

Microbiological Testing for Water and Food Samples



Hortus Technical Services can test water, and other samples for a wide range of bacteria. This information sheet summarises the main tests usually carried out for home, farm or horticultural uses. The common requests are covered below, but we have many other tests that can be carried out on water, soil and vegetation.

What are Microbes?

The term “Microbes” is commonly used to refer to bacteria, and bacteria are one of a group of microorganisms (only visible using a microscope). Other microbes include protozoa (such as amoeba, Giardia and Cryptosporidium) and viruses.

Note that there are very large numbers of microbes in the environment (soil, air and water), but only a small number of these are “Pathogenic” (ie capable of causing disease). Examples of pathogenic organisms are bacteria such as faecal streptococci and enterococci, protozoa such as Giardia and Cryptosporidium, and viruses such as those causing infectious hepatitis and polio.

Why and how do we test for Microbes?

There are many species of bacteria that could potentially be present in your water and food samples. Testing for all of these bacteria would be a time consuming and costly exercise. Generally, we focus our testing on indicator organisms.

Indicator organisms are bacteria that are used to provide evidence of poor hygiene, inadequate processing or post-process contamination of foods. They are often chosen because they are relatively quick and simple to detect. Their absence in water and food provides a degree of assurance that the hygiene and food manufacturing process has been carried out appropriately, whereas their presence usually indicates that a potential problem or failure in the process has occurred.

In addition to testing for indicator organisms, we can perform microbiological testing for specific pathogens. If any of these pathogens are detected in samples, Queensland Health may need to be notified. Pathogen testing most frequently requested at Hortus:

- Salmonella
- Listeria spp.
- Listeria monocytogenes
- Coagulase Positive Staphylococcus

Indicator Organisms

The Enterobacteriaceae and coliform bacteria represent two of the most common groups of indicator organisms used by the water and food industry. Enterobacteriaceae and Coliform bacteria are found in the intestines of animals and man, thus they are found in waste materials. These bacteria themselves are not considered pathogenic, but due to their origin they may indicate a presence of disease-causing organisms. These bacteria are used as indicator bacteria, because of their origin as well as they are easy to isolate, they tend to survive longer than disease causing organisms and they are present in larger numbers. Most importantly, these bacteria react to the natural environment and treatment processes in the same manner as pathogens.

If the Enterobacteriaceae family are isolated from foods, this indicates evidence of poor hygiene or inadequate processing (especially heat-treatment), process failure and postprocess contamination of foods. E. coli is commonly used to provide evidence of potential faecal contamination in certain foods and is used as an index organism for the presence of enteric pathogens such as Salmonella.

Enterobacteriaceae

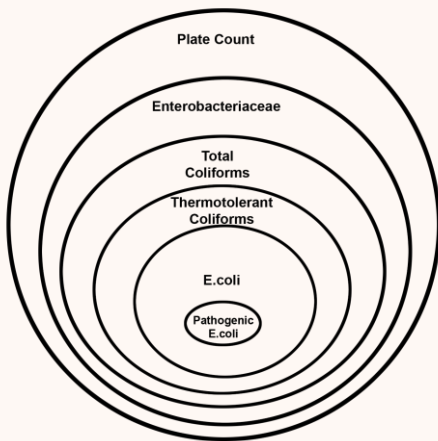


Figure 1 Diagram showing the relationship between genera within the Enterobacteriaceae family

The Enterobacteriaceae is a family of Gram-negative, non-spore-forming bacteria and is one of the most important groups of bacteria known to man. This family includes a number of important foodborne pathogens such as Salmonella, Yersinia enterocolitica, pathogenic Escherichia coli (including E. coli O157:H7), Shigella spp. and Cronobacter spp.

In addition, some members of the family are also associated with food spoilage and therefore contribute to significant economic losses for the agricultural and food industries. For example, Erwinia spp., and the more recently introduced Pectobacterium spp. and Brenneria spp., have long associations with plant and fruit diseases. Many other members of the Enterobacteriaceae are responsible for spoilage of a variety of foods including fruit and vegetables, meats, poultry, eggs, milk and dairy products, as well as fish and other seafoods.

