Pathatrix Auto™: The First AFNOR-Approved Real-Time PCR Workflow for Detecting *Salmonella* in Pooled Food Samples

LIFE TECHNOLOGIES™
FOOD SAFETY

Jason K. Wall[1], Danièle Sohier[2], and Rick Conrad[1], [1] Animal Health and Food Safety, Life Sciences Solutions Group, Thermo Fisher Scientific, Austin, Texas. [2]Adria Developpement, Quimper, France.

ABSTRACT

The Pathatrix Auto™ pathogen isolation platform provides a workflow that is able to process as many as ten individual food enrichments in the same sample pool. This sampling format has never been approved in the EU market, and would require extensive validation efforts by an expert testing lab to evidence that the approach is not only possible, but practical. In order to validate this product for food safety testing in the EU, this workflow would need to demonstrate a relevant relative detection limit, show statistical similarity to the ISO 16140 reference through accuracy, sensitivity, and specificity; and prove its robustness and practicability in the field. Adria Developpement was selected to perform the evaluation to ascertain the Pathatrix Auto's ability to detect Salmonella in pooled food sample types by Real-time PCR and selective agar plating. A Ring Trial proficiency study with 15 independent food safety testing labs was also conducted to verify that the workflow was functional and accurate with minimal training. In both the Adria Developpement study and the Ring Trial, the candidate and reference methods were found to be statistically similar. Of the 202 different food sample types tested during this evaluation, a relative accuracy of 93.1%, a relative sensitivity of 87.7%, and a relative specificity of 96.7% was attained. The relative detection limit was determined to be 0.4-1.5 log CFU/25g of sample, which was statistically similar to the reference. The selected Ring Trial labs demonstrated 100% proficiency and accuracy in performing the workflow. These results were satisfactory for approval by the AFNOR committee, and yielded the first validated method for sample pooling in the EU. The demonstrated robustness, accuracy, and ease of use of this workflow allows the user to rapidly screen for rare contamination events with high confidence, with up to a 90% cost savings over other PCR-based platforms.

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.

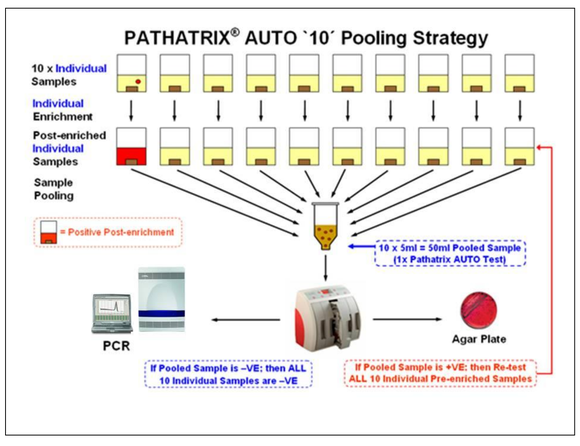

Figure 1. 10-Pooling Strategy

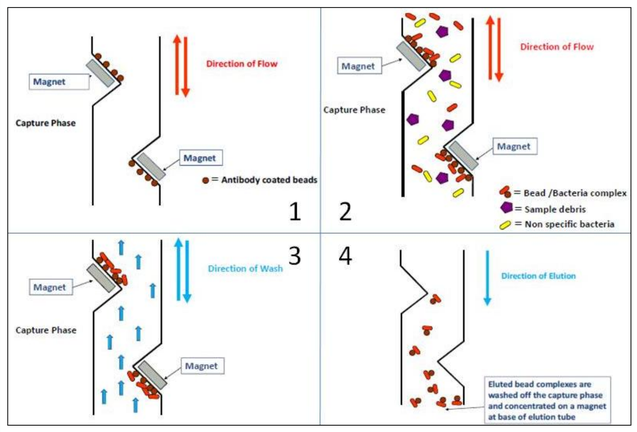

Figure 2. Automated IMS Capture Process

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.

