



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

071001

The AOAC Research Institute hereby certifies the method known as:

MicroSEQ[®] *E. coli* O157:H7 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 (November 2, 2021 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

November 2, 2021

Date

METHOD AUTHORS ORIGINAL MODIFICATION: Lily Wong, Yan Cao, Manohar Furtado, and Robert Tebbs MODIFICATION AUGUST 2018: Life Technologies, part of Thermo Fisher Scientific MODIFICATION DECEMBER 2018: Tiina Karla	SUBMITTING COMPANY Applied Biosystems (Life Technologies) 850 Lincoln Centre Drive Foster City, CA 94404	CURRENT SPONSOR Life Technologies part of Thermo Fisher Scientific Wade Road Basingstoke, Hampshire RG24 8PW, United Kingdom
---	--	---

METHOD NAME MicroSEQ® <i>E. coli</i> O157:H7 Detection Kit	CATALOG NUMBERS 4427409, 4445656, 4445657, 4407760, 4426714, 4413269, 4426715
--	---

INDEPENDENT LABORATORY Rtech Laboratories, Land-O-Lakes 1200 W. County Road F. Arden Hills, MN 55112 USA	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ^{1,4} , Edward Richter ² , Wayne Ziemer ³ ¹ U.S. Food and Drug Administration, College Park, MD 20740 ² Richter International, Columbus, OH, USA ³ Consultant, Loganville, GA, USA ⁴ Modifications: August 2018 December 2018 Modification internal AOAC Research Institute review
--	--

APPLICABILITY OF METHOD Target organism – <i>E. coli</i> O157:H7 Matrixes – Raw ground beef (375 g, 25 g) , raw beef trim (375 g, 25g), spinach, orange juice, apple juice Performance claims - The MicroSEQ® method for testing ground beef and beef trim samples demonstrated sensitivity that was equivalent to the USDA MLG 5.04 culture-based reference method; and the MicroSEQ® method for testing spinach, orange juice and apple juice samples demonstrated sensitivity that was equivalent to the ISO 16654 culture-based reference method. The test method was found to be equivalent to the reference methods based on chi-square analysis.	REFERENCE METHODS ISO 16654:2001 (E) Microbiology of food and animal feeding stuffs - Horizontal method for the detection of <i>Escherichia coli</i> O157 2001. First Edition (8) USDA Microbiology Laboratory Guidebook <i>Online</i> , Section 5.04: Detection, Isolation and Identification of <i>Escherichia coli</i> O157:H7 from Meat Products (January 2008) (9)
---	--

ORIGINAL CERTIFICATION DATE July 14, 2010	CERTIFICATION RENEWAL RECORD Renewed annually through December 2022.
---	--

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. December 2017 Level 1	1. Editorial changes on insert and labels.
2. August 2018 Level 2	2. Equivalency study for KingFisher™ Flex-96 Deep Well Magnetic Particle Processor.
3. December 2018 Level 2	3. Location change of critical raw materials from Austin, Texas to Vilnius, Lithuania.
4. December 2018 Level 1	4. User Guide updated with editorial corrections and inclusion of AOAC RI workflow.
5. November 2019 Level 1	5. Editorial/clerical changes.
6. November 2020 Level 1	6. Clerical changes to label.
7. November 2021 Level 1	7. Discontinued catalog number 44445654.

Under this AOAC® <i>Performance Tested</i> SM License Number, 071001 this method is distributed by: NONE	Under this AOAC® <i>Performance Tested</i> SM License Number, 071001 this method is distributed as: NONE
--	--

Thermocyclers characteristics to run the Real-Time PCR:
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries with the following characteristics:

Characteristics	7500 Fast Real-Time PCR Instrument
Optics	12v 75w halogen bulb
Filters	5 excitation and 5 emission filters
Sample ramp rate	Standard mode: ± 1.6°C/sec Fast mode: ± 3.5°C/sec
Thermal range	4-100°C
Thermal accuracy	± 0.5°C
Thermal uniformity	± 1°C
Format	96-well, 0.1-mL block

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 [5] 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 the 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

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 reads the individual fluorescent signals and displays them in an intuitive, easy-to-read, color-coded format.

