Fundamental Food Microbiology

Objectives

1. Identify the basic types of microbes.

2. Describe the typical bacterial growth pattern, and explain important factors affecting microbial growth.

3. Describe basic mechanisms and indications of microbial food spoilage.

4. Describe how certain microbes are used in food preservation.

5. List important pathogens of concern in meat and poultry products.

6. Describe sources of microbes in meat and poultry products.

7. Explain fundamental methods of controlling microbial contamination of meat and poultry.

Introduction

Microbiology is a specialized area of biology dealing with organisms too small to be seen without sufficient magnification. Microbiologists study bacteria, fungi, parasites, and viruses, including their interactions with humans, animals, plants, and the environment. While viruses are not a living organisms like bacteria, fungi, and parasites, they are studied by microbiologists; therefore, we will use the term microbe (any microscopic entity that exhibits the properties of life) to collectively refer to any of these biologically active and microscopic entities. In addition, there is an infectious agent responsible for numerous neurodegenerative diseases found in animals and humans known as "prions", which will be covered in this module.

Food microbiology is specifically concerned with the desirable and undesirable effects microbes can have on the quality and safety of food products. In this section, we will briefly survey the importance of microbes in food, as well as prions. We will have an overview of some the fundamental microbiological concepts and consider how microbes are involved in food spoilage, food preservation, and foodborne illness We will conclude by considering the sources of microbes and prions and the l ways in which both may be controlled in food processing environments.

Types of Microbes

Bacteria

Bacteria are small, single-celled organisms that occur in almost any natural environment. Common bacteria are too small to be seen individually without the aid of a microscope. Bacteria can multiply to form groups or colonies on a food source. After a sufficient number of replication cycles a colony of bacteria can be seen with the naked eye.

Viewed under a microscope, different kinds of bacteria will have different shapes or forms. Many bacteria have either a spherical shape or an elongated rod shape. A spherical shaped bacterium (singular) is called a coccus, and a group of spherical-shaped bacteria (plural) are called cocci. A rod shaped bacterium is called a bacillus, and a group of rod-shaped bacteria are called bacilli.

Some species of bacteria appear as individual cells microscopically. Other bacterial species may combine to form pairs (e.g., diplococci), groups of four (tetrads), grape-like clusters (e.g., staphylococci), and chains (e.g., streptococci or streptobacilli).

Many bacteria have projections from their cell walls called fimbriae (or attachment pili). These structures assist those bacteria in attaching to one another as well as to other surfaces. Some types of bacteria possess flagella that allow those bacteria to be motile.

Certain bacteria (e.g., *Clostridia* spp.) have the capacity to sporulate or form spores (also called endospores). Simply put, a spore-forming bacterium encapsulates its vital structures in a tough outer coat when environmental conditions become harsh. Spores are resistant to heat, chemicals, and other environmental conditions. Bacterial spores are unable to reproduce; however, once conditions again become favorable for growth the spores reactivate and become vegetative (reproducing) cells again.

Some bacteria can produce toxins that in sufficient doses cause foodborne illness (e.g., botulism). Toxins can be produced by spore-forming bacteria, but only in the vegetative state. Suppressing reactivation to the vegetative state, then, is important in ensuring food safety in many processes. Some non-spore forming bacteria, like *Staphylococcus aureus*, can also produce toxins.

Fungi

The fungi consist of two major groups of microbes, molds and yeasts. Molds are multi-cellular organisms. Yeasts are single-celled organisms. Molds and yeasts tend to be significantly larger than bacteria. Both molds and yeasts are widely distributed in nature, both in the soil and in dust carried by air.

Molds have a branching filamentous structure, and can develop into colonies visible as a colorful, furry or downy coating on food or surfaces. They reproduce by producing

small spores, which are not related to bacterial spores mentioned above. Mold spores can be picked up and spread by air currents. If mold spores settle on suitable surfaces, they will begin to germinate and produce new mold growth.

Yeasts are usually egg-shaped, and tend to be smaller than molds. Like molds, yeasts can be spread via air currents. They reproduce by a process known as budding. Visible colonies of yeast are generally slimy in appearance and creamy white.

Parasites

Parasites are living organisms that derive nourishment and protection from other living organisms called hosts. These organisms live and reproduce within the tissues and organs of infected human and animal hosts. There are different types of parasites, and they range in size from single-celled protozoa to multi-cellular worms. Protozoan parasites are visible only through a microscope. Many adult parasitic worms are visible without a microscope; however, a microscope is necessary for detecting eggs and pre-adult forms of some worms. Identification of the adult forms of certain parasitic worms can also require microscopy.

The respective lifecycle of different parasites also varies. While some parasites use a permanent host, others go through a series of developmental phases using different animals or human hosts. They may be transmitted from host to host through consumption of contaminated food and water. Several parasites have emerged as significant causes of foodborne and waterborne illness.

Viruses

Viruses are much smaller than bacteria. They are too small to be seen with a standard light microscope. An electron microscope is necessary to see viruses. These microbes are not true living organisms. They are composed of genetic material—either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—enclosed in a protein coat. A virus must invade a living host cell in order to replicate. Once inside the host cell, the viral genetic material directs the host cell's "machinery" to make more virus particles, which interferes with normal host cell function and may result in destruction of the host cell.

Infectious Agent – Prions

Prions are not true living organisms; they are proteinaceous infectious particle with no nucleic acid genome. The prion protein exists in every mammal in a healthy state and is called cellular prion protein (PrP_c). When misfolded they become a proteinaceous infected particle (PrP_{Sc}).

Protein molecules fold into a three-dimensional (3-D) form. The 3-D form of a particular protein is related to its particular biological function. Folding into its normal form, then,

allows a protein to be functionally active. Failure of a protein molecule to fold into its 3-D normal form renders it dysfunctional and harmful to tissue or organs. As mentioned earlier, prions are misfolded proteins (aberrant or abnormal form – PrP_{Sc}) that appear to be infectious causing disease within the animal itself or when transferred from one animal to another. The mechanism by which this change occurs is not clear. To understand the structure of prion proteins, scientist use different techniques including magnetic resonance imaging and X-ray crystallography.

