

CHAPTER 18

Testing



Overview

Testing may be used or required to demonstrate that chemical (including allergen) or microbiological hazards are being controlled. However, testing alone cannot be relied on to confirm whether produce is safe or unsafe. The main use of testing is to check the effectiveness of food safety and quality controls and to assist in identifying potential sources of contamination when they occur.

18.1 Why test?

Routine testing for chemical and microbial contaminants in fresh produce is not a reliable standalone method for ensuring food safety. While testing can support verification activities, it is inherently limited in scope, frequency and responsiveness. Sole reliance on end-product testing contradicts the preventive, systems-based approach central to HACCP principles, which emphasises identifying and controlling hazards throughout the supply chain.

It is important to note that chemical and microbial contamination generally will not be uniformly distributed across crops or postharvest environments. This uneven distribution means the likelihood of detecting food safety risks through sporadic testing is extremely low, unless sampling programmes are continuous and highly intensive, which is an impractical approach for most operations.

Greater assurance comes from conducting thorough hazard analysis and implementing preventive control measures. These proactive steps form the foundation of effective food safety management [refer Appendix 1, Chapters 3 and 4]. While regular testing remains a requirement under most assurance programmes, its primary role is to verify that identified hazards are being controlled. To meet regulatory and customer expectations, businesses should establish, implement and maintain documented testing schedule covering chemical, heavy metal, microbiological and allergen risks.

Types of verification testing that may be required include:

- growing site soil test for persistent chemicals
- growing site soil test for heavy metals
- growing site soil test for microbial contamination
- water test for irrigation water quality
- water test for postharvest water quality
- packed produce test for persistent chemicals, heavy metals and pesticide residues
- packed produce test for microbial contamination
- packed produce test for unintended allergen presence
- environmental testing of the facility and equipment to verify effectiveness of a cleaning and sanitation program.

The following guidelines are provided to assist in understanding and standardising approaches to these tests.

18.2 Sampling

Before conducting any testing, businesses need to consider:

- why they are doing the testing (i.e. routine check, incident related, regulatory or food safety standard requirement?)
- what questions they are trying to answer (i.e. is my product compliant? Do I have an issue, if so, how big could the problem be?)
- where are they going to take samples from and how many do they need to take?

Because hazards are rarely evenly distributed in a field or within a batch of produce, sampling should be conducted randomly and should reflect the characteristics of the produce grown or supplied.

The purpose of testing will influence the sampling approach and producers may need to consult with subject matter experts (i.e. chemical suppliers, microbiologists, accredited laboratories or industry consultants) to ensure appropriate methodology and interpretation. Businesses should also be aware of any minimum testing requirements specified by assurance programmes, customer specifications or regulatory authorities and ensure these are met as part of their food safety and compliance obligations.

Prior to commencing sampling, consult with your laboratory provider to confirm any specific requirements that may affect sample integrity or testing outcomes. This includes verifying whether specialised containers are required and identifying any handling protocols that should be followed during sample collection.



Image C18.1 | Using a telescopic pole allows for safe and controlled collection of water from the centre of the pond, minimising disturbance to sediment and reducing the risk of contamination from the pond perimeter.



Image C18.2 | Disposable boot covers are used to walk through designated sampling zones, to collect soil and debris for microbiological analysis of potential pathogens.



Image C18.3 | Sterile sampling bags should be used to minimise the risk of external contamination and ensures the test results accurately reflect the conditions of the sample.

18.3 Laboratory Selection

While price and convenience for sample delivery are often key factors when selecting a laboratory to test fresh produce, there are several other factors to consider when selecting a laboratory provider and most assurance programmes mandate the use of accredited laboratories.

Table C18:1 | Laboratory selection.