Total Coliforms

Coliforms are a subset of the Enterobacteriaceae family. They are Gram-negative, non-spore-forming, rod-shaped bacteria that are capable of aerobic and facultative anaerobic growth in the presence of bile salts or other surface active agents with similar growth-inhibiting properties. They are found in large numbers in the faeces of humans and other warm-blooded animals, but many species also occur in the environment. The presence of coliforms in water samples does not indicate presence of faecal contamination, as there are many environmental coliforms that are not of faecal origin.

Thermotolerant Coliforms

Thermotolerant coliforms are a sub-group of coliforms that are able to grow at $44.5 \pm 0.2^{\circ}\text{C}$. Thermotolerant coliforms, including *E. coli*, can ferment lactose (or mannitol) at $44.5 \pm 0.2^{\circ}\text{C}$ with the production of acid within 24 hours. Thermotolerant coliforms are normal inhabitants of the intestine, generally present in high numbers in human and animal faeces. However, environmental thermotolerant coliforms, can occur in natural waters. These organisms are of lesser significance.

Escherichia coli

E. coli is the most common thermotolerant coliform present in faeces and is regarded as the most specific indicator of recent faecal contamination because generally it is not capable of growth in the environment. While tests for thermotolerant coliforms can be simpler than for *E. coli*, *E. coli* is considered a superior indicator for detecting faecal contamination. *E. coli* is a normal inhabitant of the intestine, generally present in high numbers in human and animal faeces, and it generally does not grow in natural waters.

Pathogens

Salmonella

Salmonella is the name of a group of bacteria that causes the infection Salmonellosis. Salmonella is one of the most common bacterial causes of diarrhoea in the United States and the most common cause of foodborne-related hospitalisations and deaths. Salmonella is more severe in pregnant women, older adults, younger children and those with a weakened immune system. Because Salmonella bacteria can live in the intestinal tract of humans and other animals, it can be spread easily unless proper hygiene and appropriate cooking methods are used.

Sources: You can contract salmonellosis by consuming raw and undercooked eggs, undercooked poultry and meat, contaminated raw fruits and vegetables (such as sprouts and melons), as well as unpasteurized milk and other dairy products. It can also be transmitted through contact with infected animals or an infected food handler who has not washed his or her hands after using the bathroom.

Prevention: Cook foods such as eggs, poultry and ground beef thoroughly to their appropriate temperatures. Wash raw fruit and vegetables before peeling, cutting or eating. Avoid unpasteurized dairy products and raw/uncooked foods. Wash hands often, especially after handling raw meat or poultry. Clean kitchen surfaces and avoid cross-contamination by not using the same containers or utensils for raw and cooked foods.

Listeria monocytogenes

Eating food contaminated with *Listeria monocytogenes* bacteria causes listeriosis—a serious infection that primarily affects individuals who are at a high risk for food poisoning: older adults, pregnant women, young children and people with weakened immune systems. *Listeria* can grow at refrigerator temperatures where most other bacteria cannot.

Causes: *Listeria* is found in refrigerated, ready-to-eat foods, such as hot dogs and deli meats, unpasteurized milk and dairy products, and raw and undercooked meat, poultry and seafood.

Prevention: Cook all foods to proper temperatures and reheat pre-cooked foods to 165°F; wash raw fruit and vegetables before peeling, cutting or eating; separate uncooked meats and poultry from foods that are already cooked or ready-to-eat; wash hands thoroughly; store foods safely at appropriate temperatures; and maintain a clean refrigerator, kitchen area and wash reusable grocery totes regularly.