Prions are the prevailing theory for what causes a group of neurodegenerative diseases called transmissible spongiform encephalopathies (diseases of the brain). Bovine spongiform encephalopathy (BSE), also referred to as mad cow disease, is the transmissible spongiform encephalopathy that affects cattle. There are several transmissible spongiform encephalopathies that affect humans, such as Creutzfeldt–Jakob disease (CJD), Gerstmann-Straussler Scheiker Syndrome, Fatal Familial Insomnia and Kuru, but only CJD has been associated with meat consumption. The prion responsible for BSE appears to be infective to humans, and the resulting disease is called variant Creutzfeldt–Jakob disease (vCJD). Protein molecules are incapable of replicating themselves; the cells that make up the organs and tissues of animals and humans must manufacture them. As stated previously, the process by which prions are produced is not clearly understood. It is likely that prions cause other protein molecules to misfold (amplification process) or somehow induce cells to produce more misfolded proteins.


Microbial Growth

Our discussion of microbial growth will focus primarily on bacterial growth. Similar concepts apply to the growth of molds, yeasts, and some protozoa. Many parasites have complex life cycles, a discussion of which is beyond the scope of our purposes here. The most important parasites (e.g., *Trichinella spiralis* in pork) cannot reproduce in meat or poultry products. Replication of viruses will also not be discussed, because these microorganisms can only be replicated in the live animal.

If favorable environmental conditions exist, bacterial growth occurs. For our purposes, we will use the term growth to refer to an increase in microbe numbers, not an increase in size of an organism. Bacteria reproduce by dividing, a process called binary fission. When a bacterial cell is ready to divide, the material within it gradually increases until the cell's volume is almost doubled. The cocci shapes become oval while rod shapes stretch to nearly twice their length. The cell then constricts in the middle. This constriction deepens until the cell contents are held in two distinct compartments separated by a wall. These two compartments finally separate to form two new cells, which are duplicates of the former cell and each other.

Theoretical growth patterns can be represented by a graph of bacterial numbers over time and broken down into four different stages or phases.

Bacterial Growth Chart
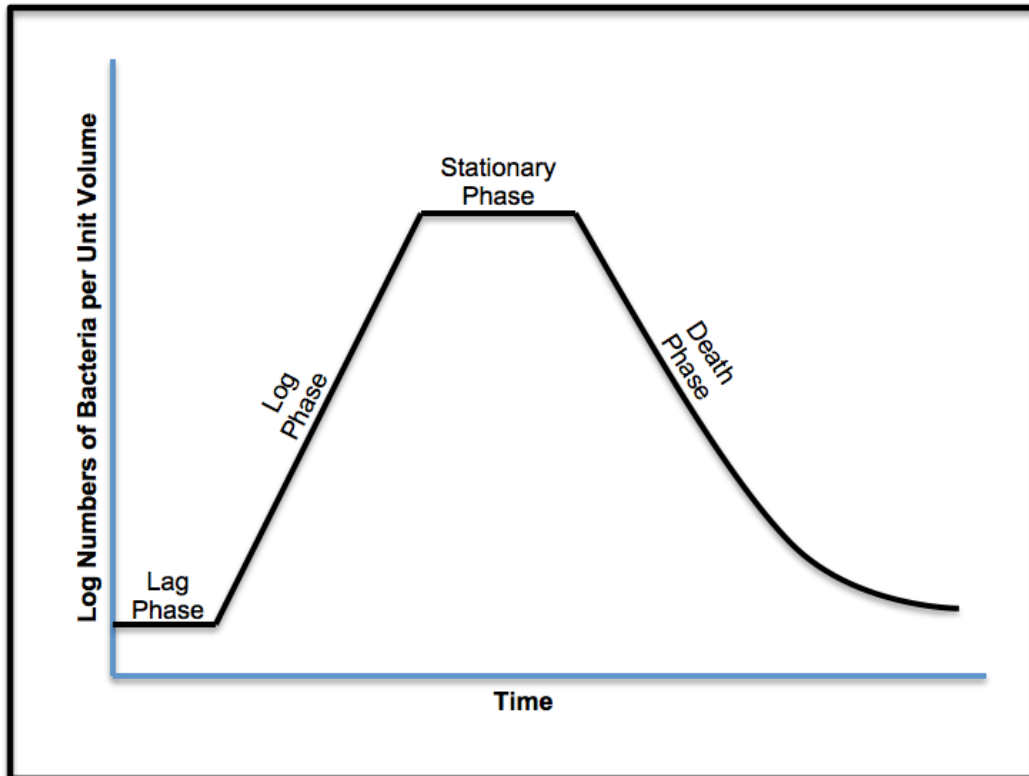
The first phase is called the **lag phase**. The lag phase occurs when a bacterial population first enters a nutrient rich environment. The rate of growth is very slow because the bacterial cells are adjusting to their new environment. In a nutrient-rich environment, such as on a meat or poultry product, the lag phase is generally short; however, the length of the lag phase is the most variable of the four phases. For example, it will take a bacterium longer to adapt to temperatures below the optimum growth range for that bacterium. Therefore, good temperature control will prolong the lag phase. Other environmental factors, including pH, water activity (a_w), and competition with other microbial species for nutrients, can also impact the length of the lag phase for a particular microbe.

After some hours or days, depending on environmental conditions and characteristics of the particular bacterial species, the bacterial cells begin to rapidly multiply. This phase is called the **log phase** because growth occurs exponentially and is depicted on a logarithmic scale on the vertical axis of the growth curve. A logarithmic scale basically allows a wide range of values to be displayed on a graph of manageable size and in a visually meaningful way. Such a scale is necessary because bacterial growth can occur at an exponential rate, i.e., 1 cell becomes 2 cells, the 2 cells become 4, then 8, then 16, then 32, then 64, etc. With each successive replication, the total number of cells doubles. The time it takes for the population of bacteria to double is referred to as doubling time or generation time. This doubling time can vary among species of

bacteria, but for most is between 10 to 30 minutes under optimal conditions for growth. It is important to note that, while the starting bacterial count may not have an effect on doubling time it will have a tremendous effect on the numbers of bacteria after each doubling. For example, the numbers of bacteria will differ tremendously after 1 doubling time if the initial count is 200 cells (become 400 cells with the first doubling) vs. 2 cells (become 4 cells with the first doubling). Effective sanitation to reduce bacterial load will limit the number of cells available to contribute to proliferation during this phase.

