Validation of the 3M™ Molecular Detection System for the Detection of *Salmonella*, *E. coli* O157 (including H7) and *Listeria* spp., in Dried Fruits, Nuts and Environmental Samples

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DFA of California, Fresno, CA: Mai Her Yang **3M Food Safety, St. Paul, MN:** Peter Benedetto

INTRODUCTION

In order to ensure the safety of consumers in the food market, a much more comprehensive farm to table approach is required. For this to be a reality, faster and more accurate testing methods for pathogens are necessary, especially since traditional microbiology methods are cumbersome and take more than four days to complete. Modern developments in molecular biology have resulted in isothermal nucleic acid amplification technology, also known as loop-mediated isothermal amplification (LAMP), which is recognized as highly robust, efficient, sensitive, specific and easy to use. The 3M™ Molecular Detection System was developed, for the rapid and specific detection of pathogens using the innovative combination of LAMP and bioluminescence to report the amplification of the target DNA in real time.

PURPOSE

This study was conducted to evaluate the performance of the 3M Molecular Detection System and its assays in the detection of *Salmonella*, *E. coli* O157:H7 and *Listeria* spp., in the presence of potential interferences from seven sample matrices. The system's performance was also assessed on environmental samples. Presumptive positives were confirmed through cultural methods.

METHOD

Salmonella, *E. coli* O157:H7 and *Listeria* spp. pathogen screen tests were performed on various nuts and dried fruits; 25g samples of each matrix were enriched with the required media (3M™ Buffered Peptone Water (ISO) or 3M™ Modified *Listeria* Recovery Broth) and incubated according to the protocol table below. One set of samples was inoculated with 25 CFU; one set was not inoculated with pathogens.

Table 1: Samples

Sample Description	Sample Size	Enrichment Media Volume
Raisins	25g	225mL
Almonds	25g	225mL
Walnuts	25g	225mL
Diced Walnuts	25g	225mL
Salted Pistachios	25g	225mL
Raw Pistachios	25g	225mL
Kernel Pistachios	25g	225mL
Environmental Surface	1 swab	100mL
Pool of Raisins, Almonds, Walnuts and Raw Pistachios	4 x 10mL (Post-enrichment)	N/A
Pool of Raisins, Almonds, Walnuts, Diced Walnuts and Salted Pistachios	5 x 10mL (Post-enrichment)	N/A
Salted Pistachios and Kernel Pistachios	2 x 10mL (Post-enrichment)	N/A
Raw Pistachios and Kernel Pistachios	2 x 10mL (Post-enrichment)	N/A
Environmental Swabs	2 x 10mL (Post-enrichment)	N/A

Table 2: Protocols

Assay	Incubation Temperature	Enrichment Time (Hours)
Salmonella	37°C	18–24
E. coli O157:H7	42°C	18–24
Listeria spp.	37°C	24–28

Environmental swabs were enriched in 100mL of the required media according to the protocol for each assay; *Salmonella*, *E. coli* O157:H7 and *Listeria* spp. environmental surface samples were ran spiked and un-spiked.

Matrix Controls were used for each matrix to check for possible interference from the sample. Kit negative controls and reagent controls (known Positive Control) were run along with each test to confirm that the kits were working as designed.

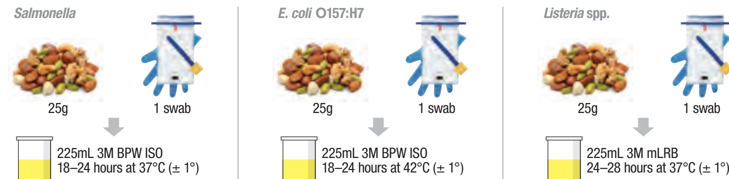
A pool was performed for spiked and un-spiked *Salmonella* samples only, 10mL aliquots were taken from each enriched sample (see Table 1); then 50µL of anti-body coated magnetic beads, specific for *Salmonella*, were added to the pooled samples and placed on the Applied Biosystems Pathatrix® Immuno-magnetic Separation unit, four individual samples were processed along with the pooled samples for comparison purposes.