DISCUSSION OF THE VALIDATION STUDY (1)

The independent validation studies taken together with the internal validation studies demonstrate that the MicroSEQ® *E. coli* O157:H7 Detection kit and the USDA MLG 5.04 reference method are statistically similar for detection of *E. coli* O157:H7 in raw ground beef and beef trim; and that the MicroSEQ *E. coli* O157:H7 Detection Kit and the ISO 16654 reference method are statistically similar for detection of *E. coli* O157:H7 in spinach, apple juice, and orange juice. The data in these studies, within their statistical uncertainty, support the product claims of the MicroSEQ® *E. coli* O157:H7 Detection Kit method in the food matrixes tested.

Table 4: Inclusivity Summary of Results (1)

Organism Reference Number	Source	MicroSEQ® Results	Organism Reference Number	Source	MicroSEQ Results	Organism Reference Number	Source	MicroSEQ Results
<i>Escherichia coli</i> O157:H7 MSU# TW00116	Human	Positive	<i>Escherichia coli</i> O157:H7 ATCC# 43895	Raw Hamburger	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC4E	Human	Positive
<i>Escherichia coli</i> O157:H7 MSU# TW00975	Human	Positive	<i>Escherichia coli</i> O157:H7 ATCC# 51657	Clinical Isolate	Positive	<i>Escherichia coli</i> O157:H7 QL# 164673	Ground Beef	Positive
<i>Escherichia coli</i> O157:H7 MSU# TW02302	Hamburger	Positive	<i>Escherichia coli</i> O157:H7 ATCC# 51658	Clinical Isolate	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-202	Ground Beef	Positive
<i>Escherichia coli</i> O157:H7 MSU# TW04863	human	Positive	<i>Escherichia coli</i> O157:H7 ATCC# 51659	Clinical Isolate	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-203	Ground Beef	Positive
<i>Escherichia coli</i> O157:H7 MSU# TW05356	Human	Positive	<i>Escherichia coli</i> O157:H7 ATCC# 700531	Clinical Isolate	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-204	Ground Beef	Positive
<i>Escherichia coli</i> O157:H7 MSU# TW07587	Human	Positive	<i>Escherichia coli</i> O157:H7 ATCC# 700599	Salami	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-205	Ground Beef	Positive
<i>Escherichia coli</i> O157:H7 ATCC# BAA-460	Human Feces	Positive	<i>Escherichia coli</i> O157:H7 ATCC# 700728	Not Available	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-206	Ground Beef	Positive
<i>Escherichia coli</i> O157:H7 NCTC# 12900	Not Available	Positive	<i>Escherichia coli</i> O157:H7 ATCC# 700927	Not Available	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-207	Ground Beef	Positive
<i>Escherichia coli</i> O157:H7 NCTC# 13125	Not Available	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC3A	Human	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-214	Beef Trim	Positive
<i>Escherichia coli</i> O157:H7 NCTC# 13126	Not Available	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC3B	Human	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-370	Beef Trim	Positive
<i>Escherichia coli</i> O157:H7 NCTC# 13127	Not Available	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC3C	Human	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-701	Beef Trim	Positive
<i>Escherichia coli</i> O157:H7 NCTC# 13128	Not Available	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC3D	Human	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-704	Beef Trim	Positive
<i>Escherichia coli</i> O157:H7 ATCC# 35150	Human Feces	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC3E	Human	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-705	Beef Trim	Positive

<i>Escherichia coli</i> O157:H7 ATCC# 43888	Human Feces	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC4A	Cow	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-706	Beef Trim	Positive
<i>Escherichia coli</i> O157:H7 ATCC# 43889	Human Feces	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC4B	Human	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-707	Beef Trim	Positive
<i>Escherichia coli</i> O157:H7 ATCC# 43890	Human Feces	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC4C	Buffalo	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-708	Beef Trim	Positive
<i>Escherichia coli</i> O157:H7 ATCC# 43894	Human Feces	Positive	<i>Escherichia coli</i> O157:H7 MSU# DEC4D	Cow	Positive	<i>Escherichia coli</i> O157:H7 QL# 2-710	Beef Trim	Positive

Table 5: Exclusivity Summary of Results (1)

