



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

061602

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

Applied BiosystemsTM RapidFinderTM STEC Detection Workflow

manufactured by

Thermo Fisher Scientific

Wade Road

Basingstoke, Hampshire RG24 8PW

UK

This method has been evaluated in the AOAC[®] *Performance Tested MethodsSM* 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 TestedSM* 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.

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

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 12, 2018

Date

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MODIFICATION AUGUST 2018: Life Technologies, part of Thermo Fisher Scientific

MODIFICATION DECEMBER 2018: Tiina Karla

SUBMITTING COMPANY

Thermo Fisher Scientific
Wade Road
Basingstoke, Hampshire, RG24 8PW UK

KIT NAME(S)

Applied Biosystems™ RapidFinder™ STEC Detection Workflow

CATALOG NUMBERS

4476886, 4476901, 4480466, 4428176, 4426715

INDEPENDENT LABORATORY

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December 2018 Modification internal AOAC Research Institute review

APPLICABILITY OF METHOD

Target organism – *E. coli* O157:H7 and “Big Six” non-*E. coli* STEC serotypes (O26, O45, O103, O111, O121, and O145)

Matrices – FSIS MLG 5.09 – fresh raw ground beef (73% lean, 375 g)

FSIS MLG 5B.05 – fresh raw beef trim (375 g)

Performance claims – Performance equivalent to that of the reference methods.

REFERENCE METHODS

USDA/FSIS-MLG 5.09: Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges. January, 2015. (3)

USDA/FSIS-MLG 5B.05: Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges. June 2014. (4)

ORIGINAL CERTIFICATION DATE

June 20, 2016

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2019

METHOD MODIFICATION RECORD

1. December 2017 Level 1
2. August 2018 Level 2
3. December 2018 Level 2

SUMMARY OF MODIFICATION

1. Updates and edits to IFUs and labels
2. Equivalency study for KingFisher™ Flex-96 Deep Well Magnetic Particle Processor
3. Location change of critical raw materials from Austin, Texas to Vilnius, Lithuania.

Under this AOAC® Performance TestedSM License Number, 061602 this method is distributed by:

NONE

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NONE

PRINCIPLE OF THE METHOD (1)

The Applied Biosystems™ RapidFinder™ STEC Detection Workflow is a suite of two kits, which, following enrichment of samples in pre-warmed (48 °C) Tryptone Soya Broth (TSB), utilize TaqMan™ Real-Time PCR technology (6). This provides two levels of specificity for confident pathogen detection by combining Polymerase Chain Reaction (PCR) amplification 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 target organism or serotype within 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 genetic signature for specific DNA sequences that are unique to the particular target serotypes of pathogenic *E. coli* have been recognized.

In addition, the RapidFinder Pathogen Detection System contains an Internal Positive Control (IPC) within the reaction mix to monitor for the presence of PCR inhibitors. Sufficient 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 within the laboratory, which could otherwise lead to false positive results.

The Real-Time PCR assay is lyophilized to improve ease of use and reproducibility by reducing the number of pipetting steps and allowing for the addition of a higher volume of sample (30 µL) to the reaction pellet. Two sample preparation methods were developed to meet the needs of testing both small (PrepSEQ™ Rapid Spin Sample Preparation Kit) and high sample numbers (PrepSEQ™ Nucleic Acid Extraction Kit).

The Applied Biosystems™ RapidFinder™ 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, RapidFinder™ 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 and negative results are displayed by the software, following an automated assessment of the individual results for the *stx*₁, *stx*₂, *eae*, O157:H7 targets and the IPC amplification result. Positive signals result in an increase of the target-specific fluorescent signal while the fluorescence of negative signals remains below the threshold applied by RapidFinder Express Software. When the reactions are complete, RapidFinder Express Software interprets the individual fluorescence signals and displays the result as an intuitive, easy-to-read, color-coded presence (+) or absence (-) format.

