Because its moisture content is lower than that of ricotta, mozzarella has a longer shelf life, but is still a suitable substrate for microbial growth. If ricotta or mozzarella cheese is left out of the refrigerator for any length of time, Listeria, Salmonella, or other bacteria (if present) could grow to dangerous levels.

**Reduced oxygen packaging**
All three of these cheeses are likely to be packaged in some kind of ROP to exclude air and prevent microbial growth.

**Part 2: Hurdle identification while assembling lasagna**

| Step | 1. Prepare noodles | 2. Prepare tomato and meat sauce | 3. Prepare cheese and spinach sauce | 4. Assemble the lasagna | 5. Bake the lasagna | 6. Store leftovers |
|---|---|---|---|---|---|---|
| Pasteurization or heating | ✔ | | ✔ | | ✔ | |
| Cooling | ✔ | | | | | ✔ |
| Refrigeration | ✔ | | ✔ | | | ✔ |
| Freezing | | | | | | ✔ |
| Time | | | ✔ | ✔ | | |

**Step 1.** Once the lasagna noodles are cooked, the $a_w$ becomes more suitable for microbial growth in general and especially for bacteria. Therefore, the noodles should be cooled and refrigerated to inhibit the growth of microorganisms if they will not be used immediately. In general, cooked noodles should be used within two hours or refrigerated before assembling.

**Step 2.** Since the tomato sauce is from a can and the meat is already fermented, these ingredients could be mixed together and not cooked any further to prepare the meat sauce. If the meat were raw, then all the sauce components should be heated to at least 74°C.

**Step 3.** Partial cooking to thicken the sauce is required during preparation of the cheese and spinach sauce. The flour is mixed with cream and heated. Eggs are mixed with ricotta cheese, and then spinach is added. Further heating may help to thicken the sauce. Once prepared the sauce should be used within two hours or refrigerated.

**Step 4.** When assembling the lasagna, the main hurdle to be aware of is time. All the food ingredients have undergone some mixing, chopping, shredding, or manipulation. Microbes may have entered any one of the ingredients or the mixes, and potentially could grow. Limiting the time for assembly will lower the risk of microbial growth.

**Step 5.** Once all the ingredients have been assembled, the lasagna and whatever invisible microbes remain is placed in the oven, one of the last hurdles of defense against any microorganisms that have survived thus far. However, in order to kill any contaminating microorganisms present, we need to ensure the lasagna reaches a suitable internal temperature of 74°C—the thermal death point for potential pathogens.

**Step 6.** There is the risk that any toxins or spores present in the assembled lasagna are not inactivated by the heating process. This is why using ingredients from reputable sources is paramount to ensuring food safety. Once the meal is finished, cooling and properly storing the lasagna in a clean refrigerator at less than 4°C becomes the next hurdle we use to minimize the opportunity for growth of any remaining pathogens, including any that were present after preparation and cooking.

## Lesson F Quiz

1. b. Hurdles or barriers
2. b. Its sight, taste and odour
3. b. Acidity
4. b. Canning
   f. Pasteurization
5. b. They minimize changes to the taste and nutritional value of the food.
6. c. It pauses microbial growth by making water unavailable while food is frozen.
7. a. It is a reminder of the importance of temperature control from farm to fork
8. a. A lack of oxygen prevents the growth of aerobic bacterium.
9. b. Carbon dioxide and oxygen levels are kept constant to reduce oxidation.
10. c. Nitrogen: carbon dioxide levels are increased from normal atmospheric air to suppress spoilage and bacterial growth.
11. Heat treatment—extrinsic
    Water activity ($a_w$)—intrinsic;
    Competitive microbes—instrinsic
12. c. worker efficiency
    f. Label design
13. d. No microbes will grow in foods that have a pH less than 4.6 because these are defined as non-potentially hazardous foods.

# Lesson G: Assessing Risk in Food Recipes and Determining Control Points

## Activity: Hazards in salmon

Chemical hazards: oil spill, PCBs, antibiotic residue

Physical hazards: bones, stones, fishing hooks

## Activity: Food flow-chart

1. Your flow chart likely reflected the recipe steps in the first version. It would have looked something like this:

| Process steps |
|---|
| Mix salt and sugar and spread half into 8 × 8 tray. ↓ |
| Crack eggs, remove yolks, and place into wells in tray. ↓ |
| Cover with remaining mix. ↓ |
| Let stand four days. ↓ |
| Refrigerate until use. |

2. The main hazard associated with this recipe is *Salmonella*. The processing itself involves quite a lot a handling (i.e., with hands), therefore *Staphylococcus aureus* and *Streptococcus* may also be considered potential hazards. The principle hazard of concern, however, with eggs is *Salmonella.*

3. There is no CCP in this recipe. Once you considered the hazard and the process, you should have realized that there was no cook step to control for *Salmonella* in the egg yolk, and there was no temperature control during the "let stand" step. Dehydrating the egg yolk in the salt/sugar mixture will draw moisture out of the egg yolk and reduce the yolk water activity. This may stop the existing *Salmonella* in the egg yolk from growing to higher numbers, but it would not kill any bacteria already present. To reduce risk in this recipe and reduce the likelihood of foodborne illness, then, adding a cook and cool step is required. This changes the food flow-diagram from the initial version to an amended version as follows.

Food flow diagram that follows the recipe:

| Process steps |
|---|
| Mix salt and sugar and spread half into 8 × 8 tray.<br>↓ |
| Crack eggs, remove yolks, and place into wells in tray.<br>↓ |
| Cover with remaining mix.<br>v |
| Let stand four days.<br>↓ |
| Refrigerate until use. |

Amended food flow diagram that includes a CCP cook step:

| Process steps |
|---|
| Mix salt and sugar and spread half into 8 × 8 tray.<br>↓ |
| Crack eggs, remove yolks, and place into wells in tray.<br>↓ |
| Cover with remaining mix.<br>↓ |
| Let stand four days, covered in the refrigerator.<br>↓ |
| Cook.*<br>↓ |
| Cool.<br>↓ |
| Refrigerate until use. |

*CCP

**Figure 1** — Raw egg yolks covered with salt and sugar mix before refrigeration and curing (dehydration)

4. This question asked you to identify critical limits and monitoring for the CCP. The outcome is to achieve a 7 log reduction of *Salmonella*. Any time and temperature combination that achieves this is acceptable. Generally, temperatures above 55°C are needed: a time/temperature table should be consulted (see CFIA link: https://www.inspection.gc.ca/food/archived-food-guidance/meat-and-poultry-products/manual-of-procedures/chapter-4/annex-d/eng/1370527526866/1370527574493.) The interior temperature of the egg yolk must be held at this temperature for the minimum time stated or longer. For example, hold at 57°C for thirty-six minutes, or at 63°C for four minutes. Either are acceptable.

