Delivering Reference Laboratory Results at the Point-of-Need

A Focus on Real-Time Polymerase Chain Reaction (RT-PCR) for Food Safety

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Abstract

Detection of pathogens is critical to human health. Equally important is the accurate diagnosis of disease (such as determining the specific organism causing an abscess) and assurance that the food supply is not contaminated (the absence of E. coli 0157:H7 in ground beef). Real-Time PCR is emerging as the gold standard in health care, providing accurate, sensitive and specific results in minutes to hours instead of days.

The ability to perform rapid accurate pathogen testing in the food supply chain has not followed as quickly as the adoption in health care. This is due to several factors including:

- Long pre-enrichment times
- Complex sample preparation
- Cumbersome Nucleotide (DNA) extraction techniques
- Expensive, delicate equipment that required extensive calibration
- Highly trained technicians
- Special laminar flow rooms within the laboratory

The next generation of smaller automated devices for RT-PCR mitigates many of these issues and allows state-of-the-art testing at the point of need. When properly implemented, this can reduce the risk of food supply contamination and avoid the delays in shipping or accepting shipments associated with traditional culture techniques.

Introduction

Despite modern processing techniques, food safety remains a critical issue. Annual estimates for the United States include over 300 Million cases of Food Borne Illness (FBI), of which 320,000 seek hospital care and 5,000 die. There are now law firms specializing in FBI, one of which (MarlerClark Law Firm LLC) has recovered over a half billion dollars on behalf of their plaintiffs.

Food producers want to ensure a safe and secure food supply and the public is increasingly concerned about food safety and food fraud. Unfortunately, the current "traditional methods" approach to testing is expensive and delays the production process. As such, products are shipped, and potentially consumed, without adequate testing. The effect of harm caused by food extends well beyond the manufacturer responsible for the outbreak. One example is the salmonella contamination of peanuts from the Peanut Corporation of America (PCA). PCA supplied less than 1% of the consumed supply in the US. Yet, the contamination, harm and recall resulted in PCA's bankruptcy and

triggered fear of peanuts that caused a reduction in sales of peanut butter by more than 10% nationwide.

Contamination and recalls are not a rare event. A review of just one month of major recalls reported to the Food and Drug Administration (FDA) yielded the following:

- May 24, 2010 - Fresh Express Recalls Romaine-based Salads with Use-by Dates of May 13-16th Due to Possible Health Risk
- May 21, 2010 - Caldwell Fresh Foods Recalls Alfalfa Sprouts Because of Possible Health Risks
- May 20, 2010 - Vanlaw Food Products, Inc. Announces Recall of Valu Time Brand Ranch Dressing
- May 06, 2010 - Imported Manouri Cheese Recalled due to Potential Listeria Contamination
- May 06, 2010 - Freshway Foods Recalls Products Containing Romaine Lettuce Because of Possible Health Risk
- April 27, 2010 - MiDAS Foods International Recalls Instant Beef Soup Mix and Instant Beef Stroganoff Sauce Mix Because Of Possible Health Risk

These likely represent a fraction of the actual lots of food that have significant levels of pathogenic contamination.

Methods to enhance food safety

Safety begins at the source; on the farm. Clean water for irrigation, avoidance of animal incursion, good worker hygiene, equipment maintenance / cleaning and avoiding soil amendment contamination must all be achieved. Testing is only the final step to ensure that the procedures for growing, collecting, processing and packaging foods have resulted in a product that will do no harm when ingested.