Presumptive positives were confirmed by the use of traditional cultural methods as follows:

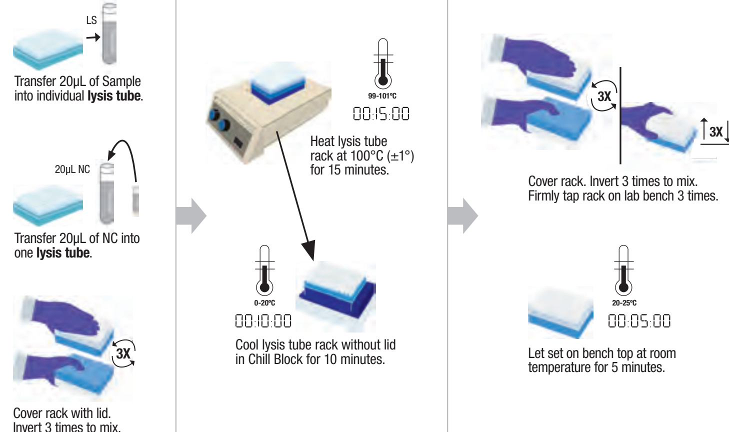
- *Salmonella*: All presumptive positive samples were streaked onto XLD, HE, BBL™ CHROMagar™ *Salmonella* and incubated at 35 ± 1°C for 24–48 hours.
- *E. coli* O157:H7: All presumptive positive samples were streaked onto CT-SMAC and incubated at 35 ± 1°C for 24–48 hours.
- *Listeria* spp.: All presumptive positive samples were streaked onto RAPID'L.mono™ (Bio-Rad) and incubated at 35 ± 1°C for 24–48 hours.

PROTOCOL REFERENCE

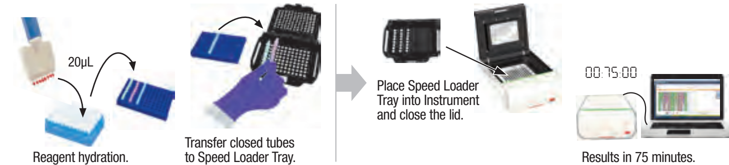
1. Enrichment



2. Lysis

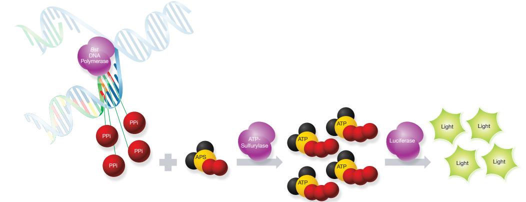


3. Amplification



TECHNOLOGY OVERVIEW

Powered by an innovative combination of unique technologies to bring molecular level accuracy expected by customers — without sacrificing productivity.



RESULTS

		Salmonella		*E. coli* O157:H7		*Listeria* spp.	
	n	% Sensitivity	% Specificity	% Sensitivity	% Specificity	% Sensitivity	% Specificity
Raisins	5	100	100	100	100	100	100
Almonds	5	100	100	100	100	100	100
Walnuts	5	100	100	100	100	100	100
Diced Walnuts	5	100	100	100	100	100	100
Salted Pistachios	5	100	100	100	100	100	100
Raw Pistachios	5	100	100	100	100	100	100
Kernel Pistachios	5	100	100	100	100	100	100
Environmental Swab	5	100	100	100	100	100	100
Pooled Samples	8	100	100	N/A	N/A	N/A	N/A

Matrix Controls were valid, confirming that there was no interference from the matrices tested.

% Sensitivity and % Specificity were calculated as follows:

$$\% \text{ Sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$\% \text{ Specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

Cultural methods confirmed all presumptive positive results from the 3M Molecular Detection System.

SIGNIFICANCE

This study demonstrates that the 3M Molecular Detection System is a highly robust, efficient, sensitive and specific detection method. Compatibility with a variety of samples was demonstrated; the absence of matrix interference was confirmed through the use of the Matrix Control for each assay.

ACKNOWLEDGMENTS

Apart from the efforts of myself, the success of any project or experiment depends largely on the encouragement and guidelines of many others. I would like to take this opportunity to express my sincere gratitude to the people who have been instrumental in the successful completion of this validation experiment. I would like to show my greatest appreciation and gratitude to 3M and Thomas Jones (DFA Laboratory Director) for allowing me the opportunity to test the 3M Molecular Detection System. I would like to express my gratitude towards Peter Benedetto for putting this validation poster together.

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