Exponential Growth Example: Let's assume a particular species of bacteria doubles every 30 minutes. After one hour, a single bacterium of that species becomes four. At the end of two hours, there will be 16 bacteria. After 15 hours, there will be 1,000,000,000 (one billion) cells. Relating this to the processing environment, consider a situation where there are 75,000 of those bacterial cells per square inch on a conveyor belt and growth conditions are ideal. By the end of one hour there could be as many as 300,000 bacteria per square inch of that belt. At the end of three hours, the bacteria count per square inch of belt surface could be 4,800,000.

The length and slope of the log phase will depend on a variety of factors, including nutrient availability, buildup of bacterial waste products, and temperature. For example, many bacteria still grow under refrigeration temperatures, just very slowly. The slope of the log phase, then, would be less steep, and the log phase prolonged. Therefore, the rate of growth would be much slower at refrigeration temperatures than under optimal conditions.

NOTE: Later we will refer to a change in bacterial numbers as a log increase (multiplication) or log decrease (reduction). The concept of a log change is mathematically different from that of exponential growth during the log phase, and the two should not be confused. In mathematical terms, a graph of the phases of bacterial growth is a plot of the *natural logarithm* of cell number against time. When referring to processes that result in a given log increase or log reduction in the numbers of bacteria, calculations will be based on the *common logarithm* ($\log_{10}$ or log base 10).

The third phase is the **stationary phase**. In this phase the rate of bacterial growth is the same as the rate of bacterial death, because the population of bacteria has reached its maximum due to limitations in the availability of nutrients and an increase in bacterial waste products.

The fourth phase is the **death phase**. In this phase, more bacterial cells are dying than those that are dividing. There is a net loss in the number of viable bacterial cells in the environment. This is the result of increasingly hostile environmental conditions associated with decreasing availability of nutrients and increasing waste products. Initially the rate of death is exponential, but it may slow down after significant numbers of bacterial cells have died. Spore-forming bacteria may begin to sporulate and remain viable but dormant. Because the nutrient supply in meat and poultry products is almost unlimited, an exponential rate of death may not occur.

Factors Affecting Microbial Growth

There are a variety of factors that affect the rate of microbial growth in food. Again, our discussion will focus on bacteria; however, much of it is applicable to yeasts and molds as well. It is not applicable to parasites or viruses.

Like all other living organisms, bacteria require favorable environment to live and grow. There are six basic environmental factors that impact bacterial growth. An easy way to remember these conditions is to use the memory device **FAT TOM**.

Food Composition	**A**cidity	**T**emperature
Time	**O**xygen	**M**oisture

Food Composition

Here we are using the word food to refer to nutrients available to the microbes, which could be a human food product, product residue on equipment, or organic debris in some non-product contact growth niche. A suitable supply of nutrients is the most important condition affecting growth of bacteria. Every living cell requires certain nutrients to multiply. These include solutions of sugars or other carbohydrates, proteins, and small amounts of other materials such as phosphates, chlorides and calcium. If the nutrient supply is removed, bacteria will not multiply.

Acidity

The degree of acidity or alkalinity of an aqueous solution is expressed on a scale between 0 and 14 referred to as the pH scale. As acidity increases, we move down on the pH scale (i.e., the pH is lower). As alkalinity increases, we move up on the pH scale (i.e., the pH is higher). The pH of pure water is 7.0, which is referred to as *neutral pH*.
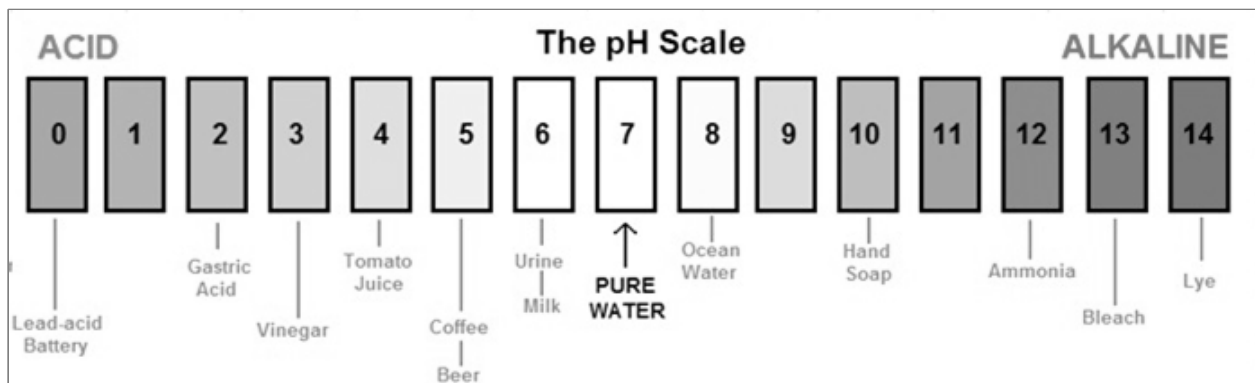
The pH of a meat or poultry product can have a profound effect on the growth and viability of microbial cells. Each species of microbe grows within an optimal range of pH values. Most microbes thrive when the pH is near neutral or slightly acidic, but there are exceptions. Most bacteria will not grow at pH levels below 4.6 because the environment

is too acidic. Many molds and yeasts can grow at a lower pH than do bacteria. On the basis of pH, food products are often grouped as high-acid foods (pH below 4.6) and low-acid foods (pH 4.6 and above). The pH of fresh meat ranges between 5.3 and 6.4 (i.e., high pH or low-acid). Meat with a pH in the 6.0 to 6.4 range spoils faster than meat in the lower pH range of 5.3 to 5.7, because spoilage microbes are more active in the pH range of 6.0 to 6.4. We will discuss later how meat and poultry processors can control pH to limit microbial growth.

Temperature

All bacteria, molds, and yeasts have an optimum, maximum, and minimum temperature for growth. These temperatures can vary among different species of microbes. Therefore, environmental temperature not only impacts the rate of growth of microbes but can determine which microbial species thrive. A temperature difference of only a few degrees may favor the growth of an entirely different population of microbes. Below approximately 41°F proliferation of spoilage microbes is slow, and growth of most pathogenic microbes stops. *Listeria monocytogenes* (Lm), a bacterial pathogen of concern in many ready-to-eat products, is a notable exception. While Lm grows optimally at temperatures in the range of 86 to 98.6°F, it is capable of growing at a temperature as low 31.3°F. Lm's rate of growth at that temperature may be slow, but can still be significant as will be discussed in a later module. At temperatures above 140°F most microbes begin to die, although the time needed for cell destruction at a particular temperature will vary for different species of microbes and may depend on other environmental factors such as humidity. In food processing, the temperature range of 41 – 140°F is commonly referred to as the **danger zone**, because the optimum, maximum, and minimum temperature for growth of most microbes will fall somewhere within that range. However, it is important to note that time is a major factor associated with the rate of growth at a particular temperature. For example, depending on other factors, the rate of growth of many pathogens may be extremely slow in the 40 to 50°F temperature range.

