Validating Pathatrix™: A Complete AOAC-Approved Workflow for the Detection of *Salmonella* spp. in Pooled Food Samples

LIFE TECHNOLOGIES™
FOOD SAFETY

Jason K. Wall[1], E. Liu[1], E. Crowley[2], and R. Conrad[1], [1]Life Technologies, Inc. Animal, Food, and Environmental Safety, Austin, Texas. [2]Q Laboratories, Inc. Cincinnati, OH.

ABSTRACT

The Pathatrix Auto™ pathogen isolation platform provides a workflow that is able to process sample 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, which 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 25g-325g 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 without the costs associated with the traditional one-sample-per-assay-well relationship. Three representative matrices were submitted for approval, of which all were analyzed in a paired study design. For all matrices tested, there were no statistically significant differences found in the number of positive test portions detected by the modified candidate method when compared to the appropriate reference method. We demonstrate here that this methodology is robust in being able to process a diverse array of sample types, and has high fidelity in correctly detecting the presence of *Salmonella* in foods.

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.

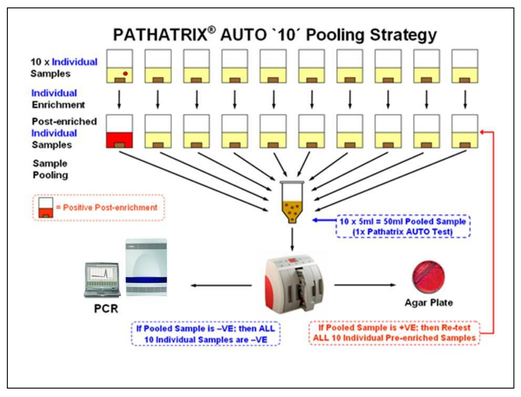

Figure 1. 10-Pooling Strategy

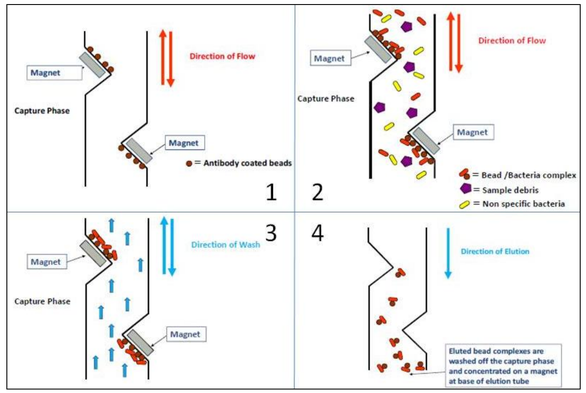

Figure 2. Automated IMS Capture Process

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.

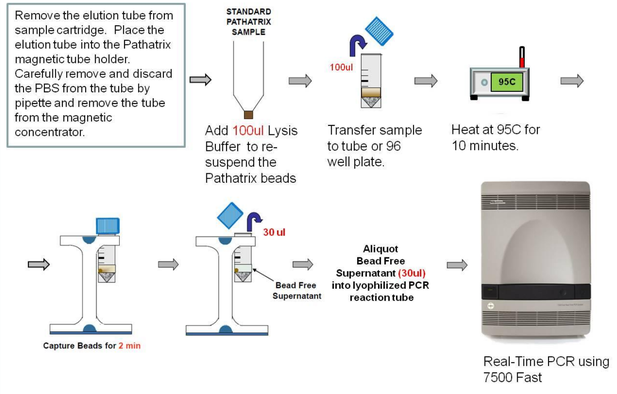

Figure 3. PCR Linking Protocol

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. For the purposes of this study, no Proteinase-K was used, as it is not necessary to lyse *Salmonella*.