5. This question asked you to identify critical limits in the process. Similar to the boiled egg example, in this process there are temperature-control points that should be addressed:

   a. The hold time "let stand for four days" must be done in refrigerated conditions so that any existing bacteria do not have an opportunity to grow,
   b. Once the yolks are cooked, they must be cooled within six hours to at or below 4°C ; and
   c. Storage must also be under refrigeration at or below 4°C.

**Figure 2** — Egg yolks after curing step

| Process steps | CCP? | Critical limit | Monitoring |
|---|---|---|---|
| Mix salt and sugar and spread half into 8 × 8 tray. ↓ | | | |
| Crack eggs, remove yolks, and place into wells in tray. ↓ | | | |
| Cover with remaining mix. ↓ | | | |
| Let stand four days covered in the refrigerator. ↓ | | Refrigerate at or below 4°C | |
| Cook. ↓ | Yes | Time and temperature to achieve 7 log reduction of *Salmonella* | Measure and record the internal temperature of an egg yolk with a probe-tip thermometer |
| Cool. ↓ | | Cool to at or below 4°C within six hours | |
| Refrigerate until use. | | Store at or below 4°C | |

## Lesson G Quiz

1. c. Microbial contamination of foods can occur at many different steps during processing.
2. d. when the menus or products have been determined
3. c. Assess whether the process step is one where microbes can be controlled.
4. c. Multiple ingredients and several handling steps.
5. c. Steps in a process may include just critical control points, just critical limits, or both.
6. b. A record of time and internal temperature kept next to cooling food
7. c. Durable life or best-before date
8. a. Operational control
9. b. Prerequisite program
10. c. Environmental control
11. a. Operational control
12. c. Checking temperatures during processing
13. d. Every process step should be evaluated for time, temperature and factors that contribute to microbial growth in food

14. c. Continuing to cook a casserole found to be undercooked

15. Step 1: Soak beans in refrigerated or acidified water, otherwise B. *cereus* toxin may form.

# Lesson H: Food Identity, Food Standards, and Certification

## Lesson H Activity: Criteria for ice cream

**Task 1:**

| Ice cream | *n* | *m* | *c* | *M* |
|---|---|---|---|---|
| Aerobic colony count (ACC) | 5 | 10^5 | 2 | 10^6 |
| Coliforms | 5 | 10^1 | 1 | 10^3 |

**Task 2:** Lot 1 fails because ACC in Sample 3 exceeds M; Lot 2 passes because two samples are permitted to be between log 5 and log 6 ACC, as long as no single sample exceeds M at log 6; Lot 3 fails because coliforms on sample 3 exceed M.

## Lesson H Activity: Criteria for *Listeria Monocytogenes* testing

| Category | Number of samples to test per lot? | Enrichment or direct plating? | Action level | Health risk |
|---|---|---|---|---|
| 1 | 5 sample units (min 100 g or ml each), which are representative of the lot and the production conditions, taken aseptically at random from each lot | Enrichment only | Detected in 125g [f, g] | Health Risk 1[h] |
| 2a | Same as above | Direct plating only | > 100 CFU/g [j, k, l] | Health Risk 2[h, l] |
| 2b | Same as above | Direct plating only | > 100 CFU/g [j, k, l] | Health Risk 2[h, l] |

## Lesson H Activity: Acceptance criteria for mushrooms

| Mushrooms | *n* | *c* | *m* | *M* |
|---|---|---|---|---|
| Maggots ≥ 2mm | 6 | 2 | 10/100 g | 20/100 g |
| Mites (dead) | 6 | 2 | 0/100 g | 75/100 g |
| Nematodes | 6 | 0 | 0/100 g | 0/100 g |

## Lesson H Quiz

1. c. The public has a right to know what is in the foods they are purchasing and consuming.

2. a. The act of deliberately using an alternate ingredient for economic gain

3. c. Halal
   e. Kosher

4. c. They determine the maximum number of lot samples that may exceed a set microbiological value.

5. c. Presence/Absence System

6. a. When the alteration is done intentionally and leads to economic gain

7. c. Certification is a requirement of global retailers and wholesalers to ensure food safety.

8. d. Standard of identity

9. a. Promotes food security and fair trade

10. b. Provides up-to-date information about pesticide use and GMO foods

11. c. Sets standards for food technology, aircraft and information technology

# Glossary

**Active packaging**: Smart or intelligent packaging that can control or display the internal environment of a packaged food beyond just covering and protecting the food; e.g., contains a chemical or agent to protect food from spoilage.

**Acute disease**: The phase of illness that is typified by short duration and recent onset (as opposed to chronic, which is long-term).

**Adulteration**: The act of deliberately making something poorer in quality by substituting another ingredient.

**Aerobic**: In the presence of oxygen or air.

**Aerotolerant**: Microbes and organisms that can grow in the presence of oxygen and air.

**Allergenic**: A substance that is capable of producing an allergenic (immune) response.

**Allergen**: A substance that causes an immune response in the host, e.g., food, dust or pollen etc.

**Antioxidant**: An agent that stops oxygen deterioration, in food it prevents spoilage.

**Anaerobic**: Absence of oxygen or air.

**Antibiotic**: A substance that kills bacteria and microbes.

**Antimicrobial**: A substance that will kill or damage microbes.

**Asymptomatic carrier**: A person who carries an illness but does not show symptoms of illness.

**Attribution**: Assigning a cause for a current disease or syndrome to a prior illness, e.g., Guillain-barre syndrome is attributed to prior *Campylobacter* food illness that may develop many years later.

**Barrier**: Obstacle or limitation.

**Backslopping**: The practice of inoculating a new batch of food with a portion of the prior batch of food culture substrate. Active microbes from the fermented batch will begin the fermentation process in the new batch of food.

**Bacteria**: A small microorganism, invisible to the human eye without the aid of a microscope. Bacteria are made of one cell that multiplies by dividing itself asexually. There are many different types of bacteria, some beneficial and some harmful.

**Bacterial succession**: Describes the changing population of microbial groups, one after the other, in a substrate. As environmental conditions in the substrate change, the types of microbes living in the substrate will change, replacing each other.

**Bacteriocidal**: The ability to kill bacteria.

**Bacteriocin**: A chemical that inhibits microbes by slowing the growth or killing the microbe. The chemical compound can be manufactured and released by one species or type of microbe to inhibit another species or type of microbe.

