CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

031001

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

MicroSEQ® *Salmonella* species Detection Kit

manufactured by

Life Technologies, part of Thermo Fisher Scientific
Wade Road
Basingstoke, Hampshire, RG24 8PW
United Kingdom

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (December 12, 2018 – December 31, 2019). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 12, 2018
Date

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Life Technologies, part of Thermo Fisher Scientific
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KIT NAME(S)
MicroSEQ® *Salmonella* species Detection Kit

CATALOG NUMBERS
4403930, 4480466, 4407760, 4413269

INDEPENDENT LABORATORY
Original Validation:
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[4] January 2011 Modification
[5] Modification review for May 2013, October 2015, November 2015, August 2016 December 2018 Modification internal AOAC Research Institute review

APPLICABILITY OF METHOD
Target organism – *Salmonella* species

Matrices – Original Validation: (25 g) - raw ground beef, raw chicken wings, raw shrimp, cantaloupe, brie, dry infant formula, chocolate, dry pet food, shell eggs, black pepper, peanut butter
MODIFICATION January 10, 2011: stainless steel, sealed concrete, plastic ceramic tile, and rubber
MODIFICATION May 2013 linked to Pathatrix® 10-Pooling *Salmonella* spp. (090203C for fresh diced tomatoes, chocolate, and deli ham
MODIFICATION November 2015: dry pet food (375 g)

Performance claims - Sensitivity was equivalent to the reference ISO culture-based method and specificity was ≥ 99% for ten food matrices. For peanut butter, the sensitivity was equivalent to the reference FDA-BAM culture-based method and specificity was ≥ 99%.

REFERENCE METHODS
ISO 6579:2002 (E) Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. 2002. Fourth Edition (6)
U.S. Food and Drug Administration, *Bacteriological Analytical Manual Online* (December 2007) Chapter 5, *Salmonella*, http://www.cfsan.fda.gov/~ebam/bam-5.html. (8)

ORIGINAL CERTIFICATION DATE
March 17, 2010

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2019

METHOD MODIFICATION RECORD
1. **January 2011**
2. **May 15, 2013**
3. **October 2015 Level 2**
4. **November 04, 2015 Level 2**
5. **December 2017 Level 1**
6. **August 2019 Level 2**
7. **December 2018 Level 2**
8. **December 2018 Level 1**

SUMMARY OF MODIFICATION
1. **Matrix extension for environmental surfaces**
2. **MicroSEQ® *Salmonella* spp. Linked to Pathatrix® 10-Pooling *Salmonella* spp. Kit for fresh diced tomatoes, chocolate, and deli ham**
3. **Pathatrix® 10-Pooling *Salmonella* spp. Kit Location change from Austin, TX to Vilnius, Lithuania approved.**
4. **Matrix extension for dry pet food (375 g)**
5. **Update IFUs and labels**
6. **Equivalency study for KingFisher™ Flex-96 Deep Well Magnetic Particle Processor**
7. **Location change of critical raw materials from Austin, Texas to Vilnius, Lithuania.**
8. **Update to User Guide AOAC RI workflow**

Under this AOAC® *Performance Tested*℠ License Number, 031001 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*℠ License Number, 031001 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)

Real-Time PCR Amplification

The MicroSEQ® Pathogen Detection System is based on TaqMan® Real-Time PCR technology [4], providing two levels of specificity for confident pathogen detection by combining Polymerase Chain Reaction (PCR) amplification [3] and signal detection in a single reaction. The first level of specificity is provided by target specific PCR primers that identify the DNA sequence of the organism in the sample. The identification of the organism is confirmed simultaneously by TaqMan® probes, which represent the second level of specificity. As a result, a fluorescent signal is emitted only if the unique genetic signature of the pathogen has been recognized. By addressing the unique genetic signature of the target organism, the Real-Time PCR system delivers results independent of environmental factors such as temperature or pH.

In addition, the MicroSEQ® Pathogen Detection System contains an Internal Positive Control (IPC) in the reaction mix to monitor the presence of inhibitors. Amplification of IPC demonstrates the absence of PCR inhibition, providing more confident negative results (reducing false negative calls). After PCR amplification and detection, reaction tubes remain sealed, thus significantly reducing the potential for contamination (false positives).