Time

We have already mentioned ways in which time can influence microbial growth. Permitting sufficient time for microbes to adapt to their environment (lag phase) is necessary before they can enter the rapid growth phase (log phase). The doubling time for most bacterial species is between 10-30 minutes under optimal conditions for growth. Generally doubling times in this range would only occur under ideal laboratory conditions. Bacteria would grow much more slowly in meat and poultry products, especially if those products are properly handled and stored. Allowing the temperature of meat and poultry products to remain in the danger zone for a sufficient period of time will promote significant proliferation of microbes and microbial toxins. In addition, time may be a factor involved in how well certain microbes adhere to the surface of a meat or poultry product. For example, it may be more difficult to eliminate bacteria from the surface of a carcass by washing or with antimicrobial sprays the longer the bacteria are allowed to remain on that carcass before washing or spraying.

Oxygen

Similar to temperature, oxygen availability can determine which microbes will be active. Microbes that have an absolute requirement for oxygen are called obligate aerobes. Those that require the total absence of oxygen are called obligate anaerobes. Some microbes are called facultative anaerobes, because they can grow in the presence or absence of oxygen. Molds require oxygen for growth. Yeasts grow best under aerobic conditions, but some can grow slowly under anaerobic conditions. The kinds of bacteria that cause food spoilage tend to be aerobes, but those that cause foodborne illness are anaerobes or facultative anaerobes.

Moisture

The availability of water in a food (referred to as water activity, or a_w) is an important factor for microbial growth. Nutrients for microbial growth must be in a soluble form for microbes to utilize them. Generally, bacteria have the highest a_w requirements, molds have the lowest, and yeasts are intermediate. It is important to note that a_w is not necessarily equivalent to measures of moisture content (e.g., Moisture Protein Ratio or MPR) in a product. Most moist food products will have greater water availability to support microbial growth than dryer food products, but there are exceptions. For example, some processing methods might incorporate certain chemical ingredients (e.g., salt) that bind to free water and in sufficient concentrations significantly reduce a_w limiting the growth of some microbes.

Interaction of Factors

We have considered each factor individually, but it is important to recognize that the rate of microbial growth will be affected by complex interactions among these factors. The precise impact of these interactions on microbial growth is often very difficult to predict. The optimal range of a factor may change when another factor is not optimal. For example, if a_w is less than optimal, the pH range at which many microbes can grow is more limited. When pH is less than optimal, a_w necessary for growth will be higher. The presence of certain chemical ingredients can also affect the pH or temperature at which some microbes grow.

Interaction of Different Microbes

A variety of microbes, including different species of bacteria, yeasts, and molds, can exist on meat and poultry products. Rarely will there be only a single species from one of these three classes of microbes on product or product contact surfaces. Competition among microbial species for vital nutrients can impact the rate of growth of microbes. Different bacterial species multiply more rapidly than others, and bacteria generally multiply more rapidly than most yeasts and molds. A rapid increase in numbers of a particular bacterial species may have a limiting effect on the growth of other bacteria, yeasts, and molds. In addition, some microbial species can produce chemical changes within the growth environment that have an inhibitory effect on other microbes.

Microbes and Food Spoilage

Spoilage is caused by physical and chemical changes in food products that result in undesirable odors, flavors, textures, or colors. Spoilage costs the food industry millions of dollars each year. There are three primary mechanisms that can result in spoilage of meat and poultry products after slaughter and during processing and storage. One is called autolytic enzymatic spoilage, and is simply due to the normal post-mortem breakdown of cellular membranes and the release of enzymes that result in deterioration of product quality. Another mechanism is called lipid oxidation, which involves reactions between oxygen and fat molecules in the product, resulting in rancidity. The third mechanism is microbial spoilage. All raw foods and even certain processed foods can contain microbes that will eventually cause spoilage unless they are controlled or destroyed.

Microbes can cause food spoilage by two basic mechanisms. The most important mechanism is related to the growth of spoilage microbes and their active metabolism of food components. The other mechanism of microbial spoilage can occur even in the absence of live microbes. As microbes die, they can release various enzymes that react with and change properties of food components, leading to spoilage.

Indications of microbial food spoilage vary with the microbe(s) involved and the time course of spoilage. Bacteria and yeasts typically result in slime formation, bad odors, rancid flavors, and discoloration (grey, brown, or green). Anaerobic spoilage bacteria, which can be an important in vacuum packaged products, can produce a distinctive souring due to the production of organic acids and gases. Molds that cause food spoilage often result in a stickiness of the product surface and eventually the formation of creamy, black, or green colonies with a fuzzy or whisker-like appearance.

Microbial food spoilage does not necessarily equate with food safety. Foods can contain dangerous microbes but still have a normal appearance, odor, flavor, and texture. Food with obvious indications of spoilage may or may not contain harmful levels of dangerous microbes. A variety of bacteria, yeasts, and molds are involved in food spoilage, but most are not pathogenic. Pathogenic microbes cause illness but generally do not affect the taste, smell, or appearance of food. Spoilage microbes may be better able to reproduce under conditions that may be less favorable to the growth of pathogens. However, some of the same conditions that accelerate spoilage, such as inadequate control of temperature and moisture, also encourage the growth of pathogenic microbes. Therefore, food spoilage is not just an issue of product quality; it represents unwholesomeness and calls into question the safety of the product.

Microbes and Food Preservation

We are generally concerned with the undesirable effects of microbes on food, including spoilage and foodborne illness. However, many microbes are beneficial to preserving certain foods, controlling pathogenic microbes, and producing certain other desirable characteristics in some products.

The primary mechanism through which certain microbes are used in food preservation is the process of fermentation. Simply put fermentation results in the conversion of sugars to simpler compounds by microbes under anaerobic conditions. The fermentative microbes gain energy through the process of fermentation, and produce substances, including organic acids, peroxides, and bacteriocins, that have an inhibitory effect on the growth and survival of other microbial species. Microbial fermentation processes are used in the production of a variety of common foods, including beer, wine, some breads, chocolate, and yogurt. Fermented meat and poultry products include many salamis, summer sausage, Lebanon bologna, Cervelat, and Thuringer. These fermented sausage products typically have a tangy flavor resulting from the process of fermentation.