**Bacteriophage**: A virus that infects bacteria.

**Best before date**: Date applied to products that indicates time for optimal quality after which olfactory quality may deteriorate; not an indication of safety of product.

**Binary fission**: Asexual reproduction of microbes that multiply themselves by dividing.

**Biofilm**: Mat or community of microbes stuck together by a slimy coating.

**Broad-spectrum**: Referring to antibiotics that have a wide range of target microbes.

**Canning**: A method of food preservation that uses thermal process of heat and pressure in an airtight container (hermetically sealed) in boiling water or steam. A *C. bot* canning process, also known as a retort, will allow for a 12-log reduction of spores, typically 240°C at 10 to 15 psi (atmospheric pressure).

**Carbohydrate**: A chemical compound made up of carbon, hydrogen, and oxygen used as an energy source by microbes.

**Carrageenan**: A chemical compound extracted from algae that forms a gelatinous structure.

**Case**: Term used to describe the ill person.

**Catabolism**: In a living organism, these are the chemical conversions of compounds into smaller compounds resulting in release of energy to the organism.

**CCP deviation response**: Another term used to describe correction actions. These corrective actions refer to those steps taken when the CCP is not achieved.

**Certification standards for industry**: Audit programs that specify criteria for food business management; businesses that meet the objectives of the program will become certified under the program.

**Cestode**: A parasitic worm characterized by ribbon-like shapes, commonly known as tapeworms.

**Chronic disease**: The phase of illness that is typified by long duration as the illness progresses (as opposed to acute, which is short-term).

**Clean and sanitize (C&S)**: The term applied to a series of activities that will reduce levels of microbial contamination to an acceptable level (steps are often described as remove soil, clean, rinse, sanitize).

**Clean in place (CIP)**: Referring to equipment that must be cleaned where it is located; often equipment that cannot be dismantled.

**Clean out of place (COP)**: Referring to equipment that can be moved and cleaned elsewhere, e.g., in a dishwasher or that can be dismantled.

**Clinical specimen**: Stool (fecal), blood, vomitus or other sample taken for testing from a case, typically during illness.

**Clinical symptoms of illness**: Description of disease manifestation, e.g., diarrhea, vomiting, fatigue, headache, etc.

**Close-to-food contact surface (C-FCS)**: These are environmental surfaces that are not in direct contact with food but are adjacent to them, for example the wheels or underside of a table that is in contact with food.

**Cocci**: Adjective used to describe a round-shaped bacteria.

**Colony forming units (CFUs)**: A method to count bacteria on an agar plate. A single bacteria will grow into a spot or colony on an agar plate. The number of colonies indicate the number of bacteria in a sample.

**Commercially sterilized**: Thermally processed food that eliminates spores and microbes to allow room temperature storage of foods (e.g., canned foods).

**Communicable illness**: An illness that can be transferred from contact with a contaminated person, animal or object through direct blood and body fluid secretion exposure, respiratory or fecal-oral routes.

**Confirmed illness**: A definition applied to a disease that normally indicates subjective and objective evidence exists. Subjective evidence could be symptoms and onset consistent with a disease, objective evidence could be a positive clinical or food sample to support the diagnosis.

**Congenital infections**: Illness and disease that is acquired from birth, meaning the baby gets the infection during pregnancy.

**Contagious**: Infectious illness that can be spread from one person to another via airborne or person-to-person contact.

**Controlled atmosphere**: Packaging that controls the amount of oxygen and normal atmosphere around a food.

**Control point**: A point at which a potential hazard may be managed or controlled.

**Corrective actions**: Steps and activities that will occur when a CCP or control point are not met. Actions taken should be thought out and planned ahead of time and are often standardized responses. Corrective actions should include three points: (1) what the action is, (2) who takes it, and (3) where the information about the corrective action is recorded.

**Critical control point**: A point at which a potential hazard must be controlled or managed because there is no other place in the process to manage the risk; after this point hazards cannot be controlled. In food processes this is often a cook (kill) step, e.g., pasteurization.

**Cross contamination**: When one surface comes into contact with another surface that allow the transference of microbes. Surfaces can refer to hands, counters, foods, hosts etc. Transference may take the form of direct or indirect contact such as through blood, aerosols, hand-contact, dust etc.

**Cyanobacteria**: A family of bacteria characterized by its ability to live anaerobically in extreme environments; a more ancient line of bacteria.

**Death phase**: The time period when many bacteria in a population of bacteria will begin to die off, due to lack of nutrients or some other factor. This phase will occur following a stationary phase.

**Dehydration**: Loss of water through drying or desiccation.

**Detergents**: Cleaning agents that by definition act as surfactants and can break up oily residues.

**Deviation**: An occurrence that is outside of the norm or standard; may refer to a food processing error.

**Differential diagnosis**: The act of comparing and contrasting between two conditions or illnesses that share the same symptoms and factors.

**Disease sequelae**: A condition arising from a prior illness or injury, often a condition arising over time.

**Disease transmission**: The pathway or method of disease going from one person to another, often person to person via hand or fluid contact.

**Disinfectants**: A chemical agent that can kill microbes, often these agents are engineered and marketed to meet prescribed levels of killing, e.g., a 7-log reduction or 99.99999% removal of virus.

**Diversion**: Movement away from intended path, in fraud the distribution of legitimate products away from intended markets.

**Durable life**: The period when a prepackaged food will retain its freshness, wholesomeness and qualities as designed by the manufacturer; date begins on day of packaging.

**Duration of illness**: The time period for the acute stage of illness.

**D-value**: Standing for decimal reduction time, this is the time required to achieve a single log reduction (90% reduction) at a given temperature or set of conditions for a specific microbe.

**Endospore**: A structure within a bacteria that is resistant to environmental extremes, such as hot and dry conditions, and is a precursor to dormancy.

**Environmental control**: The specific control policy for a prerequisite program. For example, in the processing area, temperature requirements are that room is maintained at 10°C or less.

**Ethanol fermentation**: The conversion of sugars by yeast into alcohol.

**Etiologic agent**: The microbe that causes an illness.

**Etiology**: The cause or condition for an illness.

**Exclusion policy**: A worker policy that provides guidance on how long a worker must stay home if they are ill. May also specify tests workers may need to undertake to demonstrate the illness is resolved.

**Expiry date**: The date on a food product after which nutritional or active components of the food may be compromised, e.g., baby formula or yeast.