Data Analysis

The Rapid Finder™ Express software simplifies Real-Time PCR setup and processing by providing a software-guided workflow and automated analysis of results. Designed specifically for pathogen detection in food and environmental samples, Rapid Finder™ Express software guides the user through each step of the assay and performs all required calculations. During PCR, real-time fluorescence data is collected cycle by cycle for each individual reaction. Positive signals result in an increase of the target-specific fluorescent signal while the fluorescence of negative signals remains below the threshold applied by Rapid Finder™ Express software. When the assay is complete, Rapid Finder™ Express software transforms the individual fluorescent signals and displays them in an intuitive, easy-to-read, color-coded format.

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

The independent validation studies together with the internal validation studies, within their statistical uncertainty, support the product claims for the MicroSEQ® *Salmonella* spp. Detection Kit for detecting *Salmonella* in raw ground beef, raw chicken, raw shrimp, Brie cheese, shell eggs, cantaloupe, chocolate, black pepper, dry infant formula, and dry pet food. In addition, the ERV study demonstrates that the MicroSEQ® *Salmonella* spp. detection kit and the FDA BAM reference method are statistically similar for detection of *Salmonella* in peanut butter.

Table 3. Inclusivity results using the MicroSEQ® *Salmonella* spp. Detection Kit with strains cultured in BPW (general enrichment procedure) (1)

Number	*Salmonella* Serotype	Campden code	cfu/ml	MicroSEQ® *Salmonella* spp. Detection Kit method	
				Rapid Spin	NA
1.	NA arizonae (IIIa)	16380	3.9E+04	Positive	Positive
2.	Treforest	1413	2.5E +04	Positive	Positive
3.	Utrecht	1417	4.7E +04	Positive	Positive
4.	Uccle	1416	1.9E+04	Negative	Negative
			1.1E+08 (retest)	Negative	Negative
			1.1E+04 (retest)	Positive	Positive
5.	Tranaroa	1412	5.8E+04	Negative	Positive
			2.6E+08 (retest)	Positive	NT
			2.6E+04 (retest)	Positive	NT
6.	Locarno	1386	3.8E +04	Positive	Positive
7.	Basel	1292	4.1E +04	Positive	Positive
8.	Agona	1050	3.2 E +04	Positive	Positive
9.	Brandenburg	1070	4.0E +04	Positive	Positive
10.	Bredeney	1075	3.4E +04	Positive	Positive
11.	California	1319	3.6E +04	Positive	Positive
12.	Coeln	1336	4.1E +04	Positive	Positive
13.	Derby	1352	1.9E +04	Positive	Positive
14.	Duisburg	1358	3.7E +04	Positive	Positive
15.	Essen	1370	2.7E +04	Positive	Positive
16.	Heidelberg	1025	2.4E +04	Positive	Positive
17.	Indiana	71	1.8E +04	Positive	Positive
18.	Saintpaul	1092	2.9E +04	Positive	Positive
19.	Schwarzengrund	1408	2.6E +04	Positive	Positive
20.	Stanley	1057	3.4E +04	Positive	Positive
21.	Typhimurium	1009	2.0E +04	Positive	Positive
22.	Amersfoort	1280	8.0E +03	Positive	Positive
23.	Bareilly	1291	2.0E +04	Positive	Positive
24.	Braenderup	1095	4.0E +04	Positive	Positive
25.	Edinburgh	1364	2.3E +04	Positive	Positive
26.	Infantis	1038	2.5E +04	Positive	Positive
27.	Livingstone	1385	2.6E +04	Positive	Positive
28.	Mbandaka	1391	2.3E +04	Positive	Positive
29.	Montevideo	15946	5.0E +04	Positive	Positive
30.	Norwich	1401	3.1E +04	Positive	Positive
31.	Oranienburg	1402	1.8E +04	Positive	Positive
32.	Tennessee	1411	1.7E +04	Positive	Positive
33.	Thompson	1080	2.4E +04	Positive	Positive
34.	Virchow	1011	3.6E +04	Positive	Positive
35.	Blockley	1087	3.7E +04	Positive	Positive
36.	Bovis-morbificans	1306	2.0E +04	Positive	Positive
37.	Corvallis	1341	1.7E +04	Positive	Positive
38.	Emek	1367	1.3E +04	Positive	Positive
39.	Fayed	1372	1.9E +04	Positive	Positive
40.	Hadar	1016	2.6E +04	Positive	Positive
41.	Kentucky	1382	1.5E +04	Positive	Positive
42.	Manhattan	1389	2.7E +04	Positive	Positive
43.	Newport	1041	1.2E +04	Positive	Positive
44.	Alabama	1273	1.5E +04	Positive	Positive
45.	Berta	1065	1.8E +04	Positive	Positive
46.	Canastel	1321	2.1E +04	Positive	Positive
47.	Dublin	1356	1.4E +04	Positive	Positive
48.	Enteritidis	1004	2.7E +04	Positive	Positive
49.	Gallinarum	15831	1.5E +04	Positive	Positive
50.	Miami	1393	1.9E +04	Positive	Positive

