Real-time testing of foods: the Holy Grail?

There may be a bad bug in food
Whose presence is simply no good
For the public's protection
We want fast detection
Immediate, if only we could.

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Introduction

The approach to quality assurance and control in the food industry has changed, especially with the widespread implementation of preventative, process-oriented food safety plans grounded in Hazard Analysis Critical Control Point (HACCP) and risk assessment principles. However, microbiological analysis of foods remains critical to the management of quality and safety of food products, particularly with respect to the detection of pathogens.

The time to complete tests has decreased significantly but, the required sensitivity of the test, the physiological state of the target analyte, the food matrix and associated non-target microflora, all constrain further acceleration of testing and limit the potential for achieving real-time testing of foods, particularly when testing for pathogens such as *Salmonella.* While real time testing may be the ultimate goal, is it food microbiology's Holy Grail?

Evolution of tests and times

Traditional pathogen testing typically involves the use of multiple stages of culture, with various tests taking between 2-7 days. For example, testing for *Salmonella* involves two periods of enrichment followed by plating, taking 3 days to a negative result, and a further 1-2 days to confirm a positive. In real-world terms, a short shelf-life product may have been distributed, sold or even consumed before the most lengthy tests are complete. Under such circumstances, testing is retrospective.

Figure 1. Real-time testing – is it food microbiology's Holy Grail?

The development of an array of detection methods, including those based on immunoassay or amplification of nucleic acids [1], along with improvements in enrichment, have led to significant decreases in the time to complete testing, from 5 days, to 12-24 hours, with the real possibility of testing in one shift (6-8 hours).

Constraints

The major constraints to the further acceleration of testing and to, ultimately, achieving real-time testing, are the sensitivity of the test, the initial population and physiological state of both the target and non-target microorganisms in foods, and the matrix itself. This is particularly true when testing for pathogens such as *Salmonella*.

In this case, in which the target is capable of causing disease when ingested even at a low population, a qualitative test is performed, typically aiming to detect as little as one colony forming unit (cfu) in as much as 25g of food for a single sample unit test. This equates to finding something the size of a milk carton in a swimming pool, filled not with water, but with a complex matrix, such as peanut butter or minced beef.

If the target analyte is present in a food, it typically occurs in very low numbers. While a pathogen such as Salmonella may be found in high numbers in a raw flesh food, in many foods that undergo substantial processing, the population of the organism will be reduced, potentially to zero. Also, non-replicating pathogens, especially viruses, may be present in very low numbers, but still represent a significant hazard. Nucleic-acid based amplification techniques [1-3] offer the potential for very rapid detection of a single cell or particle of an infectious agent, yet even these methods are often less sensitive in practice than in theory.

Even if a small proportion of the population survives food processing, cells of the target are then frequently injured. This issue of injury becomes increasingly crucial as the time of testing decreases. If injury occurs, recovery and initiation of vegetative growth can be delayed significantly [4-7]. Studies have shown that even minor variations in the resuscitation procedure, such as the composition of enrichment media [7, 8] can have a profound effect on the time to recovery and rate of outgrowth.

Low initial numbers and the potential impact of injury limit the time in which a target organism recovers and grows, constraining the minimum time taken for a population to reach the threshold required for some rapid methods, such as ELISA [1].

The ambiguity of the physiological state of the target calls into serious question the ability to directly detect a pathogen. As many foods are subject to one or more processes deleterious to microorganisms, the target of detection may be alive (fully vegetative or injured) or dead. If the method of detection does not assess viability, a positive result may not reflect a true health hazard associated with the food under test. On the other hand, acceptance of a false-positive result leads, at the least, to product rework or disposal.

It has been proposed that reverse-transcriptase PCR answers the question of

viability. RNA species are turned over rapidly in viable cells and are considered to degrade rapidly upon cell death. However, there is evidence that RNA may survive in cells rendered inactive by typical food processes[9].

The background microflora present in the food may interfere with testing, sometimes to a significant degree[10]. With regard to rapid testing involving some form of enrichment, overgrowth of target by background may occur. In the case of direct, nucleic acid-based detection, assay of target nucleic acid may be overwhelmed by non-target nucleic acid, should a non-specific chemical extraction method be used. Potentially, target cells may be extracted specifically using, for example, antibody-coated immuno-magnetic beads[11], though the viability of such cells may still be in doubt.

Benefits

Very rapid or, potentially, real-time testing benefits both the producer and consumer. For example, faster turnaround in testing translates into less inventory being held, improving the economics of production, including a reduction in storage space. Fast or immediate availability of test results allows for positive release; product is held while testing is in progress, and is only released when testing is completed.

Real-time testing would not only benefit the producer, lessening the impact of process failure, thus reducing rework, and potentially eliminating the need for disposal or recall, but also the consumer, by reducing the risk of spoilage or disease associated with the distribution of contaminated product.

Conclusion

In conclusion, while the time taken to complete the microbiological testing of foods has decreased significantly, factors including complexity of the food matrix, the composition of the background flora and the population and physiological state of the target microorganism constrain the development of true real-time testing. Like King Arthur's knights, food microbiologists may never find their Holy Grail.

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