How rapid food safety test methods can be used in food manufacture and comparisons with conventional testing methods

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*Food safety testing is an important part of a food business' risk-mitigation toolkit, with increasing obligations for food businesses such as the December 2023 introduction of new food safety management requirements in Standard 3.2.2A of the Australian New Zealand Food Standards Code (**Food Standards Code**). With the greater regulatory scrutiny, it is essential for food businesses to effectively and accurately test for food safety, whilst minimising cost and maximising efficiency. This article dives into various conventional and rapid food safety test methods and compares factors such as sensitivity, time and complexity.*

Food must be tested for safety, regulatory and quality purposes throughout all processes in the supply chain. Testing of foods is not only required to meet regulatory safety standards and requirements, but for food businesses to minimise the risk of food recalls and to avoid damage to business, brand and product reputation.

What is a rapid test method?

Standard 1.6.1 of the Australia New Zealand Food Standards Code (**Food Standards Code**) prescribes microbiological limits in relation to different foods by category and by microbiological hazard. The Compendium of Microbiological Criteria for Food is published by Food Standards Australia New Zealand (**FSANZ**) to provide guidance on Standard 1.6.1 of the Food Standards Code.

This document includes some information on rapid testing. However, rapid testing is generally seen as being time and cost-effective [1], while potentially being less accurate than conventional testing methods [2].

Different definitions of 'rapid testing' are used in the scientific literature. At page 56, the guidance describes 'rapid testing' as "an alternative to standard microbiological testing". Rapid testing methods usually produce immediate results, whereas standard microbiological testing takes several days [3].

The role of rapid testing in a food business context

The use of rapid testing is advantageous for a food business because it can act as an alert to a bigger safety or quality problem, and to allow precautions or quarantining of affected product without the need to trigger an immediate food recall before full microbiological testing provides more precise confirmation of the exact microbiological problem and its scope.

Rapid testing thereby also reduces food waste and helps creates avenues for waste to be reused or repurposed in a safe way. Rapid testing minimises risk whilst creating opportunities for value-adding.

Common foodborne pathogens:

Some of the pathogens that rapid testing can help protect against include:

Norovirus: Noroviruses are the most common cause of foodborne viral gastroenteritis globally, which is an illness denoting infection and inflammation in the digestive system. The source contamination is often food handlers practising poor personal hygiene.

Campylobacter and *Salmonella*: As opposed to viral gastroenteritis which is commonly caused by norovirus infections, Campylobacter and Salmonella are the common cause of bacterial gastroenteritis. Poultry products are the common contaminant.

Listeria: *Listeria* is a bacteria that has a 30% fatality rate and is often associated with dairy foods and leafy vegetables.

Escherichia coli (E. coli): Another common bacteria and source of foodborne illness. Pathogenic strains of *E. coli* are often found on cattle and therefore beef is a common source of contamination.

These common pathogens are regulated under Standard 1.6.1 of the Food Standards Code – Microbiological limits for food. Standard 1.6.1 and Schedule 27 set limits for pathogens in food categories.

A breach of the limits for pathogens will result in the food being unsafe, and will amount to a breach of the *Food Act* in the State or Territory of sale. This will result not only in substantial monetary penalties, but prevents the unsafe product being sold for human consumption.

A breach of microbiological limits can also result in the food being refused entry into Australia (if it is imported) and/or can result in the product being recalled.

The FSANZ Compendium of Microbiological Criteria for Food nevertheless encourages the use of rapid testing as it is faster and more economic than conventional testing but allows detection that can be verified by more thorough conventional testing. FSANZ has also indicated that microbiological monitoring will generally involve rapid testing to test for the presence of pathogenic microorganisms. However, the guidance also cautions about limitations of rapid testing methods such as ATP testing and protein swabs, even though the results may not determine which target organisms are present [3].

Therefore, the guidance also suggests that a food business' microbiological monitoring may also need to include subsequent non-rapid microbiological testing [3].

Conventional food safety testing methods

Conventional food safety tests prioritise high accuracy and sensitivity [4]. Common conventional methods includes broad methods such as microbiological culture methods and chromatography [4], as well as specific immunoassays such as ELISA.

Chromatography

Out of the conventional methods, chromatography is the primary non-microbiological technique.