There are four basic approaches to food safety testing including:

- Presumption of safety
- Perform rapid tests locally
- Perform rapid tests locally with confirmatory tests in reference lab
- Perform all tests in reference lab

Each of these approaches has advantages and disadvantages. Presumption of safety is the least costly, yet has the highest risk of contaminating the foods supply. If a processing plant sends all specimens to a reference laboratory, there is significant monetary and time cost. Common test methods include:

Test	Speed	Accuracy	Cost per Unit	Skill Level Required
Dry reagent Immuno-assay ("Dip-Stick")	**Fast**	**Low**	**Low**	**Low**
Culture (agar plate)	**Very Slow**	**High**	**Low-mid**	**Mid**
Culture (Isotope tagged)	**Mid**	**High**	**Mid-high**	**High**
Gel PCR	**Mid**	**High**	**Mid**	**High**
Traditional Real-Time PCR	**Fast**	**High**	**High**	**High**

Review of the table above yields three important conclusions:

1. The rapid, simple tests are not accurate
2. The accurate, simple tests are not rapid
3. The accurate, rapid tests are not simple

In addition to performance of the test, there is additional logistical complexity to sending specimens to a reference laboratory. The steps include:

- Specimen
  - Order
  - Acquisition
  - Transportation
  - Accession
  - Preparation
- Test
  - Analytic preparation
  - Procedure pooling
  - Procedure sequencing
  - Procedure performance
- Results
  - Validation
  - Communication

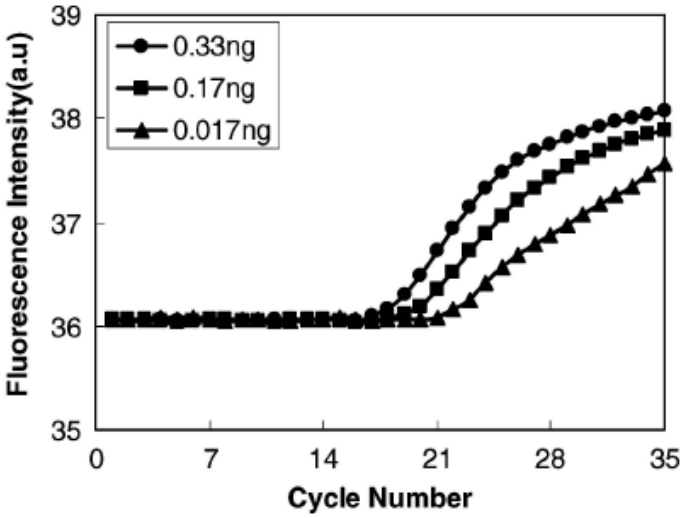
Each of these steps takes time and effort. It is entirely possible that the procedure performance of a test may take just 90 minutes, yet the total dwell time from specimen ordering to validated results communication could be days. From the perspective of the food producer, total dwell time, not procedure performance time is the critical factor.

Thus, with traditional approaches, there is no ideal. However, recent advances in RT-PCR technology have made it more accessible at the point of need and mitigated many of the barriers to adoption.

Test performance for Real-Time Polymerase Chain Reaction (RT-PCR) involves four basic steps:

1. Extract DNA from the organism
2. Amplify the target by making copies ("Thermocycling") using target specific primers.
    a. Millions of copies can be made in minutes
3. Detect the DNA amplicon using a fluorescent probe.
4. If the target was present in the sample, it will fluoresce when illuminated.

A typical RT-PCR fluorescence curve is shown below.