Things to Consider	
1	Do they operate a quality management system that complies with the requirements of international standard ISO/IEC 17025?
2	If so, are they accredited: NATA (National Association of Testing Authorities) in Australia? IANZ (International Accreditation New Zealand) in New Zealand? International Laboratory Accreditation Cooperation (ILAC)? Accredited laboratories are listed on the NATA, IANZ or ILAC websites. Laboratories accredited by NATA or IANZ to ISO/IEC 17025 are preferred when selecting a laboratory to test fresh produce.
3	Does the scope of their accreditation specifically reference the microbiological testing or analysis of residues and contaminants in fresh fruit and vegetables that your business requires? In New Zealand, the Recognised Laboratory Programme (RLP) laboratories are listed on the MPI website.

18.4 Chemical testing

Testing the soil for persistent chemicals or heavy metals should be conducted when the risk at the growing site is high, as determined by a hazard analysis [refer Appendix 1, Chapter 6 and Chapter 8].

Harvested fresh produce may be tested for residues of persistent chemicals, heavy metals or pesticides. Such tests are used to verify that these chemicals do not exceed the chemical Maximum Residue Limits (MRL) or heavy metal Maximum Levels (ML) specified in relevant legislation for harvested produce [refer Appendix 2 and Chapter 8].

Specialised plant nutrient determination and cadmium residues in produce certified laboratories are listed on the Australasian Soil and Plant Analysis Council (ASPAC).

18.4.1 Maximum Residue Limits (MRLs) for agrichemicals

The Maximum Residue Limit (MRL) is the highest concentration of a chemical legally permitted on a type of produce. The concentration is expressed in milligrams (mg) of the chemical residue per kilogram (kg) of the produce type (equivalent to parts per million, 'ppm'). Typically, MRLs are set at levels 100 or even 1000-fold lower than those that would be expected to cause symptoms of illness and consider the susceptibility of people that might be expected to be consumers of those foods.

The Maximum Level (ML) is the maximum level of heavy metal contaminant that is legally permitted to be present in a food. The concentration is also expressed in mg/kg. If the MRL for a persistent chemical or the ML for a heavy metal is exceeded, it indicates that the growing site may not be suitable for growing the produce type or that additional control measures should be implemented.

If an MRL is exceeded for a chemical used in crop protection, it normally indicates the chemical has not been used according to label directions. However, this does not normally indicate an acute public health or food safety concern. Legal prosecution for exceeding an MRL is based on the failure to follow label directions (i.e. misuse of the chemical), not for exceeding the MRL.

In Australia the MRLs for registered crop protection chemicals are established by the Australian Pesticides and Veterinary Medicines Authority (APVMA). MRLs are then adopted into Standard 1.4.2 and Schedule 20 of the Food Standards Code. A residue may meet FSANZ limits but still be non-compliant if the chemical is not authorised by the APVMA for that crop. In New Zealand the MRLs are set by Food Notice, with a default of 0.1mg/kg if no MRL set (set under section 144 (6) of the Food Regulations 2015).

Under the Trans-Tasman Mutual Recognition Arrangement (TTMRA), food imported from Australia may be legally sold in New Zealand, if it complies with Australian requirements. The converse is also true; food imported from New Zealand into Australia is legal if it complies with New Zealand requirements.

For other countries, the importing country MRLs should be checked before treatment and export. Ideally, growers should be fully aware of the MRL requirements in all likely destination markets before the growing season commences. Spray programs should be designed to meet those requirements and residue test results checked against the market MRLs. These may differ from Australian and New Zealand MRLs.

18.4.2 Maximum Limits (ML) for heavy metals

The Maximum Level (ML) is the maximum level of heavy metal contaminant that is legally permitted to be present in a food. The concentration is also expressed in mg/kg. If the MRL for a persistent chemical or the ML for a heavy metal is exceeded, it indicates that the growing site may not be suitable for growing the produce type or that additional control measures should be implemented.

MLs are specified in Standard 1.4.1 and Schedule 19 of the Food Standards Code.