The PrepSEQ™ Nucleic Acid Extraction Kit and the Applied Biosystems™ MagMAX™ Express deep Well Magnetic Particle Processor enable high throughput sample processing in a 96-well format with minimal analyst handling to prepare PCR-ready DNA. The PrepSEQ™ Rapid Spin Kit is designed to enable the low to medium throughput of samples for PCR analysis.

Real-Time PCR detection using the STEC Screening Assay includes primers and probes for the detection of *stx*₁, *stx*₂, *eae* and *E. coli* O157:H7 gene targets. Samples positive for *stx* and *eae* targets with the RapidFinder STEC Screening kit should be analyzed with the RapidFinder STEC Confirmation kit to determine if one of the “Big 6” serotypes of *E. coli* is present or not. The RapidFinder STEC Confirmation Assay is designed to confirm the presence of the “Big 6” non-O157 STEC serotypes following a positive result for *stx* and *eae* with the RapidFinder STEC Screening kit.

The RapidFinder STEC Detection Workflow assays are provided in a lyophilized PCR bead format, which includes primers, probes and internal positive controls to monitor for PCR inhibition and demonstrate that the PCR amplification process has been successful. DNA polymerase enzyme and other buffer components necessary for real-time PCR are included in the test kit. The RapidFinder Express Software is a graphical easy-to-use tool that provides step-by-step instructions to set up the real time PCR assays on the Applied Biosystems 7500 Fast Real-Time PCR instrument followed by automated data analysis.

DISCUSSION OF THE VALIDATION STUDY (1)

The data obtained during these studies, within their statistical uncertainty, support the product claims made for the Applied Biosystems RapidFinder STEC Detection workflow which is designed to enable the detection and identification of *E. coli* O157:H7 and the “big 6” STEC in both raw beef trim and raw ground beef matrices. POD analysis of the data during the validation study demonstrated that there was no statistically significant difference between the number of positive samples detected by the RapidFinder STEC Detection workflow and the USDA FSIS MLG 5.09 and 5B.05 reference methods for raw ground beef and raw beef trim.

Table 3: RapidFinder STEC Screening Kit Inclusivity Results (1)