**Extraneous materials**: Additional, unnecessary or unrelated foods.

**Extrinsic factors**: Conditions external to the substrate environment of microbes that may affect growth and survival, examples include temperature, time that conditions persist, relative humidity, nutrient availability, competition with other microbes, levels of oxygen, etc.

**Facultative anaerobe**: Bacteria and microbes that grow in the presence of oxygen although prefer to grow in the absence of oxygen.

**FAO**: Acronym for an international agency: Food and Agriculture Organization of the United Nations.

**Fecal-oral transmission**: The transference of a disease-causing agent from the feces of one host to the mouth (oral) of another host.

**Fermentation**: The conversion of complex carbohydrates and proteins through microbial activity into simpler carbohydrates, sugars, amino acids with other metabolites.

**Flatworm**: A parasitic worm characterized by an unsegmented flat body shape, called playthelminthes.

**Fluke**: A parasitic worm characterized by a complex life cycle that normally infects shellfish and vertebrates, called trematodes.

**Food contact surface (FCS)**: A surface that has direct contact with food, e.g., a table or slicer.

**Food defence:** The desire to create harm and terror, and sometimes economic gain. Harms arising from food defence issues include public health harms, such as food poisoning.

**Food fraud**: A criminal activity involving food (substitution, adulteration, diversion) for the purpose of profit.

**Food intolerance**: Difficulty in digesting some foods, does not cause an immune reaction.

**Food poisoning**: A common term used to refer to foodborne illness.

**Food microbiology**: The study of microbes in food matrices.

**Foodborne infection**: Illness caused from ingestion of harmful microbes that, once ingested, multiply in the host animal (e.g., human) and causes symptoms such as nausea, vomiting, diarrhea, etc.

**Foodborne intoxication**: Illness caused from the ingestion of a preformed toxin that is produced by a microbe, (often bacteria, but sometimes harmful algae) and cause symptoms such as vomiting, difficulty breathing, paralysis, etc.

**Foodborne pathogen**: A microbe that can be found in food and that can cause illness.

**Freeze-drying**: Sublimation of water from a food through temperature reduction and pressure.

**Fumigation**: An aerosol fog that has a disinfecting property.

**Fungi**: A microorganism that is multi-celled, visible to the human eye, although often growing underground. Fungi are made up of several families, including mushrooms, moulds, yeasts, and other types. Some fungi grow asexually and some grow sexually.

**Fungicidal**: The ability to kill fungi.

**Gastroenteritis**: Symptoms of illness that occur in the gut. Diarrhea, vomiting, abdominal cramps.

**Gastrointestinal symptoms**: See gastroenteritis.

**Generally regarded as safe (GRAS)**: A term used in the United States to define processes that have minimal risk and are generally regarded as safe.

**Glycolysis**: A chemical pathway that creates energy (ATP) from the conversion of glucose into pyruvate.

**GMO**: Acronym for genetically modified organisms.

**Gram staining**: A technique used to chemically stain bacteria to differentiate them into two groups: gram positive (purple colour) or gram negative (red colour); the colour depends on the outer cell wall structure of the bacteria.

**Gut microbiota**: The microbes (bacteria, virus, parasites, fungi etc. that naturally inhabit the digestive tract.

**Habitat**: Where a microbe resides, a place of residence.

**HACCP – hazard analysis and critical control points**: Aa system designed to manage and control risk in a food process.

**Halal**: A term for "acceptable" (permissible) in the Arabic language; in food, halal-slaughtered meat or other foods should conform to the Islamic religious law and standards.

**Haloduric**: Bacteria that grow in the presence of high salt concentrations and are capable of causing meat spoilage.

**Halophile**: Microbes that prefer to grow in salty (saline) environments, from ~2% to 30% salt.

**Halotolerant**: Microbes and organisms that can grow in the presence of salt.

**Harbourage**: Referring to a place where microbes can reside, e.g., in a biofilm or in a crevice.

**Hazard**: A biological, physical or chemical condition normally present in a food that presents risk. Hazards are potentially present; management of the process will address risk.

**Helminth**: The term used for parasitic worms that infect the gastrointestinal tracts of vertebrates. This description includes flatworms (Platyhelminthes), tapeworms (cestodes), flukes (trematodes) and roundworms (nematodes).

**Hermetically sealed**: Vacuum-packaged with a sterile seal.

**Heterofermentative (heterofermentive)**: A mixed group of bacteria that converts foods through microbial activity into fermented foods. May also refer to multiple food end-products.

**Histamine poisoning**: A toxin-mediated foodborne illness (chemical hazard). Poisoning occurs when naturally occurring muscular histidine is converted to histamine via bacterial activity. Tuna species are often implicated. Bacterial growth occurs when fish is temperature-abused. The toxin is heat-stable; therefore canning (canned tuna) can contain the toxin.

**Homofermentative (homofermentive)**: A single group of bacteria that converts foods through microbial activity into fermented foods. May also refer to single food end-products.

**Homeostasis**: Tendency to a stable equilibrium.

**Horizontal transmission**: When disease agents are transferred from one host (individual) to another host through non-genetic methods such as direct contact, droplets (aerosols) from body fluids, feces, or indirect contact.

**Host**: The animal or living organism that carries a harmful microbe. Often referring to an entity that can carry a disease.

**HTST**: High temperature/short time pasteurization method, also known as flash pasteurization. Actual temperatures and times vary according to product.

**Hurdle**: A barrier or obstacle; in food microbiology referring to a condition that challenges microbial survival.

**Hurdle technology**: The application of more than one obstacle or set of barriers that impede microbial growth.

**Immunocompromised**: A person or other host who is vulnerable to disease for one or more reasons. Having a poor immune system unable to ward off disease.

**Incubation period**: Once it enters the host, the time it takes for a microbe to cause symptoms of illness.

**Indicators and indicator tests**: These types of analytic tests look for harmless microbes in food, water or air. In addition to harmless microbes, specific enzymes or compounds are also used as indicators. A good indicator test will be easier to detect, inexpensive, and present in high numbers testing for one or more pathogens. Indicators are surrogates for pathogens. For example, coliforms and *E. coli* are used to indicate poor hygiene and sanitation in food and water. When high levels of coliforms and *E. coli* are detected the likelihood of more pathogenic fecal organisms, such as shigatoxigenic *E. coli*, *Salmonella* or *Campylobacter* is probable.

**Infectious**: Spread or transmission of disease.

**Insecticidal**: The ability to kill insects.

**Intrinsic factors**: Conditions internal to the substrate environment of microbes that affect growth and survival. Examples include pH, water activity, reduction-oxidation, salt concentration, inhibitors present, etc.