51.	Panama	1049	3.1E +04	Positive	Positive
52.	Pullorum	15832	1.1E +04	Positive	Positive
53.	Anatum	1060	3.3E +04	Positive	Positive
54.	Butantan	1316	1.6E +04	Positive	Positive
55.	Elisabethville	1366	5.0E +03	Positive	Positive
56.	Give	1374	8.5E +04	Positive	Positive
57.	Lexington	5110	5.0E +04	Positive	Positive
58.	London	1387	1.7E +04	Positive	Positive
59.	Cambridge	1320	2.7E +04	Positive	Positive
60.	Chittagong	1331	9.5E +04	Positive	Positive
61.	Krefeld	1383	8.5E +03	Positive	Positive
62.	Senftenberg	9281	2.0E +04	Positive	Positive
63.	Abaetetuba	1268	9.5E +03	Positive	Positive
64.	Aberdeen	1269	8.5E +03	Positive	Positive
65.	Pretoria	1404	5.5E +03	Positive	Positive
66.	Maastricht	9273	1.8E +04	Positive	Positive
67.	Rubislaw	1406	1.7E +04	Positive	Positive
68.	Solt	1569	1.6E +04	Positive	Positive
69.	Clifton	1334	4.0E +03	Positive	Positive
70.	Havana	3004	1.2E +04	Positive	Positive
71.	Kedougou	1021	2.5E +04	Positive	Positive
72.	Poona	725	3.7E +04	Positive	Positive
73.	Albuquerque	1276	1.5E +04	Positive	Positive
74.	Caracus	1323	3.0E +03	Positive	Positive
75.	Ferlac	1373	4.8E +04	Positive	Positive
76.	Brazil	1309	1.1E +04	Positive	Positive
77.	Nottingham	16290	2.8E +04	Positive	Positive
78.	Carmel	1324	2.5E +04	Positive	Positive
79.	Cerro	1326	1.5E +04	Positive	Positive
80.	Minnesota	1394	1.5E +04	Positive	Positive
81.	Pomona	1403	3.0E +04	Positive	Positive
82.	Urbana	1414	1.8E +04	Positive	Positive
83.	Adelaide	9766	3.9E +04	Positive	Positive
84.	Alachua	1274	2.5E +04	Positive	Positive
85.	Ealing	5449	5.3E +04	Positive	Positive
86.	Emmastad	1368	1.4E +04	Positive	Positive
87.	Inverness	9274	4.2E +04	Positive	Positive
88.	Champaign	15635	2.7E +04	Positive	Positive
89.	Allandale	1277	2.7E +04	Positive	Positive
90.	Bulawayo	1315	1.7E +04	Positive	Positive
91.	Duval	1361	2.3E +04	Positive	Positive
92.	Waycross	1885	2.3E +04	Positive	Positive
93.	Berkeley	1295	2.3E +04	Positive	Positive
94.	Houten	1376	2.4E+04	Negative	Positive
			3.1E+08 (retest)	Positive	NT
			3.1E+04 (retest)	Positive	NT
95.	Clovelly	1335	1.7E +04	Positive	Positive
96.	Dugbe	1357	3.0E +04	Positive	Positive
97.	Deversoir	1353	1.7E +04	Positive	Positive
98.	Phoenix	9280	1.9E +04	Positive	Positive
99.	Dahlem	1345	2.4E +04	Positive	Positive
100.	Wassenaar	1415	1.1E +04	Positive	Positive

NT = Not tested NA = Not applicable (subspecies)

Table 4. Exclusivity results using the MicroSEQ® *Salmonella* spp. Detection Kit (1)