18.4.3 What to test for?

Chemical residue tests for pesticides should screen for all chemicals applied during crop growth and postharvest treatment. The commonly requested chemical residue test is a multi-residue screen, meaning that they assess the levels of a range of persistent chemicals, heavy metals and commonly used chemicals for the produce type and production method. Multi-residue screen may not cover the full range of chemicals used so it is important to check the active constituents that are tested for when selecting tests. Utilising multi-residue screen will also detect any residues from chemicals not directly applied that may be present from spray drift from neighbouring sites or from pre-planting applications from nurseries.

Testing can be important on growing sites where there is a high level of risk from persistent chemicals or heavy metals. In general, it is more useful to test the fresh produce type grown on the site rather than the soil, as it is the residue on or in the harvested produce that is most relevant for regulators, customers and consumers. However, soil tests before planting can indicate the degree of contamination and this may affect the choice of crop to be grown.

18.4.4 How often to test?

The requirement for testing should be established by the hazard analysis and the frequency determined by the confidence level required to verify the chemical use program is correct. To meet the requirements of most food assurance programmes, a chemical residue test is generally undertaken once a year, but this may not be enough if different chemicals are used during different growing conditions (e.g. during warm and cool seasons for all year-round crops). Some assurance programmes and customers may require a higher frequency of testing and may prescribe which active ingredients are tested for.

18.4.5 Where to sample?

A sample for testing can be collected at several points in the supply chain:

- prior to harvest, after all withholding periods for crop protection chemicals applied to the crop have elapsed
- after application of postharvest treatments and packing, for produce that may be stored for a short period before dispatch
- before or on delivery to the first customer in the supply chain, for produce that is harvested, packed and immediately dispatched
- prior to storage, for produce that is stored for an extended period before delivery, such as apples
- after storage, where the postharvest application of chemicals for long-term storage is being verified.

18.5 Microbial testing

Microbial testing can support verification of microbial control measure and compliance with customer requirements. However, reliable results require extensive sampling, especially when contamination is low or localised, making this approach costly and often impractical. A preventative strategy, based on good agricultural and hygiene practices, is a more effective way to ensure produce safety.

There are currently no mandatory microbiological limits in the Food Standards Code for irrigation or wash water or for fresh produce not classified as ready-to-eat (RTE). However, Food Standards Australia New Zealand (FSANZ) does stipulate microbial limits for RTE foods under Standard 1.6.1 and Schedule 27. RTE produce includes items intended to be consumed without further washing, peeling, or cooking (i.e. pre-washed leafy vegetables or cut fruit). Whereas whole raw produce requiring consumer preparation is not classified as RTE. Assurance programmes and customer specifications may impose additional microbial requirements. Guidance on critical limits for human pathogens is provided in Table C18:2, with supporting details in Appendix 3 and the Compendium of Microbiological Criteria for Food, available on the FSANZ website.

18.5.1 What to test for?

There are many types of microbes that may pose a concern for food safety [refer Appendix 3]. However, testing for every possible human pathogen is impractical and prohibitively expensive. A more efficient approach is to monitor for ‘indicator organisms’ [refer Appendix 4], which are non-pathogenic but share similar growth conditions with human enteric pathogens. Their presence suggests potential faecal contamination and the possible presence of pathogens. Common indicators such as *E.coli* or generic *Listeria spp.* are included in testing programs (Table C18:2) to provide a rapid assessment of contamination risk. If indicator organisms are detected at unacceptable levels, further investigation is required to identify the contamination source and determine whether the product is suitable for sale and human consumption. It is important to note that some bacteria may enter a viable but non-culturable (VBNC) state under stress conditions (i.e. exposure to sanitisers or UV light), making them more difficult to detect through standard testing methods.



Testing requirements should be guided by regulatory obligations, food safety standards and customer specifications.

Table C18:2 | Description of microbes that may be part of a testing programme.