Organism	Serotype	Source ¹	Origin	Screening Kit Result
<i>Escherichia coli</i>	O157:H7	ATCC® 35150™	Human feces	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 12900	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 13126	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 13127	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 13128	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 35150	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	ATCC 43895	Raw Hamburger	Positive
<i>Escherichia coli</i>	O157:H7	ATCC 51657	Clinical Isolate	Positive
<i>Escherichia coli</i>	O157:H7	ATCC 51658	Clinical Isolate	Positive
<i>Escherichia coli</i>	O157:H7	ATCC 51659	Clinical Isolate	Positive
<i>Escherichia coli</i>	O157:NM	NCTC13125	Human Feces	Positive
<i>Escherichia coli</i>	O157:NM	ATCC 700376	Human Feces	Positive
<i>Escherichia coli</i>	O26	MSU TW04270	Human	Positive
<i>Escherichia coli</i>	O26	MSU TW04284	Human Child	Positive
<i>Escherichia coli</i>	O26	MSU TW08031	Human	Positive
<i>Escherichia coli</i>	O26:H11	ATCC BAA-1653	Ice cream	Positive
<i>Escherichia coli</i>	O26	MSU TW07814	Human	Positive
<i>Escherichia coli</i>	O26:H11	MSU DEC10E	Not Available	Positive
<i>Escherichia coli</i>	O45	MSU TW10121	Human	Positive
<i>Escherichia coli</i>	O45	MSU TW14003	Human	Positive
<i>Escherichia coli</i>	O45	MSU TW07947	Human	Positive
<i>Escherichia coli</i>	O45	MSU DEC 11C	Human	Positive
<i>Escherichia coli</i>	O45	UPENN 1.2622	Human	Positive
<i>Escherichia coli</i>	O45	UPENN 11.1079	Human feces	Positive
<i>Escherichia coli</i>	O55:H6	MSU DEC 1A	Not Available	Positive
<i>Escherichia coli</i>	O103	MSU TW09101	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW07971	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW11239	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW07697	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW05997	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW08101	Human	Positive
<i>Escherichia coli</i>	O111:H12	MSU DEC 6A	Human Infant	Positive
<i>Escherichia coli</i>	O111:H8	MSU DEC 6C	Human	Positive
<i>Escherichia coli</i>	O111	MSU DEC 8D	Human	Positive
<i>Escherichia coli</i>	O111	MSU TW07926	Human	Positive
<i>Escherichia coli</i>	O111	MSU TW06296	Human Infant	Positive
<i>Escherichia coli</i>	O111	MSU DEC12A	Human Child	Positive
<i>Escherichia coli</i>	O121	NCTC 9121	Human	Positive
<i>Escherichia coli</i>	O121	UPENN 5.0959	Not Available	Positive
<i>Escherichia coli</i>	O121	UPENN 7.1686	Not Available	Positive
<i>Escherichia coli</i>	O121	UPENN 7.1709	Not Available	Positive
<i>Escherichia coli</i>	O121	UPENN 10.0709	Not Available	Positive
<i>Escherichia coli</i>	O121	MSU TW07931	Human Isolate	Positive
<i>Escherichia coli</i>	O145	NCTC 10279	Not Available	Positive
<i>Escherichia coli</i>	O145	MSU TW09153	Human	Positive
<i>Escherichia coli</i>	O145	MSU TW07596	Human	Positive
<i>Escherichia coli</i>	O145	MSU TW09356	Human	Positive
<i>Escherichia coli</i>	O145	MSU TW01664	Human	Positive
<i>Escherichia coli</i>	O145	UPENN 7.1711	Human feces	Positive
<i>Escherichia coli</i>	O163	NCTC 11021	Human	Positive

¹ATCC- American Type Culture Collection; NCTC- National Type Culture Collection; MSU- Michigan State University; UPENN- University of Pennsylvania.

Table 4: RapidFinder STEC Screening Kit Exclusivity Results (1)

Organism	Source ¹	Origin	Assay Result
<i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	ATCC 8750	Not Available	Negative
<i>Citrobacter freundii</i>	ATCC 8090	Not Available	Negative
<i>Cronobacter sakazakii</i>	ATCC 29544	Child's throat	Negative
<i>Edwardsiella tarda</i>	ATCC 15947	Human feces	Negative
<i>Enterobacter aerogenes</i>	ATCC 13048	Sputum	Negative
<i>Escherichia blattae</i>	ATCC 29907	Insect	Negative
<i>Bordetella bronchiseptica</i>	ATCC 4617	Lung of Dog	Negative
<i>Brochothrix thermosphacta</i>	ATCC 11509	Animal-derived foodstuff	Negative
<i>Citrobacter farmeri</i>	ATCC 51633	Human feces	Negative
<i>Escherichia coli</i> O91	NCTC 9091	Clinical Isolate	Negative
<i>Escherichia coli</i>	ATCC 8739	Feces	Negative
<i>Kluyvera intermedia</i>	ATCC 33110	Surface water	Negative
<i>Escherichia coli</i> O113	NCTC 9113	Not Available	Negative
<i>Escherichia coli</i> O115	NCTC 10444	Mammal, Calf	Negative
<i>Escherichia coli</i> O117	NCTC 9117	Not Available	Negative
<i>Escherichia coli</i> O118	NCTC 9118	Not available	Negative
<i>Rahnella aquatilis</i>	ATCC 55046	Soil	Negative
<i>Escherichia coli</i> O142	NCTC 10089	Not Available	Negative
<i>Shigella boydii</i>	ATCC 9207	Pork liver	Negative
<i>Escherichia coli</i> O146	NCTC 10677	Not Available	Negative
<i>Shigella sonnei</i>	ATCC 9290	Human feces	Negative
<i>Escherichia fergusonii</i>	ATCC 35469	Feces, human	Negative
<i>Escherichia hermanii</i>	ATCC 33650	Mouse Brain	Negative
<i>Escherichia vulneris</i>	ATCC 29943	Human Wound	Negative
<i>Hafnia alvei</i>	ATCC 51815	Milk	Negative
<i>Klebsiella pneumoniae</i> subsp. <i>pneumonia</i>	ATCC 4352	Cow's Milk	Negative
<i>Microbacterium testaceum</i>	ATCC 15829	Paddy	Negative
<i>Pantoea agglomerans</i>	ATCC 19552	Sewage	Negative
<i>Pseudomonas aeruginosa</i>	ATCC 9027	Outer Ear Infection	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Choleraesuis</i>	ATCC 10708	Not Available	Negative

