PCR vs ELISA In-Process Test Selection for the Food Industry

Introduction

Optimal in-process food safety practices, from raw materials testing through final product release, are critical factors toward the protection of public health. Therefore, understanding the parameters for selecting the best-fit testing method for each step along the way becomes imperative.

However, the multitude of analytes, technologies, and brands available in the marketplace can make method selection a daunting task. As such, the intention of this white paper is to clarify a science-forward approach to selecting the right tools for the job.

Laboratory Detection Methods

In the food industry, laboratory methods are used to assess incoming materials quality, process efficacy, shelf-life validity, product release qualification, and most importantly, consumer safety. A number of technologies are utilized with each offering unique benefits according to the analytical goal. Here we will discuss and differentiate the two most commonly employed methods: polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA).

Method Overview

PCR: The Basics

Polymerase chain reaction (PCR) is a DNA-based method that amplifies small segments of target DNA for molecular analysis, pathogen identification and quantification. The basic technology includes the following steps:

Step 1: Denaturation

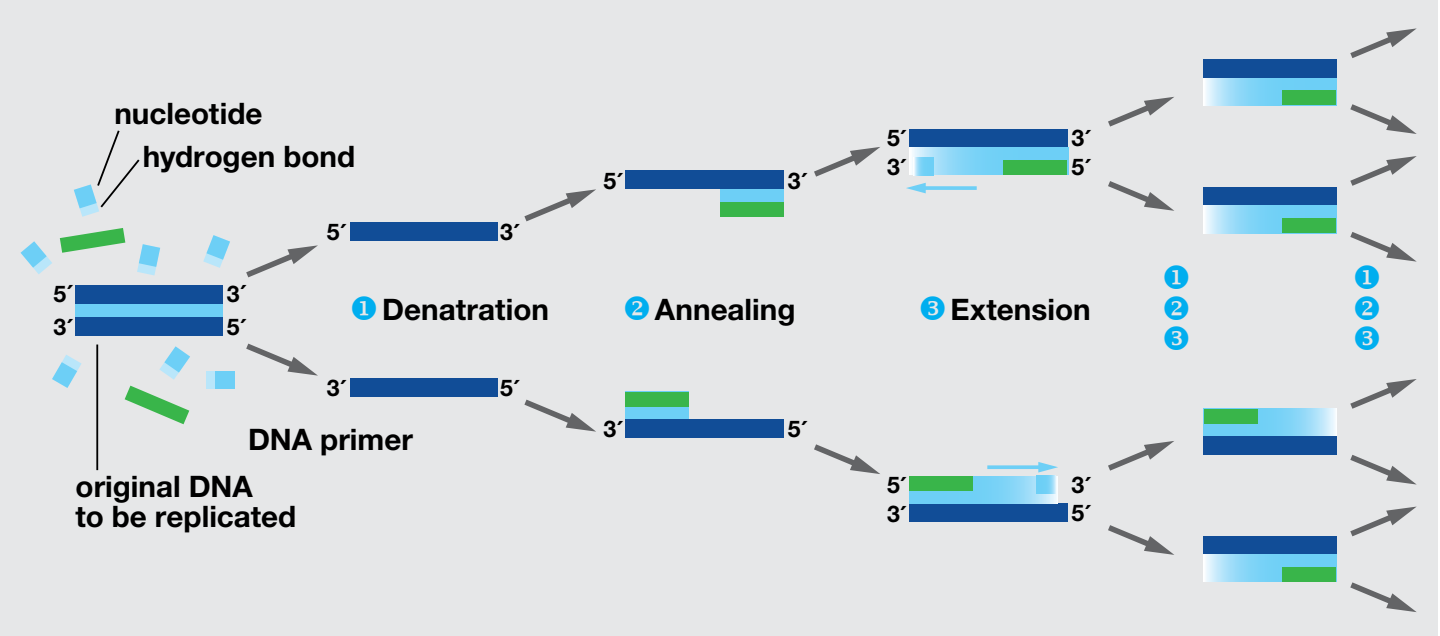
The sample is heated to separate, or denature, double-stranded DNA into two single strands.

Step 2: Annealing

Short nucleic acid sequences (primers) are added to the sample which bind specifically (anneal) to the target DNA providing a starting point for DNA synthesis (polymerization).

Step 3: Extension

New strands of DNA are made using the original strands as templates. A DNA polymerase enzyme, *Taq* polymerase (an enzyme originally isolated from *Thermus aquaticus*), is added to build two new DNA strands based on the denatured DNA template strands. This process can be repeated many times to amplify or produce sufficient DNA for analysis.