Number	Organism	Campden code	CFU/ml	MicroSEQ® *Salmonella* spp. Detection Kit method	
				Rapid Spin	NA
1.	*Aeromonas hydrophila*	4111	1.5E+08	Negative	Negative
2.	*Avibacterium avium*	8389	8.0E+07	Negative	Negative
3.	*Citrobacter freundii*	40	2.2E+08	Negative	Negative
4.	***Edwardsiella tarda***	8392	5.1E+08	Negative	Negative
5.	*Enterobacter aerogenes*	15736	2.9E+08	Negative	Negative
6.	*Enterobacter cloacae*	1472	4.1E+08	Negative	Negative
7.	*Enterobacter sakazakii*	6634	6.9E+08	Negative	Negative
8.	*Escherichia coli*	11017	3.0E+08	Negative	Negative
9.	*Hafnia alvei*	4009	1.6E+08	Negative	Negative
10.	*Klebsiella oxytoca*	8387	8.0E+07	Negative	Negative
11.	*Klebsiella pneumoniae*	6786	3.3E+08	Negative	Negative
12.	*Morganella morganii*	1542	5.1E+08	Negative	Negative
13.	*Pantoea agglomerans*	15947	1.3E+08	Negative	Negative
14.	*Pasteurella bettyae*	16395	5.9E+08	Negative	Negative
15.	*Pasteurella multocida*	16396	1.0E+08	Negative	Negative
16.	*Proteus mirabilis*	1588	3.6E+08	Negative	Negative
17.	*Proteus vulgaris*	1581	2.3E+08	Negative	Negative
18.	*Providencia rettgeri*	8386	6.5E+08	Negative	Negative
19.	*Pseudomonas aeruginosa*	8299	1.0E+08	Negative	Negative
20.	*Pseudomonas fluorescens*	15937	3.0E+08	Negative	Negative
21.	*Pseudomonas fragi*	16050	6.5E+07	Negative	Negative
22.	***Serratia liquefaciens***	1491	1.7E+08	Negative	Negative
23.	***Serratia marcescens***	1521	2.2E+08	Negative	Positive
24.	*Serratia proteamaculans* subsp. *quinovora*	16463	6.0E+07	Negative	Negative
25.	*Shigella boydii*	324	1.5E+08	Negative	Negative
26.	*Shigella flexneri*	325	1.5E+08	Negative	Negative
27.	*Shigella sonnei*	4107	2.6E+08	Negative	Negative
28.	*Vibrio mimicus*	6351	7.5E+07	Negative	Negative
29.	*Vibrio parahaemolyticus*	15737	7.6E+07	Negative	Negative
30.	*Yersinia enterocolitica*	4103	2.1E+08	Negative	Negative

Table 5. Inclusivity results using the MicroSEQ® *Salmonella* spp. Detection Kit with strains cultured in BPW with skim milk and brilliant green (chocolate enrichment procedure) (1)

Number	*Salmonella* Serotype	Campden code	MicroSEQ® *Salmonella* spp. Detection Kit method	
			Rapid Spin	NA
1.	NA arizonae (IIIa)	16380	Negative	Positive
2.	Treforest	1413	Positive	Positive
3.	Utrecht	1417	Positive	Positive
4.	Uccle	1416	Negative	Negative
5.	Tranaroa	1412	Positive	Positive
6.	Locarno	1386	Positive	Positive
7.	Basel	1292	Positive	Positive
8.	Agona	1050	Positive	Positive
9.	Brandenburg	1070	Positive	Positive
10.	Bredeney	1075	Positive	Positive
11.	California	1319	Positive	Positive
12.	Coeln	1336	Positive	Positive
13.	Derby	1352	Positive	Positive
14.	Duisburg	1358	Positive	Positive
15.	Essen	1370	Positive	Positive
16.	Heidelberg	1025	Positive	Positive
17.	Indiana	71	Positive	Positive
18.	Saintpaul	1092	Positive	Positive
19.	Schwarzengrund	1408	Positive	Positive
20.	Stanley	1057	Positive	Positive
21.	Typhimurium	1009	Positive	Positive
22.	Amersfoort	1280	Positive	Positive
23.	Bareilly	1291	Positive	Positive
24.	Braenderup	1095	Positive	Positive
25.	Edinburgh	1364	Positive	Positive
26.	Infantis	1038	Positive	Positive
27.	Livingstone	1385	Positive	Positive
28.	Mbandaka	1391	Positive	Positive
29.	Montevideo	15946	Positive	Positive
30.	Norwich	1401	Positive	Positive
31.	Oranienburg	1402	Positive	Positive
32.	Tennessee	1411	Positive	Positive
33.	Thompson	1080	Positive	Positive
34.	Virchow	1011	Positive	Positive
35.	Blockley	1087	Positive	Positive
36.	Bovis-morbificans	1306	Positive	Positive
37.	Corvallis	1341	Positive	Positive
38.	Emek	1367	Positive	Positive
39.	Fayed	1372	Positive	Positive
40.	Hadar	1016	Positive	Positive
41.	Kentucky	1382	Positive	Positive
42.	Manhattan	1389	Positive	Positive
43.	Newport	1041	Positive	Positive
44.	Alabama	1273	Positive	Positive
45.	Berta	1065	Positive	Positive
46.	Canastel	1321	Positive	Positive
47.	Dublin	1356	Positive	Positive
48.	Enteritidis	1004	Positive	Positive
49.	Gallinarum	15831	Positive	Positive
50.	Miami	1393	Positive	Positive
51.	Panama	1049	Positive	Positive
52.	Pullorum	15832	Positive	Positive
53.	Anatum	1060	Positive	Positive