¹ATCC- American Type Culture Collection; NCTC- National Type Culture Collection.

Table 5: RapidFinder STEC Confirmation Kit Inclusivity Results (1)

Organism	Serotype	Source ¹	Origin	Confirmation Kit Result
<i>Escherichia coli</i>	O157:H7	ATCC 35150	Human feces	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 12900	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 13126	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 13127	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 13128	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	NCTC 35150	Not Available	Positive
<i>Escherichia coli</i>	O157:H7	ATCC 43895	Raw Hamburger	Positive
<i>Escherichia coli</i>	O157:H7	ATCC 51657	Clinical Isolate	Positive
<i>Escherichia coli</i>	O26	MSU TW04270	Human	Positive
<i>Escherichia coli</i>	O26	MSU TW04284	Human Child	Positive
<i>Escherichia coli</i>	O26	MSU TW08031	Human	Positive
<i>Escherichia coli</i>	O26:H11	ATCC BAA-1653	Ice cream	Positive
<i>Escherichia coli</i>	O26	MSU TW07814	Human	Positive
<i>Escherichia coli</i>	O26:H11	MSU DEC10E	Not Available	Positive
<i>Escherichia coli</i>	O26	MSU TW00971	Feces, Human	Positive
<i>Escherichia coli</i>	O45	MSU TW10121	Human	Positive
<i>Escherichia coli</i>	O45	MSU TW14003	Human	Positive
<i>Escherichia coli</i>	O45	MSU TW07947	Human	Positive
<i>Escherichia coli</i>	O45	MSU DEC 11C	Human	Positive
<i>Escherichia coli</i>	O45	UPENN 1.2622	Human	Positive
<i>Escherichia coli</i>	O45	UPENN 6.3127	Not Available	Positive
<i>Escherichia coli</i>	O45	UPENN 11.1079	Human feces	Positive
<i>Escherichia coli</i>	O103	MSU TW09101	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW07971	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW11239	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW07697	Human	Positive
<i>Escherichia coli</i>	O103	MSU TW05997	Human	Positive
<i>Escherichia coli</i>	O103	UPENN 5.1658	Human, Feces	Positive
<i>Escherichia coli</i>	O103	UPENN 7.1691	Human, Feces	Positive
<i>Escherichia coli</i>	O111:H12	MSU DEC 6A	Human Infant	Positive
<i>Escherichia coli</i>	O111:H8	MSU DEC 6C	Human	Positive
<i>Escherichia coli</i>	O111	MSU DEC 8D	Human	Positive
<i>Escherichia coli</i>	O111	MSU TW07926	Human	Positive
<i>Escherichia coli</i>	O111	MSU TW00186	Human	Positive
<i>Escherichia coli</i>	O111	MSU TW01387	Human	Positive
<i>Escherichia coli</i>	O111	MSU TW07502	Human	Positive
<i>Escherichia coli</i>	O121	NCTC 9121	Human	Positive
<i>Escherichia coli</i>	O121	UPENN 5.0959	Not Available	Positive
<i>Escherichia coli</i>	O121	UPENN 7.1686	Not Available	Positive
<i>Escherichia coli</i>	O121	UPENN 7.1709	Not Available	Positive
<i>Escherichia coli</i>	O121	UPENN 10.0709	Not Available	Positive
<i>Escherichia coli</i>	O121	MSU TW07614	Human Isolate	Positive
<i>Escherichia coli</i>	O121	MSU TW08023	Human Isolate	Positive
<i>Escherichia coli</i>	O145	NCTC 10279	Not Available	Positive
<i>Escherichia coli</i>	O145	MSU TW09153	Human	Positive
<i>Escherichia coli</i>	O145	MSU W07596	Human	Positive
<i>Escherichia coli</i>	O145	MSU TW09356	Human	Positive
<i>Escherichia coli</i>	O145	MSU TW01664	Human	Positive
<i>Escherichia coli</i>	O145	UPENN 10.0707	Human Isolate	Positive
<i>Escherichia coli</i>	O145	UPENN 10.0708	Human Isolate	Positive