Notably, in the case of quantitative or real-time PCR, reliable detection and measurement of the DNA generated in each cycle are made possible using an oligonucleotide probe designed to hybridize within the target DNA sequence. Cleavage of this probe by *Taq* polymerase is then used to detect amplification in real time.

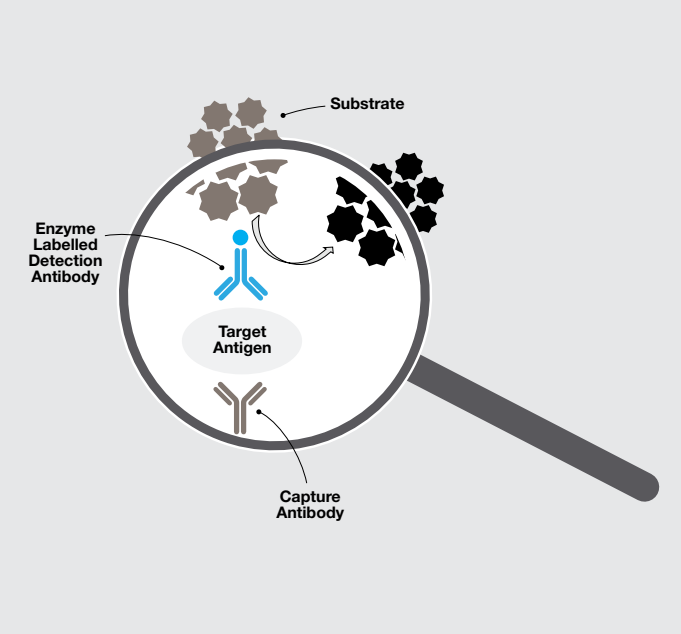
ELISA: The Basics

ELISA is an immunological-based method designed to detect a targeted antigen (protein, protein fragment epitopes, polysaccharides, lipids, and other biomolecules) by a specific antibody. The most commonly targeted antigens in the food industry include allergens and mycotoxins.

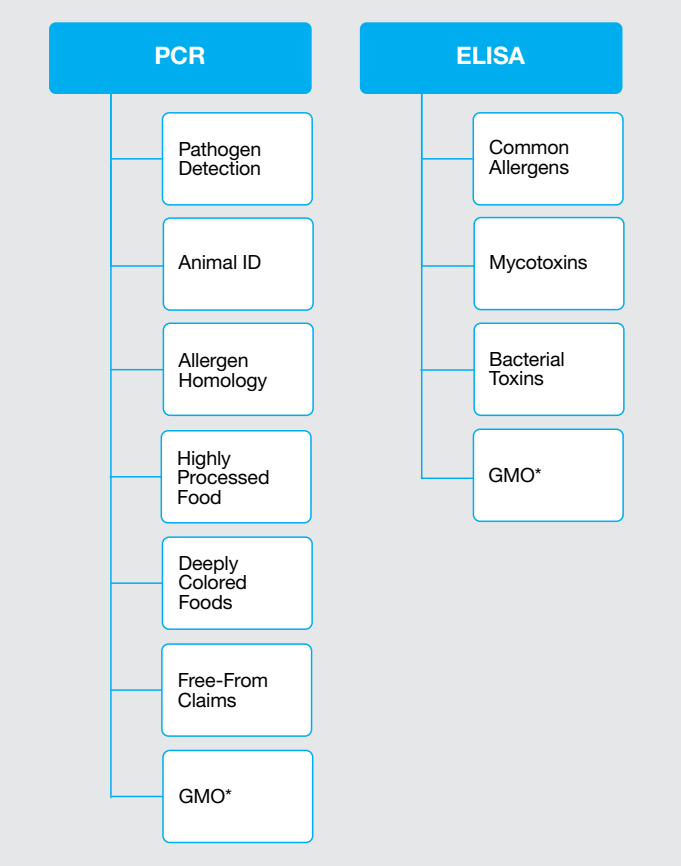
There are four types of ELISA assays: Direct, Indirect, Sandwich, and Competitive:

- The **Direct** method is the simplest and most rapid form of ELISA. In this method, the sample is added directly to the microtiter well and allowed to adsorb in a non-specific manner. An enzyme-conjugated antigen-specific antibody is then added, followed by a detection substrate. This method is limited by both the background noise of non-specific adsorption (lower specificity) and the lack of signal amplification garnered by the secondary antibody step (lower sensitivity).
- The **Indirect** method involves a similar process, but with the addition of a second antibody which provides signal amplification. Background noise can still be problematic with Indirect ELISA as is the potential for cross-reactivity with the non-specific secondary antibody.
- The **Sandwich** ELISA, as the name suggests, sandwiches the target antigen between two antibodies. The initial capture antibody is bound to the microtiter well, enhancing the specificity. And the signal amplification of the second antibody increases the assay sensitivity. This is a commonly utilized ELISA format for allergen and mycotoxin detection.
- The **Competitive** ELISA, also referred to as an inhibition or blocking ELISA, is the most complex of the four, but also mitigates the challenges of small target and single epitope antigens, providing a greater degree of both sensitivity and specificity for challenging targets.