54.	Butantan	1316	Positive	Positive
55.	Elisabethville	1366	Positive	Positive
56.	Give	1374	Positive	Positive
57.	Lexington	5110	Positive	Positive
58.	London	1387	Positive	Positive
59.	Cambridge	1320	Positive	Positive
60.	Chittagong	1331	Positive	Positive
61.	Krefeld	1383	Positive	Positive
62.	Senftenberg	9281	Positive	Positive
63.	Abaetetuba	1268	Positive	Positive
64.	Aberdeen	1269	Positive	Positive
65.	Pretoria	1404	Positive	Positive
66.	Maastricht	9273	Positive	Positive
67.	Rubislaw	1406	Positive	Positive
68.	Solt	1569	Positive	Positive
69.	Clifton	1334	Positive	Positive
70.	Havana	3004	Positive	Positive
71.	Kedougou	1021	Positive	Positive
72.	Poona	725	Positive	Positive
73.	Albuquerque	1276	Positive	Positive
74.	Caracus	1323	Positive	Positive
75.	Ferlac	1373	Negative/ Positive on retest	Positive
76.	Brazil	1309	Positive	Positive
77.	Nottingham	16290	Positive	Positive
78.	Carmel	1324	Positive	Positive
79.	Cerro	1326	Positive	Positive
80.	Minnesota	1394	Positive	Positive
81.	Pomona	1403	Positive	Positive
82.	Urbana	1414	Positive	Positive
83.	Adelaide	9766	Positive	Positive
84.	Alachua	1274	Positive	Positive
85.	Ealing	5449	Positive	Positive
86.	Emmastad	1368	Positive	Positive
87.	Inverness	9274	Positive	Positive
88.	Champaign	15635	Positive	Positive
89.	Allandale	1277	Positive	Positive
90.	Bulawayo	1315	Positive	Positive
91.	Duval	1361	Positive	Positive
92.	Waycross	1885	Positive	Positive
93.	Berkeley	1295	Positive	Positive
94.	Houten	1376	Positive	Positive
95.	Clovelly	1335	Positive	Positive
96.	Dugbe	1357	Positive	Positive
97.	Deversoir	1353	Positive	Positive
98.	Phoenix	9280	Positive	Positive
99.	Dahlem	1345	Positive	Positive
100.	Wassenaar	1415	Negative	Negative

NA = Not applicable (subspecies)

Table C: Summary of Method Comparison Results* (1)

Matrix	Raw Ground Beef				Raw Chicken Wings			Shrimp			Cantaloupe		
Method	Level	ISO	MicroSEQ®		ISO	MicroSEQ®		ISO	MicroSEQ®		ISO	MicroSEQ®	
			Rapid Spin	NAE		Rapid Spin	NAE		Rapid Spin	NAE		Rapid Spin	NAE
Total +	Low	6/20	6/20	6/20	8/20	8/20	8/20	7/20	7/20	7/20	12/20	12/20	12/20
	High	18/20	18/20	18/20	12/20	12/20	12/20	16/20	16/20	16/20	13/20	13/20	13/20
	Control	0/5	0/5	0/5	N/A	N/A	N/A	0/5	0/5	0/5	0/5	0/5	0/5