**Irradiation**: Objects exposed to ionizing radiation from natural or artificial sources; treatment of food to eliminate microbial activity.

**Kill step**: A procedure that destroys and reduces the number of microbes during processing; also known as the CCP.

**Kosher**: A term for foods that conform to the Jewish dietary regulations of dietary law.

**Label claims**: A generic term that encompasses mandatory and voluntary labelling on food products according to federal regulations; claims may be about content, origin, nutrients, health, quality, allergens etc.

**Lactic acid bacteria (LAB)**: A group of gram-positive bacteria that convert foods through microbial activity and are characterized by their production of lactic acid, lowering the pH of foods (acidifying) and outcompeting many spoilage organisms. Common bacteria found in a range of fermented foods.

**Lactic acid fermentation**: Microbial activity that creates a chemical process involving the conversion of glucose sugar to lactic acid by a number of different energy pathways.

**Lag phase**: The time period in a population of bacteria when there is a delay in population growth of bacteria. This phase occurs prior to the log phase.

**Log phase**: The time period in a population of bacteria when populations double during each replication cycle, termed exponential growth. This phase occurs following the lag phase and prior to the stationary phase.

**Lot acceptance**: Criteria that must be met for a production batch of food to be allowable for the retail market.

**Mesophile**: A microbe that prefers to grow at moderate temperatures. Optimal temperatures are between 20°C to 40°C, with minimum and maximum temperatures between 10°C and 50°C.

**Metabolic exhaustion**: A term describing the utilization of all energy sources in a cell, signalling depletion of sources of energy (ATP) and reduced ability to live or metabolize further.

**Metabolism**: Describes all the chemical activity in a living organism including creation (anabolism) of compounds for the organism and break-down (catabolism) of molecules for energy.

**Microaerophilic**: Bacteria that grow in the presence of oxygen, but in smaller amounts than what is normally found in air.

**Microbes**: A generic term used to describe micro-organisms including representatives from bacteria, viruses, fungi, protists, some worms and algae.

**Microbicidal**: The ability to kill bacteria, virus and other microbes.

**Microbiology**: The study of microbes.

**Microbiome**: A specific habitat or environmental space where microbes reside, for e.g., "human microbiome" would describe all the microbes living in and on a human versus "desert microbiome" would describe all the microbes living in a desert.

**Microcystin**: Toxins that are produced by fresh-water blue-green algae.

**Microorganism**: A generic term to describe all types of microbes such as bacteria, fungi, parasites and viruses.

**Mislabelling**: Incorrect label claims made about a food product, may be inadvertent or deliberate.

**Mitigate**: To control, correct, or make less severe; to reduce harm.

**Modified atmosphere packaging (MAP)**: Atmospheric packaging that uses specific ratios of reduced oxygen and higher nitrogen and carbon dioxide levels to slow aerobic bacterial growth (spoilage bacteria). Inert nitrogen gas is also used to flush packages of food to preserve and protect food during shipping.

**Monera**: A branch of microbes.

**Monitoring procedures**: Activities taken during processing that objectively observe the process to ensure that standards are met. Monitoring may include visual procedures (e.g., absence of mould on vegetables before processing) or measurements (e.g. temperature record of a cook process).

**Mould**: A type of fungi that can be beneficial or harmful depending on where and how it is growing. E.g., Penicillium moulds are useful in making antibiotics versus Aspergillus moulds that can cause crop damage and foodborne illness.

**Mycobactericidal**: The ability to kill mycobacteria, e.g., tuberculosis.

**Mycotoxin**: (Greek origin) Toxic substance produced by fungal organisms; can cause disease.

**Nematode**: A parasitic worm characterized by its round shape, commonly called a roundworm.

**Non food-contact-surface (N-FCS)**: Surfaces that are not near to food such as drains, floors in washrooms etc.

**Obligate**: This is an adjective meaning "required." For example, "obligate aerobe" refers to a microbe that must have oxygen to survive.

**Odds ratio**: Statistic that quantifies the strength of an association between two events, A and B.

**Operational control**: The specific control policy for a prerequisite program. For example, a shipping operational control might be to only accept foods from cold-chain certified companies. A receiving operational control would be to require of employees that they put perishable foods away with two hours of receipt.

**Organic**: In food systems, using animal or vegetable origin fertilizers and pesticides; natural vs. synthetic.

**Organoleptic**: Referring to the senses: taste, odour (scent), sight and touch.

**Osmotolerant**: Microbes that can tolerate high solutes (for example, high salt or high sugar) in the environment.

**Outbreak**: When two or more individuals become ill from a common exposure, e.g., a common food or water source. Illness is often linked by exposure in time, place and etiologic agent.

**Oxidation**: Process where atmospheric oxygen causes browning and spoilage in foods (rancidity).

**Parasite**: A multi-celled microbe that may or may not be visible to the human eye and that survives by growing in another host, where it causes damage to the host.

**Pasteurization**: A low heat method to reduce microbial numbers in foods but not eliminate spores.

**Pathogen**: An infectious agent able to inflict damage and cause disease in a host organism.

**Pathogenesis**: The ability to cause disease.

**Pathogenic**: An adjective applied to microbes that are capable of causing illness.

**Pellicle**: A generic term for an outer hard surface layer. In food, it can mean the covering on the surface of meat, fish or poultry that allows smoke to better adhere to the surface of meat during the smoking process. It can also mean the SCOBY or layer that forms on top of fermenting kombucha.

**Persistent**: Continued presence.

**Persistent diarrhea**: Condition in which there is the continued presence of gastroenteritis-type stool for longer than one week.

**Person to person transmission**: The ability of a disease agent to travel from one host (person) to another host (person) through host contact, often fecal-oral or by fluid transfer.

**Pesticidal**: The ability to kill pests (rodents, insects etc.)

**pH**: The measure of how much acid (hydrogen ions, H+) or alkalinity (hydroxyl anions, OH-) are in a food or solution, based on a scale of 0 (most acid) to 14 (most alkaline). Acid foods will have a pH below 7, alkaline foods will have a pH above 7, neutral pH is at pH 7. Food safety definitions for potentially hazardous foods are those with a pH $> 4.6$. Canning processes differentiate low-acid foods with a pH $> 4.6$ as those foods requiring botulinum cook steps to control for *C. botulinum* spores in foods. Foods with a pH $< 4.6$ are called high-acid foods and can be canned with boiling water methods.

**Potentially hazardous food (PHF)**: A food with intrinsic properties favorable to microbial growth. For example moist (high water activity), neutral (pH of 7), high-protein or high-carbohydrate-rich food (source of nutrients) such as meats and rice.

