Validating Pathatrix: A Complete AOAC-Approved Workflow for Detecting *Salmonella* spp. in Pooled Food Samples

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FOOD SAFETY

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ABSTRACT

The Pathatrix Auto™ pathogen isolation platform provides a workflow that is able to process volumes up to 50mL containing as many as ten individual food enrichments in the same sample pool. AOAC approval was recently obtained for this workflow that allows Food Safety professionals to utilize this pooling approach prior to screening by Real-Time PCR. The core of this isolation technology is the automated purification of pathogenic *Salmonella* serovars by antibody-conjugated magnetic beads specific for *Salmonella*. The captured bead-bound bacteria are then lysed and the supernatant is added to a lyophilized MicroSEQ® *Salmonella* spp. Real-Time PCR assay previously validated by AOAC and AFNOR. By combining the specificity of antibody-based capture and the sensitivity of Real-Time PCR, we are able to reliably detect 1 CFU in a 375g food sample. The ability to pool individual samples, in addition to the ease of use of this workflow, enables the processing of hundreds of samples per hour at a fraction of the cost of platforms that do not accommodate a pooled sample format. This creates an economic benefit to food producers by providing a workflow that is able to rapidly and inexpensively screen for rare contamination events. This work demonstrates to the food production industry that by using this workflow, one can attain equivalent-or-better results than traditional culture methods, in far less time, for a significantly less cost burden than other PCR-based platforms. In short, it makes available the use of PCR technology to detect pathogens without the costs associated with the traditional one-sample-per-assay-well relationship. A wide variety of sample types were tested in the course of this AOAC validation study. In all cases, this workflow correctly identified all positive and negative samples. We demonstrate here that this methodology is robust in being able to process a diverse array of sample types, has high fidelity in correctly detecting the presence of pathogens, and propose that the workflow is universal for all bacterial pathogens of interest found in food.

MATERIALS AND METHODS

The following figures summarize the pooling concept, the Pathatrix® IMS-capture process, and the DNA purification protocol which enables the IMS-captured bacteria to be lysed and subjected to Real-Time PCR analysis.

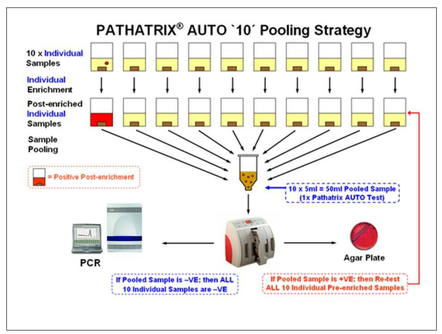


Figure 1. 10-Pooling Strategy

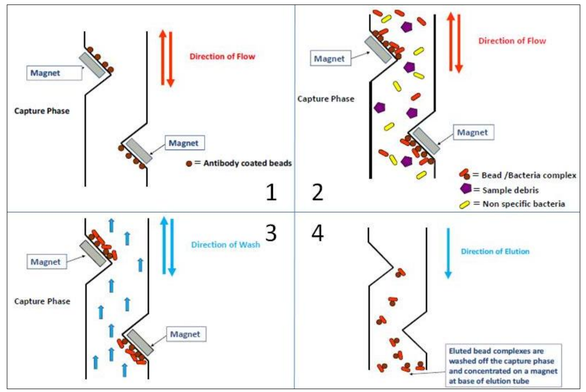


Figure 2. IMS Capture Process

Step 1 – Paramagnetic beads are bound to the inside of a capture chamber by the application of magnets to the outside of the chamber.

Step 2 – The food sample is drawn into the capture chamber and allows the antibody-coated paramagnetic beads to associate with the target.

Step 3 – The capture chamber is washed to clear away sample debris and non-specific binding events.

Step 4 – The magnets are withdrawn and the bead-target complex is able to be eluted off of the capture chamber.