Microbe Type	Description
Thermotolerant coliforms	Thermotolerant coliforms are normal bacterial inhabitants of the intestines of warm-blooded animals. They are generally present in high numbers in human and animal faeces and may be used as an indicator of faecal contamination. However, there are also types of thermotolerant coliforms that can grow in the environment in the absence of faecal contamination. Particularly <i>Klebsiella pneumoniae</i> in water contaminated with effluents from pulp and paper mills. Based on international and domestic research a limit of thermotolerant coliforms <100 cfu/g is currently set on fresh produce specifications in Australia. However, the specific thermotolerant coliform <i>E. coli</i> is the preferred indicator organism for identifying faecal contamination. For the use and limitations of faecal indicators refer Appendix 4.
<i>Escherichia coli</i> (<i>E. coli</i>)	<i>E. coli</i> is the most common thermotolerant coliform bacteria present in animal faeces and is therefore the best indicator of recent faecal contamination. It is generally not capable of independent growth on produce unless provided with an environment rich in moisture and nutrients. There are five sub-groups of <i>E. coli</i>, however, that can cause human illness (named as EHEC, ETEC, EIEC, EPEC, EAEC). They are called sub-types, and their differentiation is based on the symptoms of the illness they typically cause. Of these five sub-types, the one of most concern to the food industry is enterohaemorrhagic <i>E. coli</i> (EHEC) also called Shiga-toxin producing <i>E. coli</i> (STEC). Strains of this sub-groups can cause serious illness especially in young children and the elderly. In young children, infection can lead to lifelong kidney damage, usually requiring a transplant for the victim to then have a normal life not requiring frequent dialysis as a therapy. In the elderly, death may result. This subgroup is also the one most commonly involved in foodborne disease outbreaks, including ready to eat salad vegetables. As such, there is much attention given to them in the fresh produce industry, especially their potential presence in ready-to-eat fresh produce offerings.
<i>Listeria spp.</i> as an indicator organism	<i>Listeria</i> species are common in the environment, being found in soil, decaying plant material and other sources. Carriers also include many species of animals. The vast majority are not harmful. If <i>Listeria spp.</i> are detected, on equipment which comes into contact with produce or the produce itself, this indicates that conditions are favourable for the growth of <i>L. monocytogenes</i> also.
<i>Listeria monocytogenes</i>	A number of specific strains of <i>L. monocytogenes</i> are human pathogens. While the risk of contracting listeriosis is quite low, unless the levels on or in a food are very high [refer Appendix 3], the disease can be fatal, particularly among the young, elderly, pregnant or immunocompromised. Infection can also result in miscarriages. If <i>L. monocytogenes</i> is detected, sources of contamination should be investigated and appropriate control measures implemented.
<i>Salmonella enterica</i>	Species of <i>Salmonella</i> bacteria are found in the intestinal tracts of a wide variety of animals and are a significant public health concern. While the incidence of <i>Salmonella</i> in fresh produce is low, contamination is possible from the environment and through handling. It may also be found in organic fertilisers and composted biosolids. Most <i>Salmonella</i> do not grow at temperatures below 7°C and the optimum temperature for growth is 35-37°C. If <i>Salmonella</i> is detected in a 25 g sample of fresh produce, sources of contamination should be investigated and appropriate control measures implemented.
Viruses	While not routine, some customers may request testing for viruses e.g. Norovirus or Hepatitis A, especially for RTE products. These tests are complex and only conducted by specialist laboratories.

18.5.2 Where to test?

Table C18:3 | Example testing locations [refer Chapters 3, 5, 7, 8, 9 and 11].