Matrix	Brie Cheese				Dry Infant Formula			Chocolate			Shell Eggs		
Method	Level	ISO	MicroSEQ®		ISO	MicroSEQ®		ISO	MicroSEQ®		ISO	MicroSEQ®	
			Rapid Spin	NAE		Rapid Spin	NAE		Rapid Spin	NAE		Rapid Spin	NAE
Total +	Low	9/20	9/20	9/20	10/20	10/20	10/20	15/20	15/20	15/20	3/20	3/20	3/20
	High	18/20	18/20	18/20	19/20	19/20	19/20	18/20	18/20	18/20	12/20	12/20	12/20
	Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Matrix	Ground Black Pepper				Dry Pet Food		
Method	Level	ISO	MicroSEQ®		ISO	MicroSEQ®	
			Rapid Spin	NAE		Rapid Spin	NAE
Total +	Low	12/20	12/20	12/20	0/20	0/20	0/20
	High	18/20	18/20	18/20	8/20	8/20	8/20
	Control	0/5	0/5	0/5	0/5	0/5	0/5

*Results are confirmed positives.
Rapid Spin= Manual PrepSEQ™ Rapid Spin method
PrepSEQ NA= Automated PrepSEQ™ Nucleic Acid Extraction method

DISCUSSION OF MODIFICATION APPROVED JANUARY 2011 (12)

This matrix extension evaluation indicated that the MicroSEQ® Salmonella spp. Detection Method, using either the automated PrepSEQ™ NA Extraction Kit or the manual PrepSEQ™ Rapid Spin Sample Preparation Kit, successfully detected low numbers of Salmonella in sealed concrete, stainless steel, plastic, rubber and ceramic tile environmental surfaces. The results presented indicate that there were no statistically significant differences detected between the MicroSEQ Salmonella method (and both sample preparation methods) and the FDA BAM reference method.

For one environmental surface, sealed concrete, two false positive results were detected by the MicroSEQ Salmonella method using the PrepSEQ NA sample prep kit. The MME instrument used in this method had an instrumentation failure (head alignment error) that was repaired prior to proceeding with subsequent testing. The alignment issue was discovered after the preparation of the sealed concrete samples was completed and the preparation of the ceramic tile samples was initiated. The prepared samples for the sealed concrete were tested and the false positive results were detected. To prepare for subsequent retests, the elution plate was stored at -20°C until the repair was complete and the samples were then prepared for retesting 3 days.after those prepared by the PrepSEQ Rapid Spin Kit and assayed by the MicroSEQ method. The retest results were identical to the initial round of testing for the PrepSEQ NA prepared samples, detecting two false positives. The discrepancy observed was possibly due to cross contamination by the misaligned head on the MME while preparing samples in the deep well plate. The results were not statistically significant between the two sample preparation methods, however.

The MicroSEQ® Salmonella Detection Method is a reliable and rapid method for the detection of Salmonella in environmental samples, giving results as fast as 18 hours (including 16 h enrichment of samples). The lyophilized reagent format of the MicroSEQ® Salmonella spp. Detection Kit allows for a simpler, streamlined process for the end user to set up PCR samples. The 7500 Fast System provides rapid turnaround in less than an hour. The RapidFinder™ Express software is simple to use and provides guidance for the user through each step of PCR and instrument set up. Additionally, the RapidFinder™ Express software analyses the results automatically to enable the user to identify a positive Salmonella result at a glance, without the need to carry out manual interpretation.

Table C: Summary of Method Comparison Results* (12)

Matrix	**Sealed Concrete**				**Stainless Steel**			**Rubber**			**Plastic**		
Method	Level	FDA/BAM	MicroSEQ		FDA/BAM	MicroSEQ		FDA/BAM	MicroSEQ		FDA/BAM	MicroSEQ	
			Rapid Spin	Prep-SEQ NA		Rapid Spin	Prep-SEQ NA		Rapid Spin	Prep-SEQ NA		Rapid Spin	Prep-SEQ NA
Total +	Low	7/20	8/20	8/20	14/20	17/20	17/20	4/20	7/20	7/20	13/20	15/20	15/20
	Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Matrix	**Ceramic Tile**			
Method	Level	FDA/BAM	MicroSEQ	
			Rapid Spin	Prep-SEQ NA
Total +	Low	13/20	18/20	18/20
	Control	0/5	0/5	0/5

**Results are confirmed positives.*

Rapid Spin= Manual PrepSEQ™ Rapid Spin method

PrepSEQ NA= Automated PrepSEQ™ Nucleic Acid Extraction method

DISCUSSION OF MODIFICATION APPROVED MAY 2013 (13)

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, and are presented along with an overall summary of results for each matrix.