Chromatography involves separating and analysing complex mixtures [4]. In gas chromatography, the sample is vaporized and injected into a heated column, with allows separation based on volatility and affinity of components. Whereas liquid chromatography involves injection of the sample in liquid form.

Although these methods have the highest sensitivity, they are limited by expensive instruments and lengthy and complicated testing procedures.

Microbiological culture methods

Microbiological culture methods are the traditional means of detecting and quantifying microorganisms in food samples. The process can be broken down to six general steps [5]. Sample preparation: Food samples are collected and prepared such as through dilution for analysis.

Inoculation: The prepared sample is inoculated or transferred to culture media. The culture media can be general purpose or selective media.

Incubation: The culture is then incubated for optimal microbial growth. This process can take up to several days.

Observation of colonies: Based on the type of medium used, the colour or size of the colonies can be a qualitative indicator of the microorganisms present.

Isolation and identification: Further testing such as biochemical tests can be used to confirm microorganism identity.

Enumeration: Counting the colony number can be used to calculated microbial load.

This process can be provide highly accurate and sensitive results, however it is limited to only testing for microbes in the food safety context and it can take several days to complete.

ELISA Immunoassays

Immunoassays are tests that use antibodies to detect or measure substances such as hormones or proteins or pathogens. The antibodies of immunoassays bind to its target substance and forms a complex that can be measured and detected, such as through florescence.

Enzyme Linked Immunosorbent Assay (**ELISA**) is a conventional method commonly used by food industry and food industry agencies, such as the European Food Safety Authority [6].

In the food safety context, ELISA is used to detect substances such as pathogens, allergens and residues of restricted substances such as pesticides.

ELISA involves the following process:

Coating: Where food samples are prepared and target substances such as allergens, contaminants or pathogens are attached to a microplate well [7].

Addition of primary antibody: A primary antibody that is specific to the target substance is added to the microplate well. For example, when testing for peanut allergens, an antibody that is against peanuts

Addition of secondary antibody: A secondary antibody which is attached to an enzyme is introduced, which binds to a primary antibody.

Substrate addition: A substrate solution is then added. If the target substance is present, an enzymatic change will occur which will be detectable through a colour change for example.

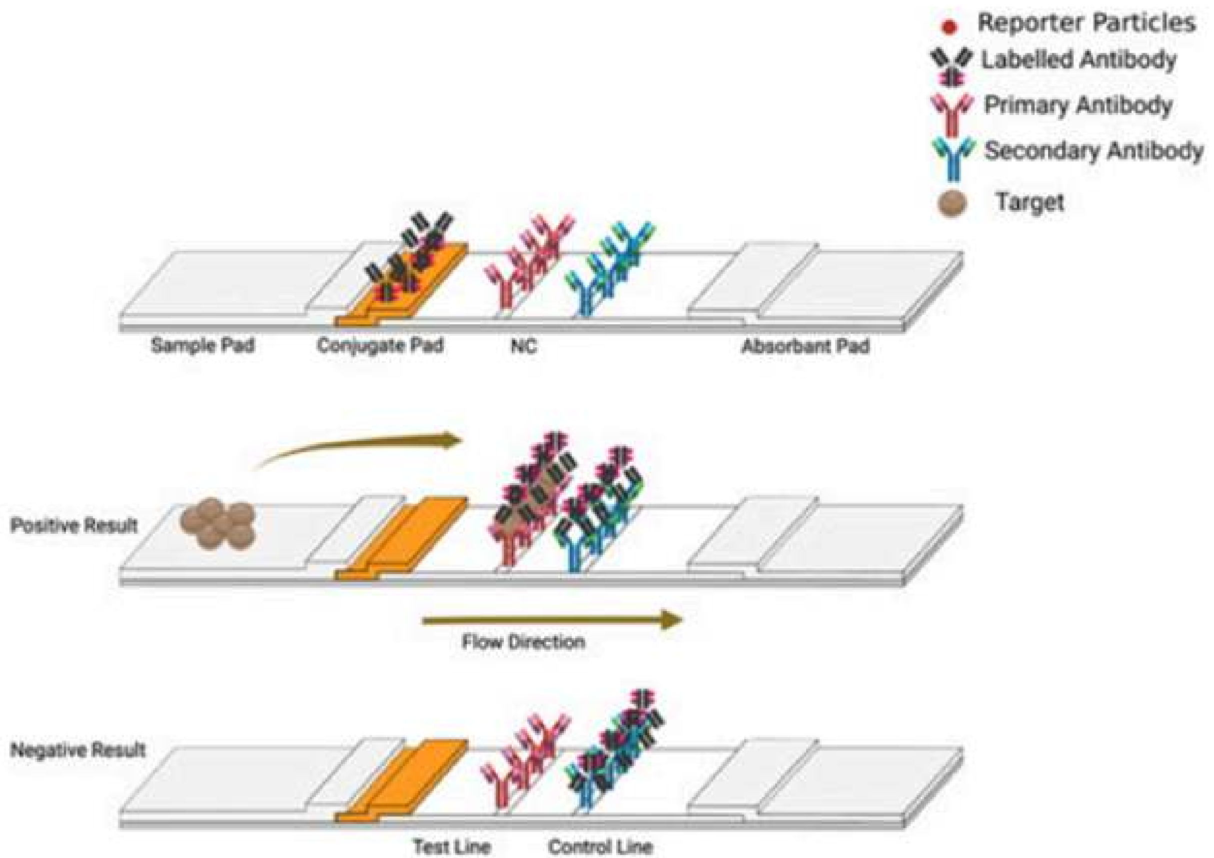
The intensity of the enzymatic change can also indicate the level of target substance present. Overall, this process can take several hours to complete.