In the case of the Sandwich ELISA, the procedure is initiated by adding a diluted test sample to an antibody-coated microwell (microtiter plate). If the targeted antigen (allergen protein) is present it will bind to the antibody. After a wash step, a second antibody linked to an enzyme (conjugate) is added. The conjugate will bind to the captured antigen if present. Following an additional wash, a substrate is added. In the presence of bound conjugate, a colored reaction becomes visible, the intensity of which is directly proportional to the amount of antigen (allergen protein) in the sample.



Preferred Method by Application



*Although PCR is the gold standard for GMO testing, it can also be conducted with lateral flow technology (not addressed here). Preferred methods are typically more common and a better fit-for-purpose, but other methods may be used.

Given the methodological differences and individual technology advantages, PCR and ELISA are complementary with both playing an important role in food safety testing. Optimal utilization by application is detailed as follows.

When to Use PCR

Pathogen Detection

The World Health Organization estimates that 1 in 10 individuals worldwide falls ill from contaminated food each year, resulting in nearly half a million deaths and the loss of 33 million healthy years of life. As such, detection of food-borne pathogens is a crucial component of in-process and final product release protocols.

Traditionally, the identification and quantification of pathogens in food was performed by microbial culture techniques utilizing a wide variety of selective, non-selective, and differential media. Considering the laborious, manual processes, subjective interpretation and additional biochemical testing, the average test turn-around time can exceed a week. Moreover, technology limitations such as viable but non-culturable (VBNC) organisms which can include: *Campylobacter jejuni, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli* (including EHEC), *Legionella pneumophila, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhi, Salmonella Typhimurium,* numerous *Shigella* species, *Streptococcus faecalis,* and various *Vibrio* species,[1] increases the potential for false-negative results and health risk. While other methodological challenges, such as too numerous to count (TNTC) scenarios, add additional cost and further delay product release.

Alternatively, real-time PCR, allows for in-process, growth-independent monitoring of DNA amplification which provides rapid, quantitative results. Independent studies have demonstrated that PCR is significantly more sensitive than culture to detect common food pathogens such as *Salmonella*[2] and *Listeria*[3] while third-party method authorities, including AOAC and AFNOR, have validated quality PCR methods for a wide variety of food-borne pathogens.

One of PCR's unique benefits to the food industry is its suitability for testing highly processed food. Because DNA is typically more stable than proteins (as detected by immunoassays like ELISA), PCR is not susceptible to false-negative results related to antigen denaturation common in food processes such as heat treatment and the addition of acidic compounds. PCR also offers the significant advantage of testing for multiple pathogens from a single sample.

Food Adulteration: Animal Identification

The Grocery Manufacturers Association estimates that fraud may cost the global food industry between $10 billion and $15 billion (USD) per year, affecting approximately 10% of all commercially sold food products.[4] In order to control and eliminate fraudulent behavior, reliable animal identification becomes an important factor for food operators, particularly those with a worldwide supply chain and broad consumer reach.

Because PCR is a DNA-based, molecular method, it is easily able to identify, differentiate and quantify swine, cattle, sheep, horse, donkey, chicken, goat and other species in food samples. Notably, however, PCR cannot differentiate between food products that share the same DNA (e.g., cow's milk and beef, hen's eggs and chicken). This same species recognition is also a challenge with immunoassay methods due to antigen homology.

Allergen Detection

Food allergy is recognized as a growing problem in most countries affecting approximately 2.5% of the general population worldwide, with reported prevalence rates ranging from 1% to 10%.[5] Accurate detection of potential food allergens is, therefore, a critical component of the allergen control plan. Although immunoassay technology is frequently the method of choice for allergen testing, and thereby more commonly utilized, there are a number of scenarios where PCR provides more accurate and reliable allergen results.