**Prebiotic**: Ingredients that stimulate the growth of beneficial microbes.

**Prerequisite program**: In the food industry, these are written policy standards for preventative programs that address subjects such as employee hygiene and wellness, training, cleaning and sanitation, facility maintenance, shipping and receiving, storage, pest control, recall programs and others.

**Pressure cooking**: See canning; thermal cooking under a vacuum to increase atmospheric pressure; also known as "canning" or "retort".

**Primary fermentation**: The first step in a multi-step fermentation pathway.

**Prion**: A very small protein particle, capable of causing illness, once undergoing a spontaneous shape change.

**Probiotic**: Microbes that are considered beneficial for health and organisms microbiome.

**Processing factor**: An activity that affects food composition. For example, grinding meat will expose more surface area for microbial growth; washing produce will reduce microbial numbers.

**Protein**: An organic compound made of amino acids, essential for cells and nutrition. Amino acids contain the chemical nitrogen, essential as a building block for protein.

**Protozoa**: A small multi-celled microbe that may or may not be visible to the human eye. Harmful protozoa include *Giardia*, *Cryptosporidium*, and Toxoplasmosis.

**Psychrophile**: A microbe that prefers to grow in cold temperatures. Optimal temperatures are between 0°C to 10°C, with minimum and maximum temperatures between -20°C and 20°C.

**Psychrotroph**: Microbes able to feed and grow at refrigeration temperatures down to freezing temperatures.

**Pyruvate**: A chemical containing 3 carbons that is a breakdown product of glucose (glucose contains 6 carbon atoms).

**Rancidity**: Spoiled foods arising from exposure to microbial activity, excess air (oxidation), light or moisture.

**Records**: Evidence that manually or electronically substantiates an activity. In food processing records may refer to temperature charts that record pasteurization or checklists that record that cleaning and sanitizing schedules are met.

**Redox**: Abbreviated term for reduction-oxidation, an intrinsic factor that affects microbial growth. Microbes are less able to grow in low redox substrates (less ability for energy reactions).

**Reduced oxygen packaging (ROP)**: Packaging that excludes or reduces oxygen; a generic term applied to all types of ROP; vacuum packaging—excluding all air; modified atmosphere packaging—excluding some air etc.

**Reservoir**: The source of an infectious organism, where the organism resides (inhabits) and multiplies.

**Residue testing**: The action of evaluating a surface for contaminants (protein, DNA, sugars, microbes).

**Retort**: See canning; usually refers to commercial canning operations designed to process low-acid foods to sterilization temperatures (above 110°C) allowing shelf-stability and room temperature holding.

**Risk**: The likelihood of an adverse event (hazard) occurring. For example, climbing a staircase there is a potential trip or fall hazard; there is lower risk of a trip or fall climbing stairs with a handrail and a higher risk of a trip or fall climbing a staircase without a handrail.

**Rods**: Adjective used to describe bacteria that have rectangular shapes.

**Roundworm**: A parasitic worm characterized by its round shape, also called a nematode.

**Sanitation**: Activities related to public health measures that ensure filth and disease is controlled through hand-washing, surface cleaning, controlling garbage and pests, etc.

**Sanitize**: Through use of chemical (e.g., bleach) or other means (e.g. UV light or steam) to reduce the levels of microbes to an acceptable level.

**Secondary fermentation**: A second or later fermentative step in a process that involves fermentation of the first substrate and the breakdown products into other substrates and compounds.

**Sensitivity and specificity**: Analytic tests are judged by two attributes. Ability to correctly identify the microbe of interest (finding the microbe when it is not there is termed a false positive); and specificity; how often they are able to detect the organism when it is there (inability is termed a false negative).

**Somatic**: A body cell or non-reproductive cell.

**Spiral shaped**: Adjective used to describe bacteria that have curvy spiral shapes.

**Spoilage**: Foods that have lost optimal quality and freshness and are said to be spoiled when they have off odours, off tastes or do not look visually fresh (bruised, damaged).

**Spore**: A structure that some bacteria have in their life-cycle when conditions become stressful, that allows them to survive in nutrient poor or harsh environments. Spores are generally resistant to heating.

**Stages of disease**: The progression of a disease within a host that describes initial infection and sequence of symptoms.

**Standard of identity**: A term that refers to the composition of a food product.

**Standard operating procedure (SOP)**: A document that describes how an activity is performed, such as how a food is made, how a piece of equipment is cleaned and sanitized (SSOP), how staff are to dress at work etc.

**Starter culture**: Describes a pure population of microbes used to start a fermentation process. May be commercially purchased and strain-specific.

**Stationary phase**: The time period in a bacterial growth phase where growth plateaus and the number of bacteria dividing into new cells is equal to the number of bacteria dying off. This phase follows the log phase.

**Stress reaction**: A change in metabolic state resulting from a barrier to hurdle; in microbes this may result in altered gene response to prepare for a new environmental condition (high acid, dryness, low nutrient availability etc.).

**Sublimation**: Transition state from solid directly to gas, without passing through liquid stage, e.g. from ice to vapour, without transition to liquid water/melted ice stage.

**Substitution**: Using an alternate (in food fraud, inferior) ingredient or food in place of the accepted claimed ingredient or food.

**Substrate**: The surface or material on which a bacteria will grow and get its food.

**Suspected illness**: A definition applied to a disease that normally indicates subjective evidence, such as typical symptoms or linkage to a common exposure.

**Symbiotic culture of bacteria and yeast (SCOBY)**: A mixed culture of microbes, typically bacteria and yeasts, that are used to ferment foods and beverages.

**Symbiosis**: Long-term and close association between two organisms.

**Systemic illness**: This is a serious type of illness that affects the entire body of the host, e.g., a bloodborne infection would be systemic (and not confined to a single organ or tissue).

**Tapeworm**: A parasite.

**Thermal death point (TDP)**: The temperature at which bacteria of a specific population (species) will be killed within a specified period of time.

**Thermalization**: Cooking of foods to a prescribed log reduction, often at lower temperatures than those used for pasteurization. Also referred to as thermization.

**Thermoduric**: Bacteria that survive the pasteurization process. These *Enterococci* and spore formers such as *Bacillus* and *Clostridium*.

**Thermophile**: A microbe that prefers to grow in hot temperatures. Optimal temperatures are between 40°C to 80°C, with minimum and maximum temperatures between 20°C and 120°C.