What	Where	Examples of Why
Environmental surfaces	Examples include product contact equipment, conveyors, scales, floors, produce bins, cool room walls, doors and produce bins.	Testing can be completed to verify controls are effective, meet customer specification requirements or to check for contamination from hazards.
Water - potable water - irrigation water - wash water	Water should be sampled at the point where it contacts produce. When investigating potential contamination, water should also be tested at the water source.	Testing can be completed to verify controls are effective, meet food safety standards, customer specification requirements or to check for contamination from hazards. To determine the risk of contamination, testing should be completed at times when the likelihood of contamination is highest and at a frequency that allows management of the potential risk. Water should be tested more often if it is from variable sources such as dams, rivers or creeks, rather than a stable source such as a deep bore. Bore water is generally considered lower risk than surface water. However, periodic testing is still recommended to verify its safety. Particularly test if the conditions changes, such as after heavy rain or during drought periods or to check whether a water treatment process is effective.
Produce - pre and post-harvest - production lot	To check the effectiveness of a postharvest practice, sample the produce immediately afterwards. To check for gross contamination, sample the produce at harvest.	Testing can be completed to verify controls are effective, meet customer specification requirements or to check for contamination from hazards. Testing of the inputs such as the water and the produce can also be performed to verify that controls implemented work effectively. For example, if sanitisers in solution on produce reduce the microbial numbers or to determine the frequency that water may need to be changed in rinse tanks. When testing to assess contamination risk, test when the likelihood of contamination is highest. This may mean testing when there is a high risk that a particular practice, inputs or weather conditions may have contaminated produce.

Customers or regulatory agencies may require additional testing for other microbes [refer Appendix 2]. This is particularly likely if produce has no subsequent pathogen reduction step or if it is destined for hospitals or aged care homes, because people in these facilities are considered as vulnerable, often having reduced immunity and are more susceptible to microbiological infections.

Testing for microbial hazards other than bacteria, such as viruses and parasites is difficult and many laboratories are not equipped to perform these tests. However, the presence of *E. coli* can indicate such organisms may be present [refer Appendix 4].

18.6 Allergen testing

While fresh produce is generally free from common food allergens, unintentional cross-contact can occur during post-harvest handling, particularly in packing or processing environments where allergen containing products are also handled. Allergen testing may be required to verify cleaning effectiveness, meet customer or food safety standard requirements or support allergen free claims.

18.6.1 What to test for?

Testing is usually focused on the most common food allergens relevant to regulatory or customer requirements. These may include peanuts, tree nuts, milk, egg, soy, wheat, gluten from wheat, rye or oats, fish, crustacea, mollusc, sesame and lupin. Generally, tests target allergenic proteins, using enzyme-linked immunosorbent assay (ELISA). Some rapid onsite test kits are also available with use the same principle. Testing can be conducted on product, food contact surfaces or rinse water from equipment.

18.6.2 How often to test?

The frequency of allergen testing should be based on the risk of allergen cross-contact identified in the hazard analysis. Testing may be required routinely as part of cleaning verification (e.g. when switching between allergen containing and allergen free products), following environmental swabbing programmes or periodically to verify allergen management programmes. Food safety standards and customer specifications may also have specific requirements for testing frequency and methods.

18.6.3 Where to sample?

Sampling may be conducted on product, food contact surfaces, equipment, water or packaging materials. Areas to consider include shared conveyors, packing lines, storage bins, cutting equipment and packaged produce. It is important to follow laboratory instructions on sample collection, handling and transport to ensure accurate results.

18.7 Facility / Environmental testing

Collecting samples from equipment surfaces, floors, walls and cool rooms at a facility is generally referred to as environmental monitoring. This type of testing may be used to investigate whether a facility is the source of a contaminant identified through produce testing. It can also be used to verify the effectiveness of cleaning and sanitation programs [refer Chapter 9].

A range of commercial testing kits are available for surface sampling and are a valuable tool for measuring cleanliness and sanitation program effectiveness over time but have their limitations.