Rapid food safety test methods

LFA Immunoassays

In comparison to ELISA, a more rapid immunoassay is that of lateral flow assays (**LFA**) [8]. LFA is where the sample is placed on a paper-like membrane strip called the sample pad. After being placed, the sample will migrate along the strip. If the target substance is present, it will bind to labelled antibodies or probes on the strip- including primary and secondary antibodies in the process described earlier for ELISA immunoassays. The final substrate-antibody complex will flow along the paper strip and form a line, and the intensity of the line will reflect the concentration of the substance. Figure 1 depicts this process [8].

Figure 1: Schematic design of a sandwich LFA (derived from Younes et al. 2023)



ELISA- the conventional method, takes several hours to complete whereas LFA produces results in under 30 minutes [9]. Moreover, ELISA requires a laboratory with specialised equipment to determine the exact quantity of the substance. On the other hand, LFA requires no specialised equipment and is suitable for use in the field. However, ELISA provides quantitative results whereas LFA provides largely qualitative results to provide a positive or negative result. LFA can indicate quantity but is not reliable and has limited precision.

PCR

Polymerase Chain Reaction (**PCR**) is a DNA amplification method that has a variety of uses, not only in the food safety context but also in other contexts such as health and epidemiology [10]. PCR methods commonly involve three basic steps:

Denaturing DNA

DNA is first 'denatured' or separated from its double-stranded state into two single strands. This involves heating the strands to 95 C.

Annealing primers

The DNA strands are then cooled to 45-60 degrees in order to 'anneal' or bind two primers to the two single-strands of DNA