¹ATCC- American Type Culture Collection; NCTC- National Type Culture Collection; MSU- Michigan State University; UPENN- University of Pennsylvania.

Table 6: RapidFinder STEC Confirmation Kit Exclusivity Results (1)

Species	Source ¹	Origin	Confirmation Kit Result
<i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	ATCC 8750	Not Available	Negative
<i>Citrobacter freundii</i>	ATCC 8090	Not Available	Negative
<i>Cronobacter sakazakii</i>	ATCC 29544	Child's throat	Negative
<i>Edwardsiella tarda</i>	ATCC 15947	Feces, human	Negative
<i>Enterobacter aerogenes</i>	ATCC 13048	Sputum	Negative
<i>Escherichia blattae</i>	ATCC 29907	Insect	Negative
<i>Bordetella bronchiseptica</i>	ATCC 4617	Lung of Dog	Negative
<i>Brochothrix thermosphacta</i>	ATCC 11509	Animal-derived foodstuff	Negative
<i>Citrobacter farmeri</i>	ATCC 51633	Human feces	Negative
<i>Escherichia coli</i> O91	NCTC 9091	Clinical Isolate	Negative
<i>Escherichia coli</i>	ATCC 8739	Feces	Negative
<i>Kluyvera intermedia</i>	ATCC 33110	Surface water	Negative
<i>Escherichia coli</i> O113	NCTC 9113	Not Available	Negative
<i>Escherichia coli</i> O115	NCTC 10444	Mammal, Calf	Negative
<i>Escherichia coli</i> O117	NCTC 9117	Not Available	Negative
<i>Escherichia coli</i> O118	NCTC 9118	Not available	Negative
<i>Rahnella aquatilis</i>	ATCC 55046	Soil	Negative
<i>Escherichia coli</i> O142	NCTC 10089	Not Available	Negative
<i>Shigella boydii</i>	ATCC 9207	Pork liver	Negative
<i>Escherichia coli</i> O146	NCTC 10677	Not Available	Negative
<i>Escherichia coli</i> O163	NCTC 11021	Feces	Negative
<i>Escherichia fergusonii</i>	ATCC 35469	Feces, human	Negative
<i>Escherichia hermanii</i>	ATCC 33650	Mouse Brain	Negative
<i>Escherichia vulneris</i>	ATCC 29943	Human Wound	Negative
<i>Hafnia alvei</i>	ATCC 51815	Milk	Negative
<i>Klebsiella pneumoniae</i> subsp. <i>pneumonia</i>	ATCC 4352	Cow's Milk	Negative
<i>Microbacterium testaceum</i>	ATCC 15829	Paddy	Negative
<i>Pantoea agglomerans</i>	ATCC 19552	Sewage	Negative
<i>Pseudomonas aeruginosa</i>	ATCC 9027	Outer Ear Infection	Negative
<i>Escherichia coli</i> O55:H6	MSU DEC1A	Not Available	Negative