Coagulase Positive Staphylococci

Coagulase positive staphylococci (staph) is a group of bacteria that's commonly found on the skin, throats and nostrils of many healthy people and animals. Therefore, it usually doesn't cause illness unless it is transmitted to food products where it can multiply and produce harmful toxins. Staphylococcal food poisoning can cause many of the same symptoms as other foodborne illnesses, such as nausea, stomach cramps, vomiting or diarrhea. The bacteria can also be found in unpasteurized dairy products, salty foods such as ham and other sliced meats, or in foods that require no cooking, such as puddings, prepared salads or sandwiches. Staphylococcal toxins are heat resistant, which means they cannot be destroyed by cooking.

Sources: Foods that are made or come in contact with the hands and require no additional cooking are at highest risk, including:

- Salads, such as ham, egg, tuna, chicken, potato and macaroni
- Bakery products, such as cream-filled pastries, cream pies and chocolate eclairs
- Sandwiches

Prevention: Wash hands with soap and water, do not prepare or serve food if you have a nose or eye infection or if you have wounds or skin infections on your hands or wrists. Keep the kitchen area clean and keep food out of the danger zone by cooling foods immediately in wide, shallow containers and refrigerating them.

Standard Plate Counts

Heterotrophic Plate Count (Water)

Heterotrophs are broadly defined as microorganisms that require organic carbon for growth. They include bacteria and fungi. HPC refer to numbers of organisms grown on non-specific culture media without inhibitory or selective agents. Even though the test is non-selective, only a small proportion of the microorganisms present in samples will be recovered. The types of organisms detected by HPC tests vary widely with location and time of year. Tests detect microorganisms that grow over a specified incubation period and at a defined temperature. Incubation periods can range from one day to weeks, and temperatures from 20°C to 40°C.

Microorganisms detected within HPC include:

- vegetative bacteria such as coliforms and other Enterobacteriaceae that are sensitive to disinfection processes;
- fungi and bacteria such as *Bacillus* spp that form disinfectant-resistant spores; and
- bacteria and fungi that grow in water.

Source and occurrence

Heterotrophic microorganisms include the naturally occurring microbial flora of water and soil environments (typically non-hazardous) and organisms present in a range of pollution sources. They occur in large numbers in raw water sources. Some drinkingwater treatment processes, such as coagulation and sedimentation, reduce the number of HPC organisms in water but growth can occur in other processes such as sand filtration. Numbers of HPC organisms are reduced significantly by disinfection processes; however, they can grow rapidly in drinking water once disinfection residuals have dissipated.

Total Plate Count (Food)

Total plate count (also referred to as aerobic plate count or standard plate count) can provide a general indication of the microbiological quality of a food. A standard plate count will not differentiate between the natural microflora of a food, spoilage microorganisms, organisms added to fermented foods or pathogenic microorganism. It cannot be used to predict the safety of the product and will be influenced by the storage conditions of the product. Depending on the product, a high standard plate count may indicate that the product may have been prepared unhygienically or stored inappropriately. When assessing standard plate count results, the processing and/or ingredients present in the foods needs to be considered. Please refer to Table 1: Microbiological Guidelines for Food.

Assessing a standard plate count result requires knowledge of the food and processing conditions. Care should be taken when assessing a single result as series of results over time generally provide a better understanding. Individual survey reports will justify the undertaking (or not) of a standard plate count test.

Yeast and Mould Count

The large and diverse group of microscopic foodborne yeasts and moulds (fungi) includes several hundred species. The ability of these organisms to attack many foods is due in large part to their relatively versatile environmental requirements. Although the majority of yeasts and moulds are obligate aerobes (require free oxygen for growth), their acid/alkaline requirement for growth is quite broad. Their temperature range is also broad, with a few species capable of growth below or above this range. Moisture requirements of foodborne molds are relatively low; most species can grow at very low moisture, although yeasts generally require a higher water activity.

Both yeasts and moulds cause various degrees of deterioration and decomposition of foods. They can invade and grow on virtually any type of food at any time; they invade crops such as grains, nuts, beans, and fruits in fields before harvesting and during storage. They also grow on processed foods and food mixtures. Their detectability in or on foods depends on food type, organisms involved, and degree of invasion; the contaminated food may be slightly blemished, severely blemished, or completely decomposed, with the actual growth manifested by rot spots of various sizes and colors, unsightly scabs, slime, white cottony mycelium, or highly colored sporulating mold. Abnormal flavors and odors may also be produced. Occasionally, a food appears mould-free but is found upon mycological examination to be contaminated. Contamination of foods by yeasts and moulds can result in substantial economic losses to producer, processor, and consumer.