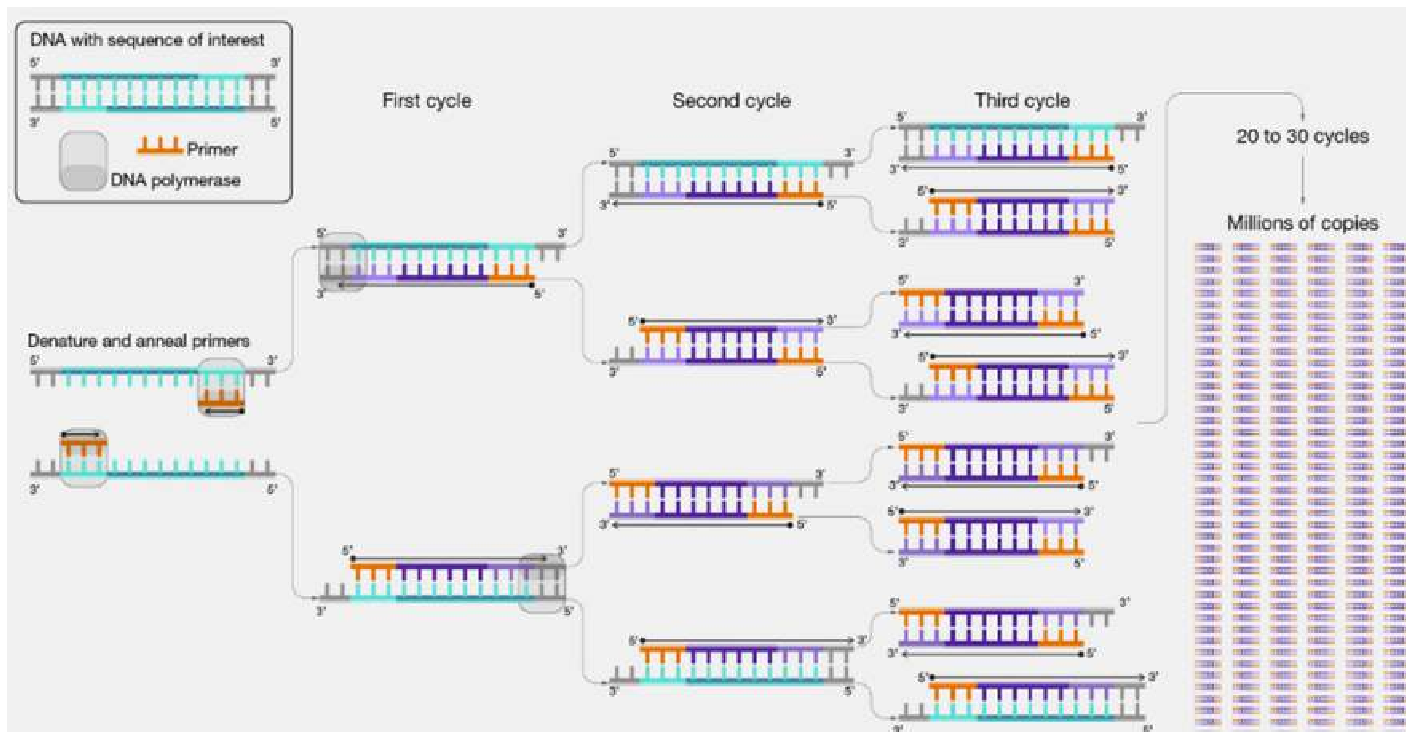
Extension of DNA

Taq polymerase enzyme is then used to extend the DNA to form double strands again. This is done at 72C. This process is then repeated which doubles the number of DNA strands per cycle.

Overall, this process of PCR amplifies the quantity of DNA which enables detection and analysis for the presence of pathogens or any other biological organism (see Figure 2) [10].

Through developments such as real time (RT) PCR, results can be produced in under an hour [11].

Figure 2: Basic PCR methodology (derived from National Human Genome Research Institute 2024) [12]



Protein swabbing

Protein swabbing is a short-form way of allergy testing. Since most allergens are food proteins, if a surface is free from proteins, then it can be established that they are allergen free. Surface testing for general protein involves swabbing an area then incubation prior to analysis. General protein swabs are not as sensitive as allergen-specific testing but are much more economical.