The process of fermentation primarily exerts a preservation effect by increasing the acidity of products. During this process, the pH level of the product is reduced by starter culture activity and by appropriate time/temperature factors. A starter culture will generally include bacteria that produce lactic acid (e.g., *Lactobacillus spp.*) along with certain other bacteria, yeasts, or molds used to impart certain flavor and color characteristics to the product. The starter culture is added to the meat or poultry mixture, and held in an environment optimum for the growth of the microbes in the culture. The temperature and humidity are carefully monitored to promote growth of the culture. The starter culture microbes actively reproduce. As they do, they compete with other microbes that might result in spoilage of the product or that might be pathogenic. The lactic acid produced by starter culture bacteria, like Lactobacillus, lowers the pH of the product. It is important that lactic acid is produced quickly, because it inhibits undesirable bacteria, like the toxin-producing *Staphylococcus aureus*. The pH is monitored over time to determine when the process is complete. Generally, the goal is to achieve a pH of 5.0 or less within a certain timeframe.

Microbes and Food Safety

Certain microbes associated with food can also be harmful. We refer to these as foodborne pathogens (see Attachment 1). There are millions of cases of foodborne illness in the U.S. each year. The cost of foodborne illness is staggering. According to the USDA's Economic Research Service (ERS), the estimated annual cost of foodborne illness caused by *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and other pathogenic serotypes of *E. coli* is approximately $6.9 billion. This figure includes the cost associated with medical expenses, lost productivity, and death. There are other significant costs to both industry and government.

In meat and poultry products, pathogenic bacteria probably account for the largest proportion of all foodborne illnesses. The most important **bacterial pathogens** include *Salmonella, Campylobacter, Listeria monocytogenes* (*Lm*)*, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, Staphylococcus aureus*, and the Shiga toxin-producing *E. coli* (STEC; includes *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145 serogroups). Of these, *Salmonella* and STEC are two of the most common causes of large outbreaks. We will discuss more details about most of these pathogens in other training modules together with process familiarization and sampling programs.

Molds and yeasts do not appear to be responsible for any meat- or poultry-related foodborne illnesses, maybe because significant mold or yeast growth is easily recognized and the food discarded. Some molds do produce toxins (e.g., aflatoxin produced by *Aspergillus spp.*), but food-related illness appears to be associated with nuts and grains not meat and poultry. Also, some molds on food products may cause allergic reactions and respiratory problems in sensitive individuals exposed to the mold.

Some **important foodborne parasites** are *Giardia duodenalis*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, *Trichinella spiralis*, *Taenia saginata* (beef tapeworm), and *Taenia solium* (pork tapeworm). It is important to note that, while *Toxoplasma gondii* is not a common cause of foodborne illness, it is among the top five foodborne pathogens that result in hospitalization and death. Trichinosis (or trichinellosis), caused by *Trichinella spiralis*, was historically an important foodborne illness resulting from the consumption of undercooked pork products. Trichinosis has largely been eliminated due to changes in swine production practices, consumer education, and prescribed treatments for destruction of trichinae in certain classes of pork products (9 CFR 318.10).

Other Pathogenic Agents

Prion-associated vCJD appears to be of relatively low incidence. BSE in cattle and vCJD in humans are slowly progressive diseases. Initial symptoms in humans are generally psychiatric, i.e., depression. As the disease progresses, neurologic signs appear and worsen to the extent that patients are unable to care for themselves, until death occurs. Cattle can initially display behavioral changes progressing to neurologic signs, the inability to rise, and ultimately death. There are certain cattle tissues considered to be of high risk for prion contamination. These tissues are referred to as specified risk materials (SRMs).

The presence of **viruses** in food is generally associated with contaminated food workers in the retail or food service arena. Norovirus is recognized as the leading cause of foodborne-disease outbreaks in the United States. It is a highly contagious gastrointestinal illness. Insufficiently cooked shellfish have been direct sources of illness, but other foods appear to be contaminated by infected food handlers. Most outbreaks are likely to arise through direct contamination of food by a food handler immediately before the food is eaten. Hepatitis A, hepatitis E, and rotavirus are other

important viral diseases that may be transmitted through food, but again, their transmission has generally been associated with contamination by infected food service workers.

Resources

More details about foodborne pathogens can be found at the websites listed below. IPP are encouraged to make themselves more knowledgeable of these pathogens and the diseases they can cause.

- **Bad Bug Book: Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins**
  www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf

- **Foodborne Illness and Disease Fact Sheets**
  www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/foodborne-illness-and-disease

- **Estimates of Foodborne Illness in the United States**
  www.cdc.gov/foodborneburden/index.html

- **Causes of Food Poisoning**
  www.foodsafety.gov/poisoning/causes/index.html

- **Pathogens 101**
  www.stopfoodborneillness.org/pathogens-101

Sources of Microbes in Food

Excluding certain areas like the gastrointestinal tract, upper respiratory tract, and lower urinary tract, the internal tissues (e.g., muscle tissue) of normal healthy livestock and poultry are generally sterile. Nevertheless, raw and many processed foods contain a variety of different bacteria, yeasts, molds, and viruses. Fortunately, relatively few of all the varieties of microbes are important with respect to food safety. Livestock and poultry, people, equipment, pests, water supplies, food ingredients, and air currents can all be important sources of microbes in the food-processing environment. A basic knowledge of the sources of microbes in food helps the inspector better understand potential vulnerable points for microbial contamination in meat and poultry processing and the importance of fundamental methods for controlling microbes in food.

Healthy animals are major sources of spoilage and pathogenic microbes in raw meat and poultry, because they shed microbes in their feces and carry microbes on their hides or feathers. Large intestinal contents of normal animals may contain as many as 10 billion bacteria per gram (a U.S. one-cent coin weighs 2.5 grams.). Soil also contains a variety of microbes that can also contaminate the hides and feathers of live animals.

While dressing animals during the slaughter process, these bacteria can easily be transferred from the hide, skin, feathers, and gastrointestinal tract to the carcass itself. Disease conditions, like mastitis, pneumonia, gastroenteritis, and uterine infections, may change the normal microbial flora and ecology in affected organs and tissues, and represent additional sources of potential contamination of the slaughter environment and carcass.