For example:

- contact plates and dip slides are semi-quantitative i.e. they do not provide an exact number and may be used for general detection but are not recommended for specific pathogen identification
- swab sticks with special nutrients are semi-quantitative and indicate the presence or absence of specific pathogens

- sponges and cloths (large swabs) provide an option for large area sampling and have a high level of sensitivity that can be useful for foodborne illness investigation
- adenosine triphosphate (ATP) based measurement devices are rapid but not specific to microbes, so ineffective if plant waste is present. They may be used to monitor cleaning and sanitation of specific areas over time, single results are of little value unless a baseline has been determined.

These testing approaches do not provide the quantitative and qualitative reliability necessary for conducting foodborne illness investigations. In some cases, the residual presence of sanitisers can interfere with testing results, as a result sampling should not be performed immediately after applying sanitiser.

When collecting sample for onsite testing or for external testing, swabs and slides should be handled carefully to avoid cross-contamination which can impact the test results.



Image C18.4 | Examples of environmental sampling tools used to monitor surfaces for microbial contamination, including swabs, sponge swabs and surface sampling cloths.

18.8 Sending samples to the laboratory

Before sending a sample for testing:

1. check that the laboratory can test for the selected chemicals or microbial test required
2. consider the sample size required and how best to transport the sample. For example, collect a sample by selecting three (3) units at random from a lot/batch. For example, collect three lettuces or apples. For smaller produce (e.g. snow peas) select three (3) x 200g samples
3. consider requesting if the laboratory has specific requirements for sampling and preparing sample for analysis
4. to mitigate potential contamination of the sample, use disposable gloves to collect the sample and change gloves between samples
5. place the sample in a clean/sterile, clearly labelled plastic bag (produce), bottle (water) or other container provided by the testing laboratory
6. clearly label the sample
7. complete all sample submission form details required by the testing laboratory
8. keep the samples cool in a refrigerator, unless instructed otherwise by the testing laboratory until ready to send
9. package the sample securely to prevent damage during transport. Include the completed analysis request form and use ice bricks or freezer sheets to keep the sample chilled until it reaches the laboratory
10. use same-day freight (and otherwise overnight) to ensure the sample gets to the laboratory promptly, ideally within 24h of sampling.

18.9 Interpreting test results

18.9.1 Chemical residue test results

Laboratories may differ in how they report chemical test results. Some provide only the active constituents detected in a multi-residue screen, reported alone or relative to the MRL (mg/kg). Others report all constituents tested, with results compared against each MRL.

When interpreting chemical test results, check all active constituents detected in the report against their MRLs. If the sample value is greater than the MRL, then the MRL has been 'breached'. This is sometimes termed an MRL violation. If an MRL has been breached the cause of the breach should be investigated and appropriate corrective/control measures implemented.

Chemical testing reports may also show a number called the Limit of Detection (LOD) or Limit of Reporting (LOR). The LOD/LOR is the lowest quantity of substance the testing Instrument/method can detect within statistical confidence. This is effectively the lowest detection limit for the substance for the test method selected.

In Australia, if a chemical residue is detected (i.e. greater than the LOD/LOR) and there is no MRL for the substance, then this is a MRL breach (i.e. the substance is not permitted (registered) for use on this type of produce [refer Chapter 8]. In New Zealand, if there is no MRL listed for use of a substance on a particular type of produce then it is considered off-label use and a default limit of 0.1 mg/kg applies. In some instances in New Zealand, the limit is set as the limit of analytical quantification (e.g. 0.01 mg/kg) meaning use of the substance on that produce is not permitted and any residue detection (i.e. greater than the LOD/LOR) is considered a breach.

18.9.2 Microbial test results

It is important to understand how the test has been completed and its purpose when interpreting the results. It is important to note that some tests are not designed to distinguish between pathogenic and non-pathogenic bacteria of the same species or it may be extremely difficult to differentiate between closely related strains without highly specialised techniques. For these reasons, a positive result does not necessarily mean the water is unsafe or the produce will be unsafe to eat. Presumptive positives should be followed up with confirmatory testing to verify if pathogens are viable. Confirmed positives are the basis for corrective action. Conversely, a negative result does not necessarily mean that the water is safe to use or produce safe to consume. In some cases, samples may also be contaminated with material or chemicals, that can interfere with the reliability of the test.