Organism Reference Number	Source	MicroSEQ Results	Organism Reference Number	Source	MicroSEQ Results	Organism Reference Number	Source	MicroSEQ Results
<i>Escherichia coli</i> O26:H11 MSU# DEC10E	Cow	Negative	<i>Escherichia coli</i> O121 NCTC# 9121	Not Available	Negative	<i>Alcaligenes faecalis</i> <i>subsp. faecalis</i> ATCC# 8750	Not Available	Negative
<i>Escherichia coli</i> O55:H6 MSU# DEC1A	Human	Negative	<i>Escherichia coli</i> O142 NCTC# 10089	Not Available	Negative	<i>Bacillus cereus</i> ATCC# 11778	Not Available	Negative
<i>Escherichia coli</i> O91 NCTC# 9091	Not Available	Negative	<i>Escherichia coli</i> O145 NCTC# 10279	Not Available	Negative	<i>Candida albicans</i> ATCC# 10231	Human	Negative
<i>Escherichia coli</i> O103 NCTC# 8196	Not Available	Negative	<i>Escherichia coli</i> O146 NCTC# 10677	Not Available	Negative	<i>Citrobacter freundii</i> ATCC# 8090	Not Available	Negative
<i>Escherichia coli</i> O113 NCTC# 9113	Not Available	Negative	* <i>Escherichia coli</i> O157:NM ATCC# 700376	Human Feces	Positive	<i>Enterobacter aerogenes</i> ATCC# 13048	Sputum	Negative
<i>Escherichia coli</i> O118 NCTC# 9118	Not Available	Negative	<i>Escherichia coli</i> O163 NCTC# 11021	Feces	Negative	<i>Edwardsiella tarda</i> ATCC# 15947	Human Feces	Negative
<i>Escherichia coli</i> O111:H12 MSU# DEC6A	Human	Negative	<i>Escherichia blattae</i> ATCC# 29907	Cockroach	Negative	<i>Klebsiella pneumoniae</i> <i>subsp. pneumonia</i> ATCC# 4352	Cow's Milk	Negative
<i>Escherichia coli</i> O111:H8 MSU# DEC6C	Human	Negative	<i>Escherichia fergusonii</i> ATCC# 35469	Human Feces	Negative	<i>Microbacterium testaceum</i> ATCC# 15829	Paddy	Negative
<i>Escherichia coli</i> O115 NCTC# 10444	Calf	Negative	<i>Escherichia hermannii</i> ATCC# 33650	Human Toe	Negative	<i>Pseudomonas aeruginosa</i> ATCC# 9027	Outer Ear Infection	Negative
<i>Escherichia coli</i> O117 NCTC# 9117	Not Available	Negative	<i>Escherichia vulneris</i> ATCC# 29943	Human Wound	Negative	<i>Salmonella enterica</i> <i>subsp. enterica</i> serovar Choleraesuis ATCC# 10708	Not Available	Negative

Table 22: MicroSEQ *E. coli* O157:H7 Detection Kit Summary Data Table (1)

Inoculation Level	Inoculating Organism	MPN /25g	Total Samples	Total + Samples	MicroSEQ <i>E. coli</i> O157:H7 Method				USDA 5.04	X ² (PrepSEQ vs. USDA 5.04)	X ² (Rapid Spin vs. USDA 5.04)	Sensitivity Rate		False Negative Rate		False Positive Rate	
					PrepSEQ NA Pres.	PrepSEQ NA Conf.	Rapid Spin Pres.	Rapid Spin Conf.				PrepSEQ NA	Rapid Spin	PrepSEQ NA	Rapid Spin	PrepSEQ NA	Rapid Spin
Ground Beef (25g) 6hr Enrichment																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 43895	0.52	20	17	11	11	11	11	15	1.71	1.71	73	73	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 43895	7.0	20	20	19	19	19	19	20	1.0	1.0	95	95	0.0	0.0	0.0	0.
Ground Beef (25g) 16hr Enrichment																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 43895	0.52	20	18	15	15	15	15	15	0.0	0.0	100	100	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 43895	7.0	20	20	20	20	20	20	20	0.0	0.0	100	100	0.0	0.0	0.0	0.0
Ground Beef (375g) 16hr Enrichment																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 43895	0.58	20	4	3	3	3	3	1	1.08	1.08	300	300	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 43895	2.3	20	17	13	13	13	13	14	0.43	0.11	86	93	8.0	0.0	14	0.0

Table 22: MicroSEQ *E. coli* O157:H7 Detection Kit Summary Data Table cont'd (1)