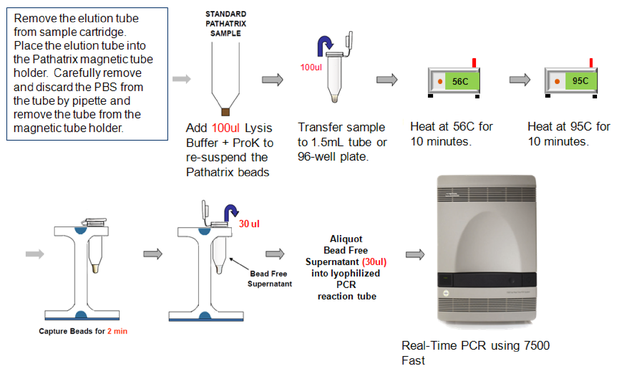


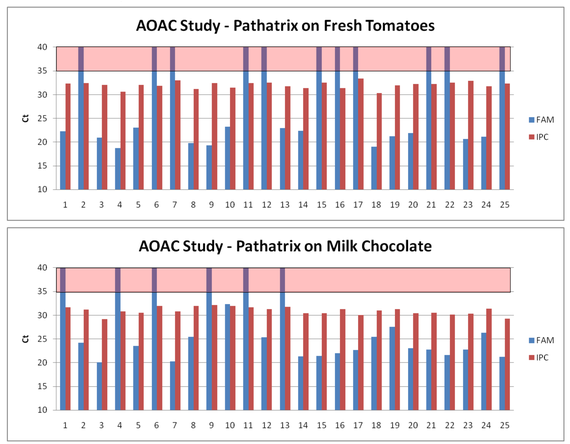
Figure 3. Linking Protocol

A Proteinase-K-mediated heat lysis method was developed in anticipation of this protocol being used for the detection of many different pathogens of interest in foods, including Gram-positive bacteria such as *Listeria*. Compared to other lysis technologies such as bead-beating and sonication, this method allows for lysis to be carried out in a 96-well plate, if desired, which increases the throughput potential for the customer.

RESULTS

Figure 4. Internal Validation on Various Food Matrices

For all graphs, FAM signal represents the direct detection of *Salmonella spp.* via our MicroSEQ® kit. VIC signal comprises the IPC (internal positive control) for that kit. The areas highlighted in red indicate the Ct region in which the sample would be identified as negative by our RapidFinder® automated sample call software.



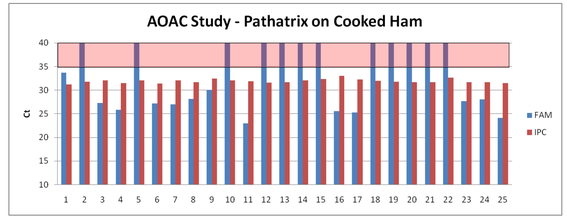


Figure 5. AOAC Study Data

Q Laboratories, Inc. (Cincinnati, OH) was contracted to perform validation studies on the Pathatrix® linked to the *Salmonella* spp. MicroSEQ® Real-Time PCR assay via the Linking Protocol in Figure 3. Validation was performed on three representative matrices – cooked ham, milk chocolate, and fresh sliced tomatoes. Of 25 unknown fractional positive inoculations on all three matrices, we were able to confirm 100% of the true positives and negatives, and detected no false positives or negatives compared to the reference method (FDA-BAM, Chapter 5). This report was submitted to AOAC and was recently approved for certification.

CONCLUSIONS

The goal of this work was to combine an AOAC-approved IMS system and an AOAC-approved assay to create a total workflow that could be certified. The result was the development of a Proteinase-K-mediated heat lysis protocol that allows for universality against all likely food pathogens. We show here that this linking protocol is a robust solution for processing a vast number of sample matrices, and is able show reliable detection of *Salmonella* spp. in a variety of food types. This is made possible through the combination of the specificity of our IMS-based target isolation, and the sensitivity of our Real-Time PCR assay, which provides a significant advantage over traditional biochemical differentiation testing in detecting rare contamination events that occur during food processing, production, and preparation. The ability of the Pathatrix® to be able to process each of the above sample types is also a significant advantage, but the true strength and prevailing differentiating factor of the platform is the cost-savings that is passed along to the customer by enabling them to pool up to 10 post-enriched samples in the same tube. Cost-per-sample is a primary concern for all food industry producers, and it is a challenge that molecular-based assay providers have had difficulty addressing. We hope to be able to show the industry that by pooling using Pathatrix®, you can get equivalent-or-better results, in far less time, and for a significantly lower price than other PCR-based offerings and even traditional biochemical methods.

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TRADEMARKS/LICENSING

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