The laboratory performing the testing can provide you the information on how the tests work and any limitations that could impact the results.

Results for microbial tests which are designed to quantify the number of bacteria present are reported as the number of colony forming units (cfu), per unit of volume (e.g. cfu/ml) or weight (e.g. cfu/g). Each colony forming units is assumed to have grown from an individual bacterium.

There are also methods which are designed to simply detect the presence of the bacteria in a certain amount of food tested. These are called qualitative methods and generally report results as Detected / Not Detected or Present / Absent per gram or other quantity of material tested.

The presence or absence of the microbe and the number of microbes present are derived through a variety of laboratory techniques.

It is important to note that some tests are not designed to distinguish between pathogenic and non-pathogenic strains of organisms.

The typical terms used in microbial test reports, based on the methods used, along with their advantages and disadvantages are described in Table C18:5.

Use Table C18:4 to determine when action is required for RTE fresh produce testing.

Note, where *Listeria monocytogenes* is of concern, testing may be conducted using larger sampling sizes (e.g. 125 g) or multiple 25 g subsamples to increase the likelihood of detecting contamination.

Seek guidance from regulators, certification bodies or technical consultants when addressing out-of-specification test results.



Image C18.5 | The streak plate techniques is used to isolate colonies of *Listeria* on selective agar for further identification and confirmation.

Table C18:4 | Guidance on critical limits on RTE foods (adapted from FSANZ Compendium of Microbiological Criteria for Food).

Hazard		Satisfactory	Marginal	Potentially hazardous
<i>E. coli</i>		<3 cfu/g	3 – 100 cfu/g	>100 cfu/g
Shiga toxin-producing <i>Escherichia coli</i> (STEC) (pathogen)		Not detected in 25 g	N/A	Detected in 25 g
<i>Salmonella spp.</i>		Not detected in 25 g	N/A	Detected in 25 g
<i>Listeria monocytogenes</i>	RTE foods that support growth of <i>L. monocytogenes</i>	Not detected in 25 g	N/A	Detected in 25 g
	RTE foods that do not support growth of <i>L. monocytogenes</i>	Absent in 25 g	<100 cfu/g	>100 cfu/g

Criteria have been agreed internationally for RTE foods that do not support the growth of *Listeria monocytogenes* where the physico-chemical characteristics fall into one of 3 ranges throughout the foods stated shelf-life, these default criteria are: pH<4.4 regardless of water activity; aw <0.92 regardless of pH, and combination of pH>5.0 and water activity <0.94 (FSANZ).

Table C18:5 | Microbiological method terms and considerations when reviewing test reports.