RESULTS

Matrix	Time Point	Strain	MPN[a]/ Test Portion	N[c]	Pathatrix®/MicroSEQ® Method[i] x[d]	Pathatrix®/MicroSEQ® Method[i] POD_C[e]	Pathatrix®/MicroSEQ® Method[i] 95% CI[h]	Reference Method x	Reference Method POD_R[f]	Reference Method 95% CI[h]	dPOD[g]	95% CI[h]
Diced Tomatoes vs. FDA/BAM Chapter 5	18 Hour Primary Enrichment	*Salmonella enterica subsp. Typhimurium ATCC 14028*	**0** (0.00, 0.18)	15	-	NA[b]	NA	0	0	0.00, 0.20	NA	NA
			0.75 (0.44, 1.20)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0	-0.28, 0.28
			3 (1.30, 6.90)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
Chocolate Enriched with NFDM vs. FDA/BAM Chapter 5	18 Hour Primary Enrichment	*Salmonella enterica subsp. Montevideo QL024-19*	**0** (0.00, 0.18)	15	NA	NA	NA	0	0	0.00, 0.20	NA	NA
			0.9 (0.54, 1.50)	20	14	0.7	0.48, 0.85	14	0.7	0.48, 0.85	0	-0.27, 0.27
			4.4 (1.70, 11.0)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
Chocolate Enriched with UHTM vs. FDA/BAM Chapter 5	18 Hour Primary Enrichment	*Salmonella enterica subsp. Montevideo QL024-19*	**0** (0.00, 0.18)	15	NA	NA	NA	0	0	0.00, 0.20	NA	NA
			0.9 (0.54, 1.50)	20	13	0.65	0.43, 0.82	14	0.7	0.48, 0.85	-0.05	-0.32, 0.23
			4.4 (1.70, 11.0)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
Deli Ham vs. USDA/FSIS-MLG 4.05	18 Hour Primary Enrichment	*Salmonella enterica subsp. Enteritidis ATCC 13076*	**0** (0.00, 0.18)	15	NA	NA	NA	0	0	0.00, 0.20	NA	NA
			0.43 (0.21, 0.75)	20	8	0.4	0.22, 0.61	8	0.4	0.22, 0.61	0	-0.28, 0.28
			2.3 (1.04, 5.02)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43

Table 1. Matrix Summary Table - Pathatrix®/MicroSEQ® Method vs. Reference Method

[a]MPN =MPN was calculated for each matrix using five 50g, five 10g and the 20 reference method samples and the Least Cost Formulations MPN Calculator
[b]N/A = Not applicable
[c]N = Number of test potions
[d]x = Number of positive test portions
[e]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[f]POD_r = Reference method confirmed positive outcomes divided by the total number of trials
[g]dPOD = Difference between the candidate method confirmed result and reference method confirmed result POD values
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level, calculated following the current AOAC guidelines and using the Least Cost Formulations AOAC Binary Workbook
[i]Test Method = Test Method results represent both Individual and 10-Pooling sample sets

Matrix	Time Point	Strain	MPN[a]/ Test Portion	N[c]	Pathatrix®/MicroSEQ® Method Presumptive[j] x[d]	Pathatrix®/MicroSEQ® Method Presumptive[j] POD_C[e]	Pathatrix®/MicroSEQ® Method Presumptive[j] 95% CI[h]	Pathatrix®/MicroSEQ® Method Confirmed x	Pathatrix®/MicroSEQ® Method Confirmed POD_R[f]	Pathatrix®/MicroSEQ® Method Confirmed 95% CI[h]	dPOD[g]	95% CI[h]
Chocolate Enriched with UHTM Presumptive Vs. Confirmed	18 Hour Primary Enrichment	*Salmonella enterica subsp. Montevideo QL024-19*	0 (0.00, 0.18)	15	NA	NA	NA	0	0	0.00, 0.20	NA	NA
			0.9 (0.54, 1.50)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.21 0.21
			4.4 (1.70, 11.0)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.45, 0.45

Table 2. Unpaired Summary - Pathatrix®/MicroSEQ® Method Presumptive vs. Confirmed

[a-h]See Table 1.
[j]Candidate Method Presumptive = Candidate Method Presumptive results represent both Individual and 10-Pooling sample sets
.

DISCUSSION

For both the low inoculation level and the high inoculation level of each matrix, the probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials. POD analyses were conducted for both the individual and 10-Pooling sample sets. The POD was calculated for the candidate presumptive results, POD_C, the reference method confirmatory results, POD_R, and the difference in the candidate presumptive and reference confirmatory results, $dPOD_C$. The POD values and 95% confidence intervals were calculated following the current AOAC guidelines and using the Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook.

For the method comparison, the POD analyses between the candidate and the reference methods for all three matrices indicated that there was no statistically significant difference between the number of positive results obtained by the two methods being compared. Fractionally positive results (5-15 positives out of 20 replicates) were obtained for each of the matrices analyzed in this study. MPN results for each matrix were determined using the Least Cost Formulations MPN Calculator [5], and are presented along with an overall summary of results for each matrix in Tables 1 and 2.

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, which is able to process a varied number of sample matrices, and show reliable detection of *Salmonella* spp. in food. Furthermore, we show that this level of robustness and reliability is possible when pooling up to 10 post-enriched samples. 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 during food 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 through the use of pooling. 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. Through the use of pooling, we hope to be able to show the industry that by 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.

ACKNOWLEDGEMENTS

Dan Kephart – AFE R&D Leader
Kim Hahn – AFE Project Manager for Pathatrix®
The Animal, Food, and Environmental Safety R&D Team
The Staff and Scientists of Q Laboratories, Inc. for their fine work and collaboration.
Maria Nelson – AOAC Consultant to Life Technologies, Inc.

TRADEMARKS/LICENSING