Table 1: Matrix Summary Table - Pathatrix®/MicroSEQ® Method vs. Reference Method (13)

Matrix	Time Point	Strain	MPN[a]/ Test Portion	N[c]	Pathatrix®/MicroSEQ® Method[i]			Reference Method			dPOD$_C$[g]	95% CI[h]
					x[d]	POD$_C$[e]	95% CI[h]	x	POD$_R$[f]	95% CI[h]		
Diced Tomatoes Vs. FDA/ BAM Chapter 5	18 Hour Primary Enrichment	*Salmonella enterica* subsp. Typhimurium ATCC 14028	0.00 (0.00, 0.18)	15	-	NA[b]	NA	0	0.00	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	0.00	-0.28, 0.28
			3.00 (1.30, 6.90)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Chocolate Enriched with NFDM Vs. FDA/ BAM Chapter 5	18 Hour Primary Enrichment	*Salmonella enterica* subsp. Montevideo QL024-19	0.00 (0.00, 0.18)	15	NA	NA	NA	0	0.00	0.00, 0.20	NA	NA
			0.90 (0.54, 1.50)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
			4.40 (1.70, 11.0)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Chocolate Enriched with UHTM Vs. FDA/ BAM Chapter 5	18 Hour Primary Enrichment	*Salmonella enterica* subsp. Montevideo QL024-19	0.00 (0.00, 0.18)	15	NA	NA	NA	0	0.00	0.00, 0.20	NA	NA
			0.90 (0.54, 1.50)	20	13	0.65	0.43, 0.82	14	0.70	0.48, 0.85	-0.05	-0.32, 0.23
			4.40 (1.70, 11.0)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Deli Ham Vs. USDA/ FSIS-MLG 4.05	18 Hour Primary Enrichment	*Salmonella enterica* subsp. Enteritidis ATCC 13076	0.00 (0.00, 0.18)	15	NA	NA	NA	0	0.00	0.00, 0.20	NA	NA
			0.43 (0.21, 0.75)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			2.30 (1.04, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[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

DISCUSSION OF MODIFICATION STUDY APPROVED NOVEMBER 2015 (14)

Results obtained during the study demonstrated that the MicroSEQ™ *Salmonella* spp. detection kit successfully detected *Salmonella* from 375 g samples of dried pet food. Using POD statistical analysis at 95% confidence levels, no statistically significant difference was observed between the number of positive samples detected by the MicroSEQ *Salmonella* spp. method or the FDA BAM reference method.

For the low level of contamination, there were 11 presumptive positive results with the MicroSEQ™ *Salmonella* spp. detection kit and 10 confirmed positive results once the FDA BAM reference method confirmation procedure had been performed (Table 1). In comparison there were 7 positive results for the low spiking level with the for the reference method. The high level of contamination, gave 5 presumptive positive results with the MicroSEQ *Salmonella* spp. method and 5 confirmed positive results with the FDA BAM Chapter 5 reference method confirmation procedure. In addition, the FDA BAM reference method identified 5 confirmed positive results for the high spiked samples. When comparing the presumptive and confirmed MicroSEQ method results a dPOD value of 0.05 was obtained. At the 95% confidence interval levels, the dPOD value showed there was no significant difference between the presumptive and confirmed MicroSEQ *Salmonella* spp. method results.

When comparing the MicroSEQ method results to the FDA-BAM reference method, there were 10 confirmed positive results for the PCR kit and 7 positive results with the reference method for the low level of contamination. This gave a dPOD value of 0.15 which at 95% confidence interval range of -0.15 to 0.41, indicating that there was no significant difference in performance between the MicroSEQ *Salmonella* spp. method and the FDA BAM reference method (Table 2). For the high level of contamination, a dPOD value of 0.00 was obtained for the statistical comparison of the MicroSEQ *Salmonella* spp. method confirmed and the FDA BAM reference method results. The 95% confidence interval range for this dPOD result was -0.43 to 0.43, indicating there was no statistically significant difference in the results for these two methods.

The MicroSEQ *Salmonella* spp. Detection Kit was demonstrated to be a simple and easy alternative method to perform, with the use of pre-warmed enrichment media allowing a user to begin testing in as little as 20 h following enrichment and incubation of the larger 375 g sample size. The PrepSEQ Rapid Spin Sample Preparation Kit simplifies DNA extraction, reducing the possibility of accidental contamination, compared with other sample preparation methods.