Inoculation Level	Inoculating Organism	MPN /25g	Total Samples	Total + Samples	MicroSEQ <i>E. coli</i> O157:H7 Method				USDA 5.04	X ² (PrepSEQ vs. USDA 5.04)	X ² (RapidSpin vs. USDA 5.04)	Sensitivity Rate		False Negative Rate		False Positive Rate	
					Automated PrepSEQ NA Pres.	Automated PrepSEQ NA Conf.	Rapid Spin Pres.	RapidSpin Conf.				Automated NA PrepSEQ	Rapid Spin	Automated NA PrepSEQ	Rapid Spin	Automated NA PrepSEQ	Rapid Spin
Beef Trim (25g) 6hr Enrichment																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 35150	1.1	20	18	14	14	14	14	13	0.11	0.11	108	108	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 35150	27.5	20	20	20	20	20	20	20	0.0	0.0	100	100	0.0	0.0	0.0	0.0
Beef Trim (25g) 16hr Enrichment																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 35150	1.1	20	15	12	12	12	12	13	0.44	0.44	92	92	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 35150	27.5	20	20	20	20	20	20	20	0.0	0.0	100	100	0.0	0.0	0.0	0.0
Beef Trim (375g) 16hr Enrichment																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 35150	1.1	20	16	16	16	16	16	13	1.1	1.1	123	123	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 35150	27.5	20	20	20	20	20	20	20	0.0	0.0	100	100	0.0	0.0	0.0	0.0

Table 22: MicroSEQ *E. coli* O157:H7 Detection Kit Summary Data Table cont'd (1)

Inoculation Level	Inoculating Organism	MPN /25g	Total Samples	Total + Samples	MicroSEQ <i>E. coli</i> O157:H7 Method				ISO 16654 Automated NA PrepSEQ	X ² (PrepSEQ vs. ISO 16654) Rapid Spin	X ² (Rapid Spin vs. ISO 16654)	Sensitivity Rate		False Negative Rate		False Positive Rate	
					Automated PrepSEQ NA Presumptive	Automated PrepSEQ NA Confirmed	Rapid Spin Presumptive	Rapid Spin Confirmed				Automated NA PrepSEQ	Rapid Spin	Automated NA PrepSEQ	Rapid Spin	Automated NA PrepSEQ	Rapid Spin
Orange Juice (25g) 8hr Enrichment vs. 24 hr ISO 16654																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	0.23	20	6	3	3	3	3	3	0.0	0.0	100	100	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	2.3	20	20	16	16	16	16	13	1.1	1.1	123	123	0.0	0.0	0.0	0.0
Orange Juice (25g) 16hr Enrichment vs. 24 hr ISO 16654																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	0.23	20	17	16	16	16	16	10	3.86	3.86	160	160	0.0	0.0	0.0	0.0
2-5CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	6.0	20	20	20	20	20	20	20	0.0	0.0	100	100	0.0	0.0	0.0	0.0

Table 22: MicroSEQ *E. coli* O157:H7 Detection Kit Summary Data Table cont'd (1)

Inoculation Level	Inoculating Organism	MPN /25g	Total Samples	Total + Samples	MicroSEQ <i>E. coli</i> O157:H7 Method				ISO 16654	X ² (PrepSEQ vs. ISO 16654)	X ² (Rapid Spin vs. ISO 16654)	Sensitivity Rate		False Negative Rate		False Positive Rate	
					Automated PrepSEQ NA Presumptive	Automated PrepSEQ NA Confirmed	Rapid Spin Presumptive	Rapid Spin Confirmed				Automated NA PrepSEQ	Rapid Spin	Automated NA PrepSEQ	Rapid Spin	Automated NA PrepSEQ	Rapid Spin
Spinach (25g) 6hr Enrichment vs. 6 hr ISO 16654																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	0.09	20	1	0	0	0	0	1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	2.3	20	15	12	12	12	12	8	1.56	1.56	150	150	0.0	0.0	0.0	0.0
Spinach (25g) 6hr Enrichment vs. 24 hr ISO 16654																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	0.0	0.0
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	0.09	20	2	0	0	0	0	2	2.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	2.3	20	16	12	12	12	12	10	0.39	0.39	120	120	0.0	0.0	0.0	0.0
Spinach (25g) 16hr Enrichment vs. 24 hr ISO 16654																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	-	-
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	0.09	20	4	2	2	2	2	2	0.0	0.0	100	100	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700599	2.3	20	13	10	10	11	10	10	0.0	0.0	100	100	0.0	0.0	0.0	10