Antigen Homology

Homology between a target antigen and a common, unrelated antigen can result in cross-reactivity and false-positive ELISA results. Such is the case with celery where antigen similarity across other common ingredients including parsley, coriander, carrot and fennel, will result in inaccurate positive findings. As such, when testing for celery allergen, PCR is the preferred method.

Highly Processed and Deeply Colored Foods

Highly processed and deeply colored food products and ingredients (e.g., chili powder) also pose problems for immunoassay technology. Because PCR detects target DNA, rather than proteins susceptible to heat denaturation, it is a much more reliable allergen detection method for highly processed food products. Although in cases of extreme chemical treatment, both the target protein and the DNA itself may be altered. Furthermore, since ELISA is a colorimetric method read by spectrophotometer, deep sample coloration can cause interference, making this method unreliable. Because PCR is unaffected by sample color, it is the preferred option.

Allergen-Free Claims

Another case where allergen control best practices would indicate the use of PCR is one where product specifications call for the complete absence of a specific ingredient rather than just its most common allergenic antigen/s. For example, such a stringent requirement may be found when the final product label indicates an *allergen-free* claim. In such a case, immunoassay testing would be insufficient thereby requiring a PCR DNA detection method.

PCR: Technology Considerations

Despite its utility in the industry, there are limitations with PCR technology of which food operators should be aware:

1. Some multiplex (multi-target) PCR methods can encounter a reduction in sensitivity due to competition between primers and amplification efficiency differences between targets. This shortcoming can be mitigated through assay optimization which is easily verified by independent agency evaluation (e.g., AOAC, NordVal, etc). These quality validation processes investigate the potential for cross-reactivity by conducting a wide variety of inclusivity, exclusivity, sensitivity and specificity tests across matrices to demonstrate real-world test accuracy.
2. Because PCR amplifies and measures total target DNA, the technology itself cannot differentiate between viable (live) and non-viable (dead) cells. This can be a problematic limitation for highly processed foods in particular. The common adoption of bacteriophage treatment to control bacterial contamination in food products also introduces DNA remnants. Furthermore, certain sterilization procedures and spices can increase the amount of residual DNA in a product. However, PCR manufacturers can address this shortcoming with enzymatic enrichments, specialized reagents, and/or sample "clean-up" kits which are highly valuable for at-risk product lines. Therefore, this concern can be mitigated.

When to Use ELISA

Pathogen Detection

Although automated ELISA methods are used to detect pathogens, this technology has demonstrated higher levels of false-positive results compared to traditional culture. Because ELISA detects protein (antigen) if a non-pathogenic organism with a similar protein complex is present, it has the potential to cross-react.[6] To avoid this limitation, some ELISA tests will target a bacteria-specific toxin, but this is only effective in the presence of the specific toxin (antigen) which can also limit the overall test sensitivity. Moreover, individual ELISA test results may vary dependent on the specificity of the antibodies utilized. Because PCR detects DNA, these limitations are irrelevant.

Allergen Detection

As one of the most common methods in the food industry, ELISA provides a sensitive, specific, and automatable option for the detection of most allergens, under most circumstances. Exceptions include deeply colored food samples which interfere with the method's colorimetric read as well as the detection of celery. Because celery is homologous with other common ingredients like parsley and carrot, PCR provides much more consistent accuracy. It is also worth noting that in cases where a label claim indicates *allergen-free*, ELISA limits of detection are inadequate. In such a case, PCR should be used to verify the absence of a specific allergenic ingredient.

Mycotoxins

Mycotoxins are secondary metabolites produced by filamentous fungi which are found in staple foods such as corn, cereals, ground nuts, tree nuts, meat, milk and eggs. Prevalent worldwide, mycotoxins are most frequently detected in warm, humid climates. The adverse effects of mycotoxins on human health are both acute and chronic and can include liver cancer, immune dysfunction, respiratory problems and convulsions, among others.[7]

In the field, simplified (lateral flow) immunoassays are often utilized for screening purposes. Whereas ELISA technology provides a robust in-laboratory method for the detection of mycotoxins, as well as other bacterial toxins, using highly specific antibodies. Given the method cost and complexity, and the commodity nature of mycotoxin vulnerable products, the PCR method is not typically utilized by food operators.

ELISA: Technology Considerations

Certain food production processes such as hydrolyzation and fermentation can alter the native, allergen target protein (antigen). As such, without adequate assay optimization, ELISA may fail to detect allergenic peptides, despite their presence in a sample. One particularly challenging example is found with gluten testing for beer. When evaluating an ELISA manufacturer in this case, validation data should be reviewed to ensure adequate detection sensitivity.