Table 1. MicroSEQ™ *Salmonella* spp. Detection Kit Presumptive result vs. Confirmed result – POD Analysis (14)

Matrix/Test Portion	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	MicroSEQ Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
375 g Dry pet food	*Salmonella* Typhimurium ATCC 14028	20 h	-	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.49 (0.25, 0.87)	20	11	0.55	(0.34, 0.74)	10	0.50	(0.30, 0.70)	0.05	(-0.24, 0.33)
			3.01 (1.31, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator version 1.6 provided by AOAC RI, with 95% confidence interval.
[b]N = Number of test portions.
[c]x = Number of positive test portions.
[d]POD$_{CP}$ = Candidate Presumptive method outcomes divided by the total number of trials.
[e]POD$_{CC}$ = Candidate confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 2. MicroSEQ™ *Salmonella* spp. Detection Kit Confirmed result vs. Reference Method result – POD Analysis

Matrix/Test Portion	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	MicroSEQ Confirmed			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
375 g Dry pet food	*Salmonella* Typhimurium ATCC 14028	20 h	-	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.49 (0.25, 0.87)	20	10	0.50	(0.30, 0.70)	7	0.35	(0.18, 0.57)	0.15	(-0.15, 0.41)
			3.01 (1.31, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator version 1.6 provided by AOAC RI, with 95% confidence interval.
[b]N = Number of test portions.
[c]x = Number of positive test portions.
[d]POD$_C$ = Presumptive Candidate method samples that confirmed positive divided by the total number of trials.
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

DISCUSSION OF MODIFICATION APPROVED AUGUST 2018 (15)

The purpose of this study was to compare performance of MagMAX Express-96 Deep Well and KingFisher Flex-96 Deep Well magnetic particle processors. Currently, users the assay are using MagMAX Express-96 during the sample preparation in their workflows. To provide an alternative sample preparation workflow, this study was set up to investigate the possibility to use KingFisher Flex-96 instruments with these kits. A successful comparison study could facilitate transfer of the protocols and provide evidence to AOAC-RI on the suitability of these protocols with KingFisher Flex-96 instrument.

Both instrument types produced similar results from both the tested assays in terms of number of positive calls returned from the sample set. When a sample set near or beyond the limit of detection of a method is analysed, variance is seen within the method but also between methods. In this study, the number of positive calls generated with MagMAX Express-96 instruments varied within the desired amount of positive results (2-8 from 10 test replicates), still indicating that these two instruments performed similarly. When the same sample sets were analysed with KingFisher Flex-96 instruments, not only did the amount of positive results remain similar with little difference but also variance within instrument type remained similar to the MagMAX Express-96 results further indicating the similarity between these two instruments. An instrument not reaching the fractional positivity level with this sample set would have indicated a significant difference in sensitivity between the nucleic acid extraction platforms and the total workflows between the instruments.

There was one case with KingFisher Flex-96 and MicroSEQ Salmonella species Detection Kit where one instrument returned nine positives results out of the ten replicate tested. All in all, the instruments returned comparable results in terms of amount of positives generated from the spiked samples.

Table 1. Results for *Salmonella* spiked samples ran with two MagMAX Express-96 and two KingFisher Flex-96 instruments and interpreted by RapidFinder Express 2.0 software using the MicroSEQ Salmonella species Detection Kit. (15)

Test replicate	*MagMAX Express-96*		*KingFisher Flex-96*	
	Instrument 1	*Instrument 2*	*Instrument 1*	*Instrument 2*
1	+	+	+	+
2	+	-	-	+
3	+	-	-	+
4	-	-	+	+
5	+	-	-	+
6	-	-	+	+
7	+	+	-	-
8	+	-	+	+
9	-	-	-	+
10	+	-	+	+
Σ Positive results	**7**	**2**	**5**	**9**

DISCUSSION OF THE MODIFICATION DECEMBER 2018 (16)

Samples were prepared near the limit of detection in ten replicates to compare the kit lots produced at the Austin, Texas and Vilnius, Lithuania manufacturing sites. Fractional positivity level (2-8) was reached with all kits and POD values were calculated for all targets. POD values of the kit lots from the old (Austin, Texas) and new (Vilnius, Lithuania) AmpliTaq™ UP manufacturing sites were evaluated through paired comparison. POD analysis showed that there is no statistical differences between lots produced at the Austin, Texas and Vilnius, Lithuania manufacturing sites.

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