**Three class attribute plans**: A sampling plan for food with measurable criteria for the presence of microbes; a three class plan includes a tolerable level (m) and unacceptable level (M) based on sampling of a specific number of units (n) tested with the number of units not exceeding the tolerable level (c) and no units exceeding the unacceptable level.

**Tolerance (allowable and zero tolerance)**: In food, the limit at which a pathogen or indicator microbe of concern is permitted or allowable in the food.

**Toxigenic**: A protein often elaborated by a bacteria that causes disease in the host.

**Toxin**: A poisonous substance, produced by some microbes. Some toxins are generally resistant to heating.

**Trematode**: A parasitic worm characterized by a complex life cycle that normally infects shellfish and vertebrates, commonly called flukes.

**Two class attribute plans**: A sampling plan for food with measurable criteria for the presence of microbes; a two class plan includes a tolerable level (m) based on sampling of a specific number of units (n) tested with the number of units not exceeding the tolerable level (c) and no units exceeding the unacceptable level.

**UHT**: Standing for ultra high pasteurization, this involves a heat treatment at very high temperature above 130°C for a few seconds.

**Verification procedures**: Activities that ensure the HACCP process is adequate. In food processing this may refer to microbial testing of foods to ensure they meet recognized standards.

**Verification**: The activity of confirming or checking the accuracy of a process.

**Virus**: Small infectious particles that can only reproduce inside another organism; it cannot reproduce by itself.

**Vacuum packaging**: Sealed non-permeable packaging that excludes all air.

**Vegetative**: A term used to describe bacteria that are actively growing and not in a spore form.

**Vertical transmission:** When disease agents are transferred from a parent host (individual) to an offspring or progeny host (egg, embryo, infant) through genetic means or other means, e.g., milk or placental transfer.

**Viable but non-culturable**: This term describes a bacteria or microbe that is alive but fails to grow in standard microbial culture conditions in the laboratory. It is an issue as microbiologists may either fail to find a microbe of concern, or underestimate the numbers of this microbe.

**Virion**: A single viral particle. A very small microbe visible only with a special microscope.

**Virucidal**: The ability to kill virus.

**Vulnerable population**: The group of individuals in a population that are more susceptible to disease and illness.

**Water activity**: This describes the available water in a given substrate. It is not a measure of % moisture, rather how much water is unbound in a substrate. A liquid high-sucrose solution would have low water activity as the water molecules in the solution are bound by the sucrose molecules.

**Wild fermentation**: A spontaneous activity arising from naturally occurring microbes on a food substrate. Does not involve use of commercially purchased starter culture.

**Xerotolerant**: Microbes that can survive in dry environments, typically with a water activity below 0.8.

**Yeast**: A single-celled fungus that divides asexually by dividing itself; in some microbes like yeast the smaller replicate will bud off of the parent cell.

**YOPI (young, old pregnant, immunocompromised)**: The acronym applied to individuals within a population that have underlying factors that increase their susceptibility to disease.

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The FOODSAFE Secretariat gratefully acknowledges the contributions of the following individuals and organizations.

**Lesson A: Food Microbiology: Introduction**
**Figure 2.** This can, obviously under pressure, was received by the Vancouver Food Bank.
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**Figure 8.** Nutrient agar plates
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 9.** Bacteria of different sizes and colours growing on a nutrient agar plate.
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**Figure 20.** *Giardia* (upper left) and *Cryptosporidium* (lower right)
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 21.** *Trichinella* worms retrieved from bear meat
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 28.** Harmful algae in British Columbia: a) *Alexandrium catenella*, b) *Dinophysis acuminata*, and c) *Pseudonitzschia pungens* and *australis*. Photo source: Nicky Haigh, Microthalassia Consultants Inc., used with permission.

**Lesson B: Microbial Growth**
**Figure 1.** Bacteria growing on a nutrient agar plate
Photo source: Camosun College, used with permission

**Figure 9.** Growth of bacteria in the DANGER ZONE
Photo source: BCCDC, used with permission

**Figure 21.** Counts on semi-solid nutrient agar following a serial dilution (1X dilution left; 0.1X dilution, right).
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**Figure 22.** Growth of *E. coli* in letheen broth: positive reaction (left tube), negative reaction (right tube)
Photo source: L. McIntyre, BCCDC, used with permission

**Lesson C: Fermentation and Spoilage**
**Figure 8.** Enzymatic spoilage from temperature abuse causing gaping in a chum filet
Photo taken from booklet, *BC Salmon: Quest for Quality. On-Board Quality Guidelines*. Vancouver, Canada. (BC Salmon Marketing Council, 1995), used with permission

**Figure 13.** Oxidization (freezer burn) of prawns from prolonged storage
Photo source: L. McIntyre, BCCDC, used with permission

**Lesson D: Foodborne Illnesses**
**Figure 3.** Causes of foodborne illness in the Canadian population
Adapted from Thomas et al., 2013 (see Lesson D References)

**Figure 7.** Collection bottle and instructions for fecal (stool) samples for diagnosing foodborne illness
Photo source: L. McIntyre, BCCDC, used with permission

**Lesson E: Sanitation, Personal Hygiene, Pests, and Cross-Contamination**
**Figure 4.** Smoked meat processor before cleaning
Photo source: Graham Monda, used with permission

**Figure 5.** Smoked meat processor after cleaning
Photo source: Graham Monda, used with permission

**Figure 6.** Biofilm build-up on meat plant equipment before cleaning
Photo source: Graham Monda, used with permission

**Figure 7.** Meat plant equipment after cleaning
Photo source: Graham Monda, used with permission

**Figure 8.** Rusted equipment
Photo source: Graham Monda, used with permission

**Figure 9.** 5 stages of biofilm development
Photo source: *D. Monroe*. "Looking for Chinks in the Armor of Bacterial Biofilms". *PLoS Biology 5 (11, e307)*. DOI:10.1371/journal.pbio.0050307. *Wikimedia Commons.* Licensed under a Creative Commons Attribution 2.0 Generic (CC BY 2.5) license

**Figure 10.** Biofilm formations missed during cleaning
Photo source: Graham Monda, used with permission

**Figure 11.** Food batter found in food distribution pipe dead end
Photo source: Graham Monda, used with permission

**Figure 12.** Domestic bleach bottle with no concentration given on packaging. Domestic bleach is assumed to be have a 5.25% chlorine.
Photo source: L. McIntyre, BCCDC

**Figure 13.** Verification of sanitizer concentration at premises: bleach (left) and QUATs (right)
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 15.** Dirty chillers and drip trays are harbourage sites for moulds and Listeria. Fans will spread these microbes on air currents though-out the processing plant.
Photo source: Graham Monda, used with permission