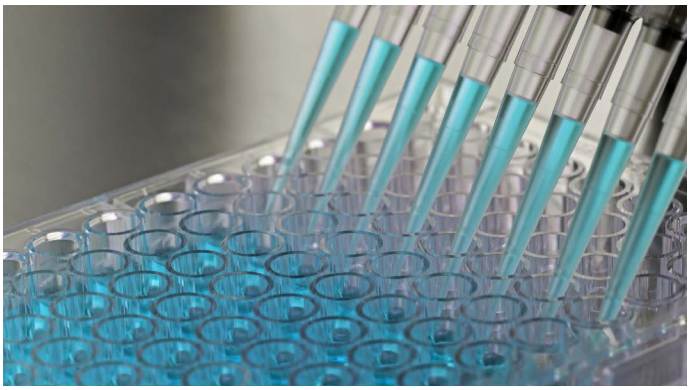
Also, as mentioned previously, allergen control best practices would indicate the use of PCR when product labeling indicates an *allergen-free* claim because ELISA will only detect specific target antigens and the limit of detection cannot assure a true zero value. Alternatively, PCR can determine the presence or absence of the allergenic ingredient itself (e.g., *free of nuts*).

Advantages, Limitations and Applications: PCR vs. ELISA Summary

Method	Technology Advantages	Technology Limitations	Applications
RT PCR	• High sensitivity • High specificity • High precision/reproducibility • DNA detection independent of antigen homology • One universal sample preparation technique • Ability to multiplex (multiple parameters in a single test) • Extracted DNA may be used for additional testing (e.g., GMO) • Fully automatable	• Subject to PCR Inhibitors • Requires pretreatment to eliminate detection of non-viable cells • Requires intact DNA for detection • Requires trained personnel • Higher cost per result	• Pathogen Detection • Highly Processed Foods • Deeply Colored Foods • Homologous Allergens • Allergen-Free Claims • Animal Identification
ELISA	• High specificity • Antigen detection allows for the identification of toxins, protein fragments • Fully automatable • Lower cost per result versus PCR	• Variable sensitivity (lower than PCR) • May require multiple sample preparation methods for different tests • Potential for cross-reactivity with homologous antigens and secondary antibodies • Potential for interference from deeply colored samples • Hydrolyzation/Fermentation can result in false-negative results in the absence of assay optimization	• Common Allergen Detection • Mycotoxin Detection • Bacterial Toxin Detection

Conclusion

Although no single test addresses every analytical challenge, food operators can employ a science-first approach to method selection. In most scenarios, the data supports adoption of a high-quality ELISA for the majority of allergen and toxin detection needs. ELISA provides the sensitivity, specificity and quantification capabilities necessary for robust food safety while easily integrating into laboratory workflow and available ELISA automation. In the case of pathogen detection, PCR is the superior tool as it is not susceptible to homologous protein cross-reactivities. This automatable DNA-based method is also preferred for highly processed foods, deeply colored samples, animal identification, and cases where a '*free-from*' claim must be substantiated.



References

[1] Fakruddin M, Mannan KS, Andrews S. Viable but nonculturable bacteria: food safety and public health perspective. *ISRN Microbiol.* 2013;2013:703813. Published 2013 Sep 26. doi:10.1155/2013/703813

[2] Lungu B, Waltman WD, Berghaus RD, Hofacre CL. Comparison of a real-time PCR method with a culture method for the detection of *Salmonella enterica* serotype enteritidis in naturally contaminated environmental samples from integrated poultry houses. *Journal of Food Protection.* 2012;75:743-747. DOI: 10.4315/0362-028X. JFP-11-297

[3] Kim DH, Chon JW, Kim H, Kim HS, Choi D, Kim YJ, et al. Comparison of culture, conventional and real-time PCR methods for *Listeria monocytogenes* in foods. *Korean Journal for Food Science of Animal Resources*. 2014;34:665-673. DOI: 10.5851/kosfa.2014.34.5.665

[4] https://sgp.fas.org/crs/misc/R43358.pdf

[5] https://www.worldallergy.org/education-and-programs/education/ allergic-disease-resource-center/professionals/food-allergy

[6] "A Summary Profile of Pathogen Detection." A. Crispin Philpott. *Food Safety Magazine.* April/May 2009.

[7] Freire, F.D.C.O., da Rocha, M.E.B. (2016). Impact of Mycotoxins on Human Health. In: Mérillon, JM., Ramawat, K. (eds) *Fungal Metabolites. Reference Series in Phytochemistry.* Springer, Cham. https://doi.org/10.1007/978-3-319-19456-1_21-1