People can be a major source of microbial contamination in the processing environment. This may result from people trafficking microbes throughout a processing area due to poor hygienic practices, including inadequate hand washing, wearing soiled clothing, and working around product while sick with an infectious disease. People are also responsible for the design and implementation of procedures and controls intended to minimize microbial contamination of products. Failure to adequately design or implement such procedures and controls creates insanitary conditions with the potential to contaminate product. For example, maintenance personnel may fail to properly sanitize tools or protect product from contamination when working on equipment during production shifts.

A variety of different kinds of equipment may be involved in slaughtering, processing, storing, and transporting meat and poultry products. Many types of microbes from other sources can get into equipment and contaminate product. Equipment can serve as niches (hiding places) for the growth of certain microbes if environmental conditions are conducive to growth and sanitation practices inadequate. Some bacteria, including many pathogens, can form biofilms on equipment surfaces as multiple bacteria attach to the surface and produce a protective matrix. Once formed, biofilms can be difficult to remove with routine cleaning and sanitizing procedures. Bacteria embedded in a biofilm can be up to a 1,000 times more resistant to many sanitizers. During operations, portions of a biofilm on moving equipment can shear off, seeding the equipment with bacteria. Equipment contaminated by any means can lead to widespread contamination of product. Good sanitation and maintenance procedures are critical to preventing this.

Just like live animals presented for slaughter, pests can carry an assortment of microbes on their surface and in their gastrointestinal tracts. Inadequate pest management may lead to the contamination of product, equipment, ingredients, and packaging materials. Allowing house pets (e.g., cats) to enter production related areas, including dry storage areas, could be a source of contamination as they can also harbor pathogens.

There are numerous uses for water in food processing facilities, from clean up of facilities and equipment, to washing of product and product formulation. Obviously non-potable or contaminated supplies of water could be sources of microbial contamination. Less obvious, though, might be water overspray from washing equipment or splashing of contaminated water onto product or food contact surfaces. In addition, standing water and damp areas of the facility could promote microbial growth and increase the possibility of cross contamination.

Non-meat and non-poultry food ingredients are possible sources of contamination. Spices and seasonings may be contaminated with pathogens if improperly processed or stored and handled under insanitary conditions. Dried herbs and spices as well as soy and milk protein ingredients can be a primary source of bacterial spores, since the spores will survive for extended times in these dry ingredients. Dried, shelf-stable non-meat ingredients have also been identified as sources of *Salmonella, Listeria* and STEC infections in multistate outbreaks. Vegetables and vegetable products that are inadequately washed and sanitized can be another source of contamination in the processing of meat and poultry products that include these ingredients.

Microbes, including bacterial spores, may be present in dust and moisture droplets in air. Obviously as air currents move dust through a processing facility, the dust can be deposited onto surfaces of the facility, equipment and utensils, employee clothing, and product. Microscopic moisture droplets traveling in air currents can condense out onto cooler surfaces, leading to contamination of those surfaces and forming condensate that potentially drips onto product or food contact surfaces.

Controlling Microbes in Food and Food Processing Environments

Pathogenic microbes are significant threats to public health. There are two fundamental ways to control microbial contamination of products and processing environments. Both are important. The first involves reducing opportunities for microbes to enter processing environments and come into contact with products. The second involves making the environment for microbes as inhospitable as possible to reduce their numbers and minimize their growth. Note that "control" is a keyword here. It is impossible to completely eliminate all microbes from processing environments and food products. However, it is possible for establishments to implement effective control strategies designed to protect against pathogens and the undesirable effects of spoilage organisms. Other portions of this training program will familiarize you with specific processes, including specific methods of controlling microbes in those processes. For now, we will broadly consider ways in which the levels of microbes in processing environments and on foods may be controlled.

Prevention of Contamination

It is important to avoid the contamination of meat and poultry whenever possible. This includes inadvertent contamination or cross-contamination from the live animal, processing procedures and equipment, employees, and the environment. Contamination can be minimized or avoided altogether by following appropriate sanitation procedures, good manufacturing procedures (GMPs), and procedures for employee hygiene. Good sanitary dressing process control measures in slaughter processes not only minimize contamination of carcasses, but also reduce the level of processing environment contamination. Effective pest control can help prevent the introduction of many microbes into the processing environment. Sound construction of the facility and maintaining its construction will reduce opportunities for microbial

contamination of the processing environment. For example, ventilation systems that provide fresh air in and throughout the establishment should be designed and constructed so as to preclude the transfer of contaminated air from the outside to the inside of the establishment. Ensuring appropriate hygienic standards and traffic patterns are followed by all employees working in and around processing areas protects against excessive contamination of the processing environment and product.

NOTE: The term *cross-contamination* generally refers to the transfer of microbes from a contaminated source to a previously cleaned/sanitized surface.

Restriction of Growth

Recognizing that bacteria will be present on meat and poultry, it is important to keep the overall number of bacteria very low to minimize concern about bacterial pathogens as well as spoilage organisms. Making a microbe's environment as inhospitable as possible can involve a variety of control measures, all of which relate to the FAT TOM factors impacting microbial growth. Effective procedures for cleaning and sanitizing the facility provide the foundation for controlling microbes. In addition, temperature, acidity, salting and drying, or some combination of these can be used to restrict the growth of pathogens.

Sanitation – Microbes can be found in processing environments, which emphasizes the need for effective sanitation procedures for equipment and floors. Adequate cleaning and sanitizing procedures will help to ensure that little organic matter is available to support microbial growth. Altering the pH of a microbe's environment may involve the use (and rotation) of acid and alkaline sanitizing agents. Moisture control in the processing environment is an important means of protecting against microbial proliferation. This may occur through measures designed to keep the environment dry, adequate ventilation, or adequate plumbing to properly convey liquid waste out of the processing area. Employee hygiene, airflow, and traffic flow of people and equipment between areas are also important to protect against cross-contamination.

Temperature – Temperature controls are important in all classes of product. Maintaining products under refrigeration is one of the most important ways to inhibit microbial growth. Cooking product to temperatures adequate enough to eliminate pathogens of concern is another way to control microbes. Many pathogens are fairly easily destroyed with relatively mild cooking. Bacterial spores and toxins, though, can be very heat resistant. Inactivation of spores and some toxins requires thermal processing to high temperatures under pressure as in canning operations. The time it takes for products to reach a particular temperature is also important in inhibiting microbial growth. Chilling raw, heat-treated, and fully-cooked products as rapidly as possible helps to ensure products do not linger in the "danger zone" for too long, which could result in the outgrowth of bacteria, including spore-forming bacteria and toxin-producing bacteria.