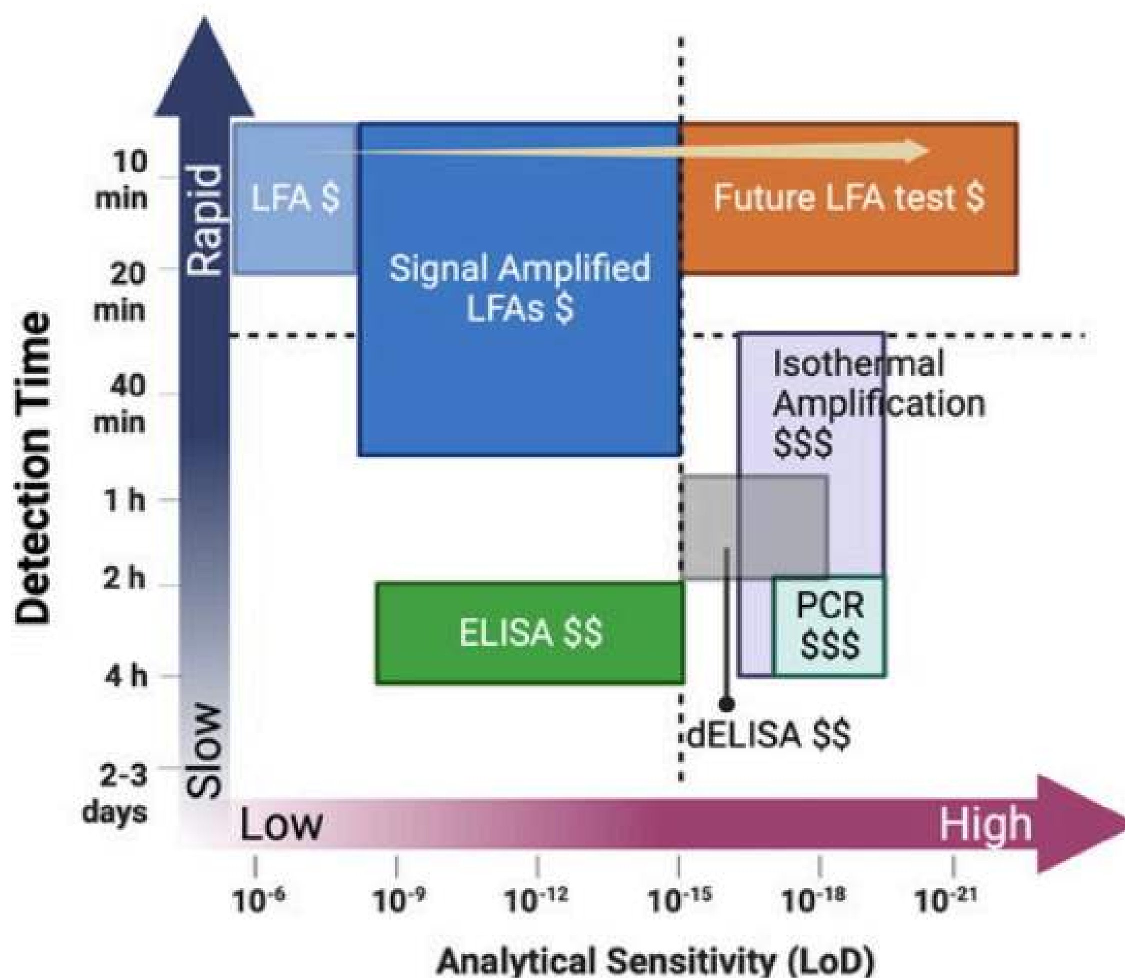
They can also provide fast results, within 30 minutes depending on incubation time [13].

Further comparison between conventional testing and rapid testing

Conventional food safety test prioritise high accuracy and sensitivity whereas rapid testing prioritises efficiency and cost [4]. As a result, conventional testing methods take much longer and rapid testing generally is not as sensitive or accurate.

This is because, generally speaking, there is a trade-off between detection time and the sensitivity of the test [9]. Even more recent conventional methods such as ELISA and conventional PCR testing- which is faster than microbiological culture methods, are nonetheless slower than rapid testing such as LFAs or RT-PCR. However, as detection time decreases, analytical sensitivity decreases and therefore false negatives are more likely (see Figure 3). There is also a relationship between greater analytical sensitivity and greater cost- even if the detection time is the same- such as between conventional PCR and ELISA (Figure 3).

Figure 3: Comparison of the sensitivity, price and detection time of food safety testing techniques (derived from Younes et al. 2023) [9]



Another point of comparison is that conventional food safety testing tends to provide quantitative results whereas rapid food safety testing provides qualitative results. Qualitative results, such as through protein swabbing or through LFAs are more useful to determine whether a microorganism is present either as a preliminary test prior to quantitative testing or where only the mere presence needs to be known. Those test methods are also useful in the field where it is impossible or impractical to use tests that require laboratory equipment such as ELISA.

Therefore, food businesses should use a combination of conventional and rapid test methods, depending on the specific circumstances. Foodlegal can advise on economic and efficient ways to test food safety and provide a food safety plan that minimises costs whilst meeting regulatory requirements.

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