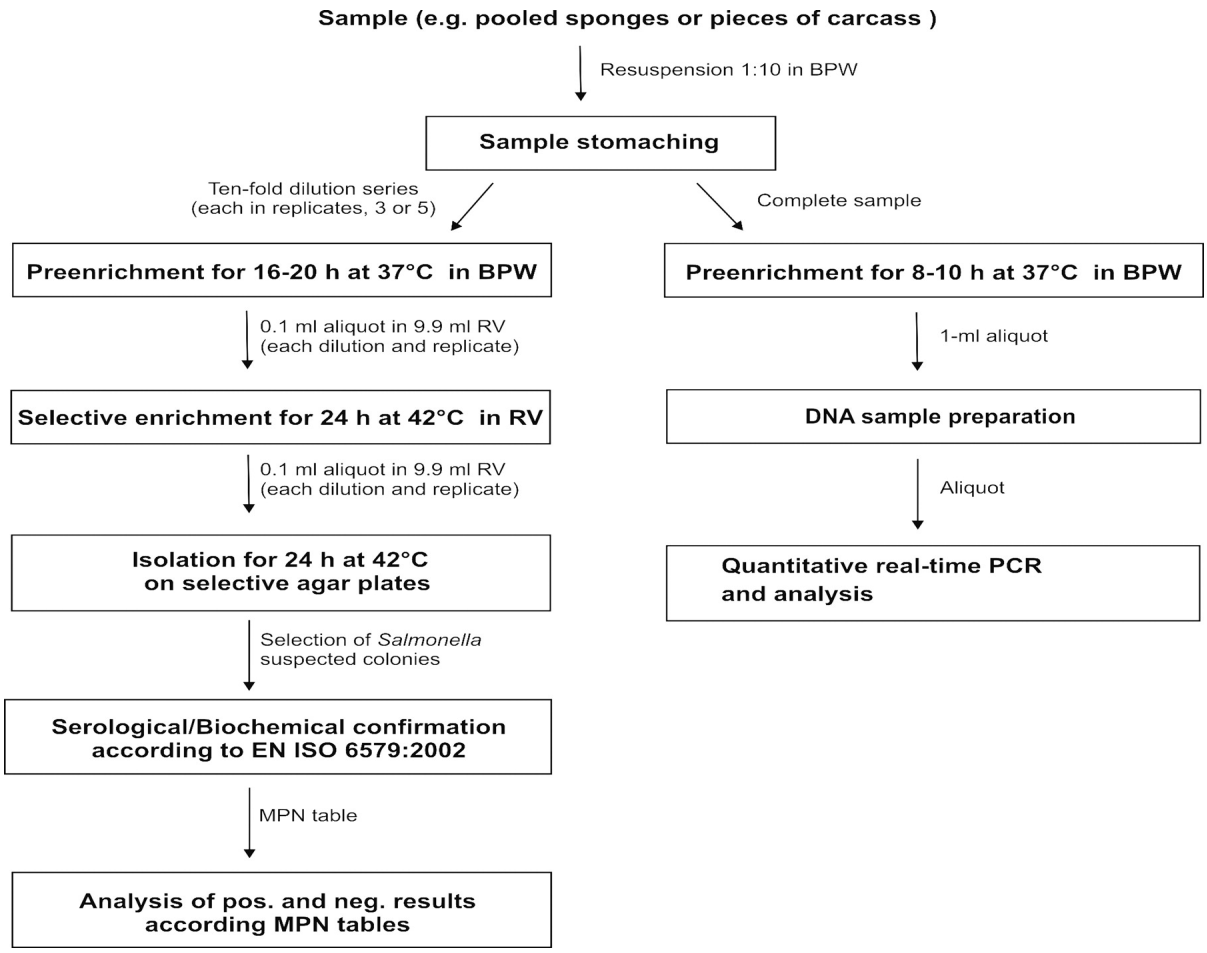


As can be seen, the curves are straightforward to interpret, detect at very low levels of contamination (0.017ng) and provide results quickly (cycle 20 = 40 minutes).

Unfortunately, with traditional approaches, these results require highly trained staff, a laminar flow laboratory, expensive equipment and delicate reagents that require refrigeration and have a short shelf life. These factors have ensured that traditional RT-PCR is performed only in reference laboratories. Thus, all the complexities and delays of transport and communication become a necessary, but not value add part of the process.

The next generation RT-PCR units have miniaturized and automated the process. They are simple to use, inexpensive, require little infrastructure and have procedures that can be done outside of a reference lab. Today there are instruments that can take pre-enriched sample, automatically extract the DNA in 30 minutes, and transfer the sample to a nano-thermocycler that provides results in less than an hour.

A comparison of traditional culture for Salmonella and RT-PCR follows:



We can now transform a procedure that used to take 3-5 days for definitive results to one that can be completed in less than 24 hours in a decentralized lab.

Conclusion

Real-Time PCR provides rapid, highly sensitive and specific results for pathogen detection. This gold standard, previously limited to the reference laboratory is now available in small labs and can be performed by basic technicians. This technique has the potential to revolutionize the speed, efficiency and quality of food testing worldwide.