**Figure 16.** Soil and food debris was found behind the panel cover of this equipment
Photo source: Graham Monda, used with permission

**Figure 17.** Zone concept diagram for environmental monitoring
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 22.** Rodents of concern in BC and identifying feces
Photo source: "FDO Guidelines with Grocery or Meal Programs," BCCDC, used with permission

**Figure 23.** Suffocated (dead) mouse protruding from bag of flour in a restaurant dry-goods storage room
Photo source: L. McIntyre, BCCDC, used with permission

**Lesson F: Microbial Barriers for Food Preservation**
**Figure 4.** Anasakid worms in sockeye salmon
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 5.** *Henneguya* sp. in pink salmon
Photo source: L. McIntyre, BCCDC, used with permission

**Lesson G: Assessing Risk in Food Recipes and Determining Control Points**
**Figure 8.** Raw egg yolks on salt and sugar mixture
Photo source: Dianna Vuu, used with permission

**Figure 9.** Raw egg yolks covered with salt and sugar mix before refrigeration and curing (dehydration)
Photo source: Dianna Vuu, used with permission

**Figure 10.** Egg yolks after curing step
Photo source: Dianna Vuu, used with permission

**Lesson G Answer Key—Activity: Food flow-chart**
**Figure 1.** Raw egg yolks covered with salt and sugar mix before refrigeration and curing (dehydration)
Photo source: Dianna Vuu, used with permission

**Figure 2.** Egg yolks after curing step
Photo source: Dianna Vuu, used with permission

**Lesson H: Food Identity, Food Standards, and Certification**
**Figure 5.** COR Kosher symbol card
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**Appendix A: Techniques for Counting and Characterizing Bacteria**
**Figure 1.** Food sample after mixing in a stomacher
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 2.** Serial dilution of food sample and inoculation into nutrient broth
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 3.** Plating of food samples and serial dilutions onto nutrient agar to establish standard plate count
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 4.** Counts from the nutrient agar serial dilutions (10 × dilution left; 100 × dilution, right)
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 5.** Growth of *E. coli* in letheen broth: positive reaction (left tube), negative reaction (right tube)
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 6.** *E. coli* O157:H7 bacteria growing on MAC agar (left) and S-MAC agar (right)
Photo source: L. McIntyre, BCCDC, used with permission

**Figure 7.** Types of nutrient agar used in a food poisoning investigation
Photo source: L. McIntyre, BCCDC, used with permission

**Appendix C: Pathogens**
*Cyclospora cayetanensis oocyts*
Photo source: BCCDC, used with permission

*E. coli* bacteria
Photo source: BCCDC, used with permission

*Listeria monocytogenes* bacteria
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*Salmonella* bacteria
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*Shigella* bacteria
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*Toxoplasma* parasites in tissue
Photo source: BCCDC, used with permission

*Yersinia* bacteria
Photo source: BCCDC, used with permission

**Appendix F: Food Production Chain**
**Figure 9.** Typical pH ranges for fruits
Photo source: L. McIntyre, BCCDC, used with permission

**Answer Key**
**Lesson G: Assessing Risk in Food Recipes and Determining Control Points**
**Figure 1.** Raw egg yolks covered with salt and sugar mix before refrigeration and curing (dehydration)
Photo source: Dianna Vuu, used with permission

**Figure 2.** Egg yolks after curing step
Photo source: Dianna Vuu, used with permission

# Contributors

## Lorraine McIntyre, Lead Writer

Lorraine McIntyre received her undergraduate degree from the University of British Columbia (1987). While working full time, she received her Master's degree in Public Health from the University of Hertfordshire in the United Kingdom (2005).

Lorraine has always been interested in science, beginning her career with several different research postings. In northern Alberta she investigated pathologies associated with canola before moving back to Vancouver to conduct *Giardia* and *Cryptosporidium* research. She joined the BC Centre for Disease Control in 1993, where she's held various positions. During this time she also worked part-time as a medical assistant setting up clinical specimens, and as an instructional science demonstrator in the science program at the Vancouver Community College, where she met her husband.

In the BCCDC Public Health Laboratory division, Lorraine supervised and coordinated water, food and gastroenteritis outbreaks, implementing BCCDC's first molecular norovirus diagnostic testing method. Her interest in food and drinking water issues facilitated her desire to work with Environmental Health. Lorraine investigates food issues and outbreaks, conducts applied research, and serves as a technical specialist providing advice to Environmental Health Officers (EHOs) in BC. She has led several multi-stakeholder groups to create guidance on a range of topics such as best practices for food distribution organizations, sous-vide-style cooking safety, and causes of environmental transmission of norovirus into oysters.

Lorraine has created a wide variety of food issue notes on the BCCDC website, and continues to investigate and publish on topical food issues ranging from ethanol issues in kombucha to *Salmonella* in frozen breaded chicken products, authoring over 30 peer-reviewed publications. Lorraine is currently (2022) the seafood/shellfish/meat food safety specialist at BCCDC, is chairing a national working group to create fermented food guidance and serves on the Health Canada food expert advisory group.

## Alex Montgomery, Subject Matter Expert Reviewer

Alex has over 20 years of experience in microbiology laboratories, covering both private and public institutions . His experience in this setting, from working with food producers in product development and quality control, to participating in food outbreak investigations, has taught him the value and impact of microbes on food quality and safety. Alex currently works as a microbiologist for the Canadian Food Inspection Agency.

## Kevin Touchet, Subject Matter Expert Reviewer

Kevin is a graduate of the BCIT's Environmental Health Program and holds a Graduate Certificate in Public Health Leadership from Concordia University of Edmonton. He started as a Public Health Inspector and has held various positions, including Manager of Food Safety, before his current role as Manager of Environmental Health for Interior Health.

Kevin is a certified FOODSAFE Instructor and taught FOODSAFE at Thompson Rivers University for 30 years. He has assisted with numerous FOODSAFE Program content reviews and updates during this time.

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Lis worked as an Environmental Health Officer with Vancouver Coastal Health for 26 years, inspecting all types of food facilities and other premises under the Public Health Act. She spent 10 years as a Senior EHO specialist in Communicable Disease Control, Pest Control, Personal Services, Special Events and Quality Assurance.

Lis received a BSc from UBC in 1989, BTech from BCIT in 1991, and a MSc from the University of London in 2010.

She continues to be passionate about teaching FOODSAFE courses.

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ISBN: 978-0-7726-8076-1
9 780772 680761

7960003987