Term	Description	Advantages/disadvantages
Colony Forming Units (cfu)	Obtained by conducting a series of dilutions, plating on selective or non-selective agar plates and incubating for a standard time and temperature. The number of cfu in the original sample is mathematically derived from the dilution series result. Expressed in units of cfu/g or cfu/ml.	This method provides a reliable estimate of the number of viable microorganisms in a sample and is widely accepted. It is relatively inexpensive and standardised. However results typically take 24-48 hours to obtain and may require additional confirmation testing for specific pathogens.
Enumeration	The determination of the number of viable microbes in a sample. The sample is prepared and then a portion tested using agar designed to grow a diverse or specific group of bacteria. Enumeration tests may also be carried out following a presumptive positive identification for the presence of a food safety pathogen, to determine the number of viable pathogen organisms in the sample i.e. a confirmed presumptive positive identification provides the qualitative result whereas enumeration provides the quantitative result.	Should be used when a number is required to meet the food safety limits set by the business. Bacterial growth is dependent on the agar used which contains specific nutrients, and the time and temperature use to grow the bacteria. In some cases, methods looking for the same bacterial group could give different results if different growth conditions have been used.
Most Probable Number (MPN)	MPN is a statistical method used to estimate the concentration of viable microorganisms in a sample by observing the number of positive growth responses in a series of dilutions. The MPN is the most likely concentration of viable pathogens in the sample.	Most Probable Number (MPN) methods are now rarely used for microbial testing due to concerns about accuracy and reliability. Where they are still applied, it is typically for soil and water analysis rather than for fresh produce or other food products. The result from an MPN method cannot be compared to a quantitative result from a plating method (i.e. cfu/g).
Polymerase Chain Reaction (PCR) methods	These methods are highly specific for a bacterial group or even species (i.e. <i>Salmonella enterica</i> or <i>L. monocytogenes</i>). After the sample has been incubated in a specific nutrient broth to increase the number of cells that might be present, a sample is prepared to detect DNA which is specific to the bacteria.	Highly sensitive and specific for the bacteria being tested. While PCR is highly sensitive and specific, it may detect DNA from non-viable bacteria, resulting in positive findings even when viable pathogens are no longer present. Additionally, presumptive positive results should be confirmed through isolation and culture (Figure C18.5). This is particularly important for STEC testing, where multiple virulence genes may be found across different <i>E.coli</i> strains (including non-pathogenic) in the same sample.

Term	Description	Advantages/disadvantages
Whole Genome Sequencing (WGS)	WGS analyses the entire DNA sequence of a microorganism to identify its exact strain, virulence genes, antimicrobial resistance and evolutionary relatedness to other isolates. Commonly used in outbreak investigations and source tracing.	WGS provides the highest level of genetic detail available for a microorganism. This method can link isolates from food and patients to identify contamination sources with high confidence. WGS is more expensive than routine microbiological testing and not typically used for routine monitoring. It may also detect non-viable organisms, similar to PCR, where results often need to be confirmed with culture. Given that WGS can provide such valuable insights it is recommended that, if there are cost or time barriers, the micro-organism is stored (by the testing laboratory) frozen to enable WGS to be carried out at a later date.
Presence/Absence	Presence or absence tests are designed to detect whether a specific microorganism is present in a given sample (e.g. 25g), without estimating how many bacteria are present. These tests are often used for regulatory compliance, especially for pathogens like <i>Salmonella</i> , <i>Listeria</i> or <i>E. coli</i> O157:H7. Results are typically reported as 'Detected' or 'Not detected'	These tests are generally cost-effective, simple to perform and suitable for routine monitoring. A key limitation is that they do not quantify the level of contamination. If a positive result is obtained, additional testing may be required to confirm organism viability and to determine contamination levels through enumeration.
Presumptive positive	The words 'presumptive positive' on a test report mean the test has potentially found the pathogen present in the sample. However, further confirmation testing is required to determine whether the pathogen is alive or non-viable or if there may have been another similar bacteria present which was wrongly identified by the kit. If the 'presumptive positive' is confirmed a further enumeration test can be carried out to estimate population size.	All methods for pathogen testing will have an initial step, where a presumptive positive is the first alert of a potential problem.

The type and frequency of testing should be based on risk assessments, applicable regulations, assurance programme requirements and specific customer requirements.

Resources

Australasian Soil and Plant Analysis Council (ASPAC) (n.d.) *ASPAC website*.

Australian Pesticides and Veterinary Medicines Authority (APVMA) (n.d.) *APVMA website*.

Food Standards Australia New Zealand (FSANZ) (n.d.) *Safe Horticulture Australia: A guide to the primary production and processing standard for horticulture*.

Food Standards Australia New Zealand (FSANZ) (n.d.) *Australia New Zealand Food Standards Code – Schedule 27: Microbiological limits for food*.

Food Standards Australia New Zealand (FSANZ) (2025) *Compendium of microbiological criteria for food*, July.

Ministry for Primary Industries (MPI) (n.d.) *MPI website*.