Acidity – Product pH can also be manipulated, though, to inhibit certain microbes in certain products. For example acidifying agents (acidulants) may be added to certain

products to reduce pH. Similarly, some products are naturally acidified by the addition of fermentative microbes. Some bacteria can survive in acidic conditions, so fermentation alone cannot be relied upon to completely eliminate all harmful bacteria.

Salting and Drying – Certain production processes involve steps to reduce the water available for microbial growth through the addition of high concentrations of salt or actual drying of the product. Salt and low water activity in a product can be very effective in controlling the growth of some harmful bacteria, but some organisms (e.g., *Staphylococcus aureus*) can survive in high salt environments.

Vacuum Packaging – Reducing the oxygen level through vacuum packaging processes is a common method of enhancing the shelf life of food products. However, vacuum packaging reduces the growth of mainly spoilage microbes. Pathogenic bacteria, such as *Clostridium botulinum* and *Listeria monocytogenes* can still grow in vacuum packaged products. Given the enhanced shelf life, it is important for processors to consider other measures necessary to protect consumers from pathogens in vacuum packaged products.

Ultimately there is no single method of preventing or controlling microbes in food. It requires a so-called multiple hurdle approach. Fundamentally, this can be represented by compliance with the Sanitation Performance Standards, maintaining effective Sanitation SOPs, and designing and implementing an effective HACCP plan. Within a particular process, a multiple hurdle approach may get more specific. For example, the use of antimicrobial agents or processes, altering the pH of the product, and temperature controls may together be a system of hurdles for ensuring effective antimicrobial control in some processes.

Attachment 1

CDC Estimates of Foodborne Illness in the United States

FINDINGS

CDC 2011 Estimates

CDC estimates that each year roughly 1 in 6 Americans (or 48 million people) gets sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases. The 2011 estimates provide the most accurate picture yet of which foodborne bacteria, viruses, microbes ("pathogens") are causing the most illnesses in the United States, as well as estimating the number of foodborne illnesses without a known cause.* The estimates show that there is still much work to be done—specifically in focusing efforts on the top known pathogens and identifying the causes of foodborne illness and death without a known cause.

Reducing foodborne illness by 10% would keep about 5 million Americans from getting sick each year.

CDC has estimates for two major groups of foodborne illnesses:

Known foodborne pathogens— 31 pathogens known to cause foodborne illness. Many of these pathogens are tracked by public health systems that track diseases and outbreaks.

***Unspecified agents**— Agents with insufficient data to estimate agent-specific burden; known agents not yet identified as causing foodborne illness; microbes, chemicals, or other substances known to be in food whose ability to cause illness is unproven; and agents not yet identified. Because you can't "track" what isn't yet identified, estimates for this group of agents started with the health effects or symptoms that they are most likely to cause— acute gastroenteritis.

To estimate the total number of foodborne illnesses, CDC estimated the number of illnesses caused by both known and unspecified agents. We also estimated the number of hospitalizations and deaths caused by these illnesses. Table 1 provides the estimates due to known pathogens, unspecified agents, and the total burden.

Table 1. Estimated annual number of domestically acquired foodborne illnesses, hospitalizations, and deaths due to 31 pathogens and unspecified agents transmitted through food, United States

Foodborne agents	Estimated annual number of **illnesses** (90% credible interval)	%	Estimated annual number of **hospitalizations** (90% credible interval)	%	Estimated annual number of **deaths** (90% credible interval)	%
31 known pathogens	9.4 million (6.6–12.7 million)	20	55,961 (39,534–75,741)	44	1,351 (712–2,268)	44
Unspecified agents	38.4 million (19.8–61.2 million)	80	71,878 (9,924–157,340)	56	1,686 (369–3,338)	56
Total	47.8 million (28.7–71.1 million)	100	127,839 (62,529–215,562)	100	3,037 (1,492–4,983)	100

National Center for Emerging & Zoonotic Infectious Diseases
Division of Foodborne, Waterborne, and Environmental Diseases

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Pathogens causing the most illnesses, hospitalizations, and deaths each year

Eight known pathogens account for the vast majority of illnesses, hospitalizations, and deaths. Tables 2–4 list the top five pathogens causing illness, hospitalization, and death.

Table 2. Top five pathogens causing domestically acquired foodborne illnesses

Pathogen	Estimated annual number of illnesses	90% Credible Interval	%
Norovirus	5,461,731	3,227,078–8,309,480	58
Salmonella, nontyphoidal	1,027,561	644,786–1,679,667	11
Clostridium perfringens	965,958	192,316–2,483,309	10
Campylobacter spp.	845,024	337,031–1,611,083	9
Staphylococcus aureus	241,148	72,341–529,417	3
Subtotal			91

Table 3. Top five pathogens causing domestically acquired foodborne illnesses resulting in hospitalization

Pathogen	Estimated annual number of hospitalizations	90% Credible Interval	%
Salmonella, nontyphoidal	19,336	8,545–37,490	35
Norovirus	14,663	8,097–23,323	26
Campylobacter spp.	8,463	4,300–15,227	15
Toxoplasma gondii	4,428	3,060–7,146	8
E. coli (STEC) O157	2,138	549–4,614	4
Subtotal			88

Table 4. Top five pathogens causing domestically acquired foodborne illnesses resulting in death

Pathogen	Estimated annual number of deaths	90% Credible Interval	%
Salmonella, nontyphoidal	378	0–1,011	28
Toxoplasma gondii	327	200–482	24
Listeria monocytogenes	255	0–733	19
Norovirus	149	84–237	11
Campylobacter spp.	76	0–332	6
Subtotal			88

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Attachment 2
A Glossary of Food Microbiology

Aerobic - Bacteria that require oxygen to grow or will grow in the presence of oxygen.

Anaerobic – Bacteria that do not utilize oxygen to grow, or will not grow in the presence of oxygen.

Bacteriocin – A proteinaceous substance produced by specific bacteria that is toxic to closely related strains of the same specific bacteria and either kills or slows the growth of those other specific bacteria.

Coliform – Bacteria that most often inhabit the intestine of animals, do not utilize oxygen, but can grow in its presence. Bacteria that are classified as coliforms have the same shape, and many of the same characteristics. These bacteria are used as indicators of sanitary quality in many food products.