GUIDELINES FOR DETERMINING THE MICROBIOLOGICAL QUALITY OF FOOD AND WATER

Disclaimer

It should be noted that these guidelines are not standards under existing legislation. The guidelines have been developed using information from Food Standards Australia New Zealand, Freshcare and Hortus Technical Services industry expertise. The guidelines are not formal standards and no liability is accepted by Hortus Technical Services for any occurrence arising from the adoption of these guidelines.

Table 1: MICROBIOLOGICAL GUIDELINES FOR FOOD

Ready to eat foods in which all the components have been cooked				
The final product is fully cooked with no additional handling after cooking therefore microbial counts should be low				
Test	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Standard Plate Count (SPC)	<10,000/g	<100,000/g	>100,000/g	
Escherichia coli (E.coli)	<3/g	3 to 10/g	>10/g	Pathogenic strains of E.coli should be absent
Coagulase positive Staphylococci	<100/g	100 to 1,000/g	1,000 to 10,000/g	>10,000/g
Salmonella spp	Not Detected in 25g			Detected
Listeria monocytogenes	Not Detected in 25g			Detected
Ready to eat foods which contain some components that have been cooked				
The final product may contain items that have been handled after cooking (sliced, mixed, piped) or may contain some components where no cooking process has been used				
Test	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Standard Plate Count (SPC)	<1,000,000/g	<10,000,000/g	>10,000,000/g	
Escherichia coli (E.coli)	<3/g	3 to 10/g	>100/g	Pathogenic strains of E.coli should be absent
Coagulase positive Staphylococci	<100/g	100 to 1,000/g	1,000 to 10,000/g	>10,000/g
Salmonella spp	Not Detected in 25g			Detected
Listeria monocytogenes	Not Detected in 25g			Detected
Fresh fruits and vegetables, salads and fermented foods				
The microbial levels are generally higher than for fully cooked products due to the normal microbial flora that may be present. SPC not used to evaluate microbial quality.				
Test	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Escherichia coli (E.coli)	<3/g	3 to 10/g	>100/g	Pathogenic strains of E.coli should be absent
Coagulase positive Staphylococci	<100/g	100 to 1,000/g	1,000 to 10,000/g	>10,000/g
Salmonella spp	Not Detected in 25g			Detected
Listeria monocytogenes	Not Detected in 25g			Detected

Table 1: MICROBIOLOGICAL GUIDELINES FOR WATER

Water Uses	Acceptable Limit
Preharvest water contacting the harvestable part of a crop that has an edible skin and is generally eaten uncooked	E.coli ≤ 126/100mL
Preharvest water contacting the harvestable part of a crop that is peeled or generally eaten cooked	thermotolerant coliforms < 1000/100mL
Preharvest water that does not contact the harvestable part of the crop	thermotolerant coliforms < 1000/100mL
Postharvest water used for water dumps and initial washing of product that is peeled or generally eaten cooked	thermotolerant coliforms < 1000/100mL
Postharvest water used for final washing or treatment of produce that is peeled or generally eaten cooked	E.coli < 1/ 100mL (potable water) Thermotolerant coliforms < 1/100mL
Postharvest water contacting produce with an edible skin and that is generally eaten uncooked	E.coli < 1/ 100mL (potable water) Thermotolerant coliforms < 1/100mL
Handwashing and cleaning water	E.coli < 1/ 100mL (potable water) Thermotolerant coliforms < 1/100mL

The Australian Drinking Water Guidelines have no specific detection limit for Standard Plate Count or Total Coliforms, however the following is recommended as minimum standards for a good water supply: Plate Count

< = less than > = greater than g = gram