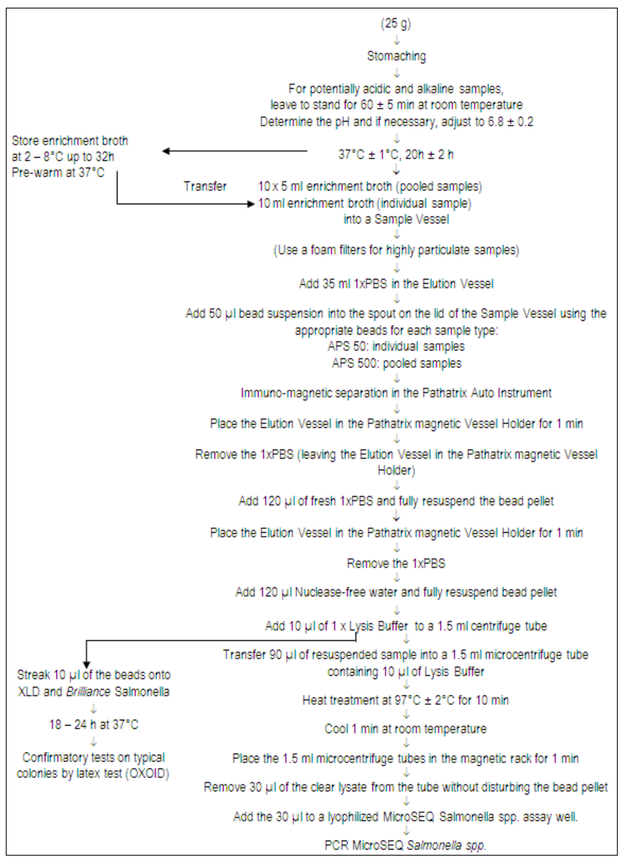

Figure 3. Study Workflow

A heat lysis method (with an optional Proteinase-K step) was developed in anticipation of this workflow being used for the detection of many different food-borne pathogens of interest, including Gram-positive bacteria such as *Listeria*. Shown here is the complete workflow used by Adria Developpement from enrichment to PCR. Also shown are the steps where aliquots are taken for selective agar plate analysis.

RESULTS

Category	Sample Types	Enrichment Time	MPN (CFUs/25g)	N	Positive Calls	
					MicroSEQ	Reference
Meat Products	Ground Beef, Beef Trim	18 hours in BPW	0	42	0	0
	Cooked Deli		x≤5	32	27	27
	RTE Foods					
Dairy Products	Milk Powder and Infant Formula	18 hours in BPW+Brilliant Green (0.002%)	0	47	0	0
	Fermented Milk and Yogurts		x≤5	32	27	29
	Pasteurized Milk Products					

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

The most probable number (MPN) represented a low level contamination in the positive samples. 153 different sample matrices were tested in the course of this study. The results of the study comparing the alternative and reference methods were that Adria found the methods to be statistically similar.

Category	Sample Method	Relative Accuracy	Relative Sensitivity	Relative Specificity
Meat Products	Pooled	93.20%	87.10%	97.70%
	Individual	95.90%	93.50%	97.70%
Dairy Products	Pooled	93.70%	90%	95.90%
	Individual	93.70%	90%	95.90%

Table 2. Statistical Findings by Sample Pooling Format - Pathatrix/MicroSEQ® Method vs. Reference Method

All samples were evaluated under a pooling and individual sample format. In all cases, Adria found that the results showed no statistical difference between the alternative method and the reference method for both pooled and individual formats. The above calculations were made according to Annex F of the ISO 16140 standard.

Lab Code	Reference Method			Alternative Method (Pooling)			Alternative Method (Individual)		
	L0	L1	L2	L0	L1	L2	L0	L1	L2
A	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
B	0/8	7/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
D	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
E	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
F	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
G	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
H	0/8	8/8	8/8	0/8	8/8	7/8	0/8	8/8	8/8
I	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
J	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
M	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
N	0/8	7/8	8/8	0/8	6/8	8/8	0/8	6/8	8/8
Expert	0/8	8/8	8/8	0/8	8/8	8/8	0/8	8/8	8/8
Concordance									
	100%	96%	100%	100%	97%	98%	100%	97%	100%

Table 3. Results of Independent Lab Study

15 labs in the EU were asked to participate in this study, in which they would perform testing on 24 samples of three inoculation levels. Four labs were disqualified from the study for various logistical and technical reasons, but the concordance for the remaining 11 labs is shown. This level of concordance and method robustness was acceptable to the AFNOR committee for approval.

DISCUSSION

The Pathatrix® Auto Salmonella spp. 10-pooling protocol linked to Applied Biosystems Real-Time PCR showed satisfying relative accuracy, specificity, and sensitivity results. The statistical tests conclude equivalence between the reference method and the two protocols (pooled and individual sample analysis) of the alternative method for the studied scope of food groups. These included Meat Products (raw beef meats, ready-to-eat meats, ready-to-reheat meats, and poultry) and heat-treated milk and dairy products. The relative detection limits of the alternative method and the ISO 16140 standard were found to be similar. The alternative method also showed satisfying inclusivity and exclusivity results. The observed data and results from the Independent Laboratory Study confirmed that the alternative method and reference method show equivalent performances in accordance, concordance, and odds ratio. The Pathatrix Auto Salmonella 10 pooling protocol linked to Applied Biosystems Real-Time PCR allows the screening of negative samples within one day, while 2 or 3 days are required for the positive samples.

CONCLUSIONS

The goal of this work was to extend our AOAC-approved workflow into the European market by way of certifying it through the AFNOR committee. We show here that the tested workflow 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 into the same test, which is a technique that had not been approved in the European market prior to this study. Over 150 sample matrices were tested at low inoculation levels during the course of this study, and satisfactory results were attained for accuracy, sensitivity, and specificity. Additionally, the workflow conducted by the expert lab was then extended into the hands of 15 independent food testing labs to show its robustness and reliability. The level of concordance between those labs during the study was found to be acceptable to AFNOR. The ability of the Pathatrix® to be able to process each of the above sample types is a significant advantage to food producers with a variety of sample types, 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, the producer can attain 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

Eric Liu – Product Manager for Pathatrix®
Kimberly Hahn – Project Manager for Pathatrix®
Dan Kephart – Animal Health and Food Safety R&D Leader
Katrien Vanhonacker – EMEA Marketing Lead
The Animal Health and Food Safety R&D Team
The Staff and Scientists of Adria Developpement for their fine work and collaboration

TRADEMARKS/LICENSING
© 2014 Life Technologies Corporation. All rights reserved.
The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.