Table 22: MicroSEQ <i>E. coli</i> O157:H7 Detection Kit Summary Data Table cont'd (1)																	
Inoculation Level	Inoculating Organism	MPN /25g	Total Samples	Total + Samples	MicroSEQ <i>E. coli</i> O157:H7 Method				ISO 16654	χ ² (PrepSEQ vs. ISO 16654)	χ ² (Rapid Spin vs. ISO 16654)	Sensitivity Rate		False Negative Rate		False Positive Rate	
					Automated PrepSEQ NA Presumptive	Automated PrepSEQ NA Confirmed	Rapid Spin Presumptive	Rapid Spin Confirmed				Automated NA PrepSEQ	Rapid Spin	Automated NA PrepSEQ	Rapid Spin	Automated NA PrepSEQ	Rapid Spin
Apple Juice (25g) 16hr Enrichment vs. 24 hr ISO 16654																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	-	-
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700728	0.23	20	13	11	11	11	11	9	0.39	0.39	122	122	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700728	11.5	20	20	20	20	20	20	20	0.0	0.0	100	100	0.0	0.0	0.0	0.0
Apple Juice (25g) 8hr Enrichment vs. 24 hr ISO 16654																	
Control	N/A	<0.075	5	0	0	0	0	0	0	-	-	-	-	-	-	-	-
0.2-2 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700728	0.38	20	7	3	3	3	3	4	0.16	0.16	75	75	0.0	0.0	0.0	0.0
2-5 CFU/25g	<i>E. coli</i> O157:H7 ATCC 700728	1.1	20	14	10	10	10	10	9	0.9	0.9	111	111	0.0	0.0	0.0	0.0

DISCUSSION OF MODIFICATION AUGUST 2018 (14)

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. The instruments returned comparable results in terms of amount of positives generated from the spiked samples. None of the *E. coli* spiked samples gave positive results with any of the tested instruments.

DISCUSSION OF THE MODIFICATION DECEMBER 2018 (15)

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.

REFERENCES CITED

1. Wong, Lily, Cao, Yan., Furtado, Manohar, and Tebbs, Robert., Evaluation of the MicroSEQ® *E. coli* O157:H7 Detection Kit, AOAC® Performance TestedSM certification number 071001.
2. AOAC Research Institute Validation Outline for MicroSEQ® *E. coli* O157:H7 Detection Kit, Approved – July 2010.
3. Feldsine, P., Abeyta, C., & Andrews, W. H. (2002) *J. AOAC International*. **85** (5):1187-1200.
4. Livak, K.J., Flood, S.J., Marmaro, J., Giusti, W., & Deetz, K. (1995) *PCR Methods Appl.* **4** (6):357-362.
5. Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., & Arnheim, N. (1985) *Science*. **230**, 1350-1354.
6. Lim, J.Y., Yoon, J.W and Hovde, C.J. (2010) *J. Microbiol. Biotechnol.* **20** (1): 1-10.
7. Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe, R.V. (1999) *Emerg. Infect. Dis.* **5** (5): 607-625.
8. ISO 16654:2001 (E) Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 2001. First Edition
9. USDA Microbiology Laboratory Guidebook Online, Section 5.04: Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products (January 2008)
10. Anderson, G. & Scott, M. (1991) *Clinical Chemistry*. **37** (3):398-402.
11. U.S. Food and Drug Administration, *Bacteriological Analytical Manual Online* (January 2001) Appendix 1, *Rapid Methods for Detecting Foodborne Pathogens*, <http://www.cfsan.fda.gov/~ebam/bam-a1.html>.
12. Maddox, C.W. (2003) in *Microbial Food Safety in Animal Agriculture: Current Topics*, M.E. Torrence & R.E. Isaacson (Eds), Blackwell Publishing Co., pp 83-88
13. Johansson, A., Berglind, L., Eriksson, U., Goransson, I., Wollin, R., Forsman, M., Tarnvik, A. & Sjostedt, A. (2000), *J. Clin. Microbiol.*, **38**, 22-26.
14. Life Technologies, Inc., Evaluation of Proposed change to MicroSEQ™ Salmonella species, MicroSEQ *E. coli* O157:H7, MicroSEQ *Listeria monocytogenes*, MicroSEQ *Listeria* species and RapidFinder STEC workflows, AOAC® Performance TestedSM certification number 031001 Approved August 2018
15. Karla, T., Evaluation of Thermo Scientific™ RapidFinder™ STEC and MicroSEQ™ assays: Critical raw material manufacturing site change, AOAC® Performance TestedSM certification number 071001 Approved December 2018