Detection limit – The lowest threshold amount of bacteria that must be present in a sample to be found. Detection level depends upon analytical methods used.

Direct plating – The application of a sample, or dilution thereof, to solid media usually containing agar and other material used to grow and enumerate bacteria.

D-value – The amount of time needed to destroy one log unit of a specific bacterial pathogen at a specific temperature in a specific medium.

Enrichment – Addition of nutrient rich broth so that certain bacteria or type of bacteria increase in number to result in a bacterial cell count that is higher than the detection limit. This is used to detect only the presence or absence of the bacteria, not the amount present.

Enterobacteriaceae – A large group of bacteria that are closely related and are commonly found in fecal material of warm-blooded animals. They include coliforms and pathogens such as *Salmonella*.

F-value – Measured in minutes, the D-value of a specific organism at 250˚F (121˚C) multiplied by the desired log reduction.

Germination – The process of a spore becoming a vegetative cell.

Inhibition – The slowing or stopping of bacterial growth.

Lag time – Time that bacteria take to become acclimated to a new environment before starting to multiply exponentially.

Lethality – The effectiveness of a treatment to destroy or kill bacteria.

Log unit – 'Log' is short for logarithm, a mathematical term for a power to which a number can be raised (exponent of 10). A unit of 10^x is typically used to count bacteria. Therefore, a 1-$\log_{10}$ change stands for a 10-fold increase (multiplication) or decrease (reduction) in numbers of recoverable bacteria. For example, the difference between 10^6 (1,000,000) and 10^7 (10,000,000) is one log unit (10-fold increase); the difference between 10^6 and 10^4 (10,000) is two log unit (Log-2 represents 10^2 or 10x10 or 100; 100-fold increase or decrease). See Attachment 3 for additional information.

Mesophiles – Bacteria that have optimum growing temperatures between 77ºF (25ºC) and 104ºF (40ºC).

Microflora – the community of microorganisms, including bacteria, molds and yeasts that live in or on another living organism or in a particular habitat.

Pathogen – Organisms that cause illness. These organisms include bacteria, protozoa, or viruses.

pH - Level of acidity or alkalinity in a product. The pH scale ranges from 1 to 14 with pH 7 considered neutral; pH 1 is the most acidic and pH 14 is the most alkaline. Fresh meat usually has a pH near 5.6.

Psychrotrophs - Bacteria that have optimum growing temperatures between 68ºF (20ºC) and 86ºF (30ºC) but can grow at temperatures as low as 32ºF (0ºC).

Shocked (heat shocked) – Occurs when a product is heated but the temperature is not high enough to destroy the bacteria. This result in bacteria that are injured for a while but, in most cases, can repair themselves and are more resistant to heat the next time the product is heated. Heat shocked can also refer to the process by which a bacterial spore is induced into germination. When a product is heated thoroughly the vegetative cells are destroyed, but the spores are undamaged by the heat. The spores then germinate into vegetative cells once the temperature has decreased to an optimum level.

Spore – A highly resistant, dormant structure formed in response to adverse environmental conditions. Bacterial spores are usually very resistant to heat, long periods of dryness, and other adverse conditions that normal vegetative cells cannot survive. When the environment becomes more favorable then the spore can reactivates to the vegetative state. Most of the time spore-forming bacteria have a toxin associated with them. When reactivation (germination) of the spore occurs metabolic activity begins that converts the germinated spore into a growing vegetative cell, thus producing the associated toxin.

Strain – Is a genetic variant or subtype of a microorganism. For example, E. coli O157:H7 strain EC57: *Escherichia* is the genus, *coli* is the species, **O157:H7** is the

serotype, and strain EC57 (assigned number or identifier) is the *genotype,* i.e., the genetic nomenclature involving specific gene variations (heat resistant).

Thermotolerant – Bacteria that can withstand higher than normal temperatures.

Toxin (enterotoxin, mycotoxin, neurotoxin) – A compound produced by a bacterium or fungi (molds and yeasts) that can cause illness in other living organisms. Specific examples include enterotoxins that affect the intestine, mycotoxins are those toxins produced by fungi, and neurotoxins attack the nervous system.

Transdermal synergists – Compounds that work with other compounds against bacteria when applied to the surface of a carcass.

Treatment – The method of processing that is being tested. A good research study will compare various treatments, such as levels of salt in a product, to a control; in this example the control maybe no salt added. All other conditions should remain the same for all samples tested except the specific treatment.

Vegetative cell – The normal bacteria cell. This is in contrast to a spore. Vegetative cells are susceptible to destruction or damage from heat, additives, and other factors that can damage and destroy them relatively easily.

Attachment 3

Log Reduction & Multiplication

"Log" stands for logarithm, which is the exponent of 10 (10^1 = 1-log$_{10}$, 10^2 = 2-log$_{10}$, 10^3 = 3-log$_{10}$, etc.). A 1-log$_{10}$ change stands for a 10-fold increase (multiplication) or decrease (reduction) in numbers of recoverable bacteria on a food product.

The table below indicates the percentage decrease in bacteria with each successive log reduction.

Log Reduction	Decrease in Bacteria
1	90%
2	99%
3	99.9%
4	99.99%
5	99.999%
6	99.9999%
7	99.99999%

Therefore, a 1-log reduction would reduce the number of bacteria by tenfold or 90%. This means that 100 bacteria would be reduced to 10, or 10 bacteria would be reduced to 1. Note that the change in the absolute number of bacteria depends on the number of bacteria before the change. If 1,000,000 bacteria are reduced by 3-logs (or 99.9%) for example, then approximately 1,000 bacteria will remain.

1,000,000	x	0.999	=	999,000
Starting bacteria		3-log Reduction		Bacteria killed
1,000,000	–	999,000	=	1,000
Starting bacteria		Bacteria killed		Bacteria that remain

The table below indicates the factor by which bacteria increase with each successive log multiplication.

Log Multiplication	Increase in Bacteria
1	10 times
2	100 times
3	1,000 times
4	10,000 times
5	100,000 times
6	1,000,000 times
7	10,000,000 times

Therefore, if 10 bacteria increase by 1-log, those 10 bacteria multiply by a factor of 10 to 100 bacteria (i.e., 10 x 10 = 100). If 10 bacteria increase by 2-logs, those 10 bacteria multiply by a factor of 100 to 1,000 bacteria (i.e., 10 x 100 = 1,000).