¹ATCC- American Type Culture Collection; NCTC- National Type Culture Collection; MSU- Michigan State University

Table 7: RapidFinder STEC PCR Screening Assay Presumptive PCR vs RapidFinder STEC Detection Workflow Confirmation Method Confirmed POD Results (1)

Matrix	Strain	Enrichment Time Point/sample preparation method	MPN ^a /test portion	N ^b	Screening Kit Presumptive Result (CP)			Confirmation Result (CC)			dPOD _{CP} ^f	95% CI ^g
					X ^c	POD _{CP} ^d	95% CI	X ^c	POD _{CC} ^e	95% CI		
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	18 h PrepSEQ Rapid Spin ^h	N/A ^j	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.55 (0.29, 0.93)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
			2.58 (1.15, 5.78)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	10 h PrepSEQ Nucleic Acid Extraction ⁱ	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.61 (0.33, 1.04)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
			3.70 (1.521, 9.20)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	18 h PrepSEQ Nucleic Acid Extraction	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.61 (0.33, 1.04)	20	10	0.50	(0.30, 0.70)	7	0.35	(0.18, 0.57)	0.15	(-0.15, 0.41)
			3.70 (1.521, 9.20)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw beef trim	<i>E. coli</i> O103 MSU TW08101	10 h PrepSEQ Nucleic Acid Extraction	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.69 (0.40, 1.14)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
			1.97 (0.91, 4.27)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw beef trim	<i>E. coli</i> O103 MSU TW08101	18 h PrepSEQ Rapid Spin	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.69 (0.40, 1.14)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
			1.97 (0.91, 4.27)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cX = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Confirmation method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the presumptive positive candidate method result and confirmed reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hPrepSEQ Rapid Spin = Enrichments analyzed using the PrepSEQ Rapid Spin sample preparation kit followed by PCR analysis with the RapidFinder STEC Detection Workflow

ⁱPrepSEQ Nucleic Acid Extraction = Enrichments analyzed using the PrepSEQ Nucleic Acid Extraction sample preparation kit followed by PCR analysis with the RapidFinder STEC Detection Workflow

^jNot applicable

Table 8: POD Analysis of Results of RapidFinder STEC Screening Assay Confirmed vs USDA Reference Method (1)

Matrix	Strain	Enrichment Time Point/sample preparation method	MPN ^a /test portion	N ^b	Screening Kit Confirmed Result			USDA FSIS MLG 5.09 or 5B.05 Reference Method			dPOD _c ^f	95% CI ^g
					X ^c	POD _c ^d	95% CI	X ^c	POD _R ^e	95% CI		
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	18 h PrepSEQ Rapid Spin ^h	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.55 (0.29, 0.93)	20	9	0.45	(0.26, 0.66)	8	0.40	(0.22, 0.61)	0.05	(-0.24, 0.33)
			2.58 (1.15, 5.78)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	10 h PrepSEQ Nucleic Acid Extraction ⁱ	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.61 (0.33, 1.04)	20	7	0.35	(0.18, 0.57)	9	0.45	(0.26, 0.66)	-0.10	(-0.37, 0.19)
			3.70 (1.521, 9.20)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	18 h PrepSEQ Nucleic Acid Extraction	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.61 (0.33, 1.04)	20	7	0.35	(0.18, 0.57)	9	0.45	(0.26, 0.66)	-0.10	(-0.37, 0.19)
			3.70 (1.521, 9.20)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw beef trim	<i>E. coli</i> O103 MSU TW08101	10 h PrepSEQ Nucleic Acid Extraction	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.69 (0.40, 1.14)	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.33, 0.24)
			1.97 (0.91, 4.27)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw beef trim	<i>E. coli</i> O103 MSU TW08101	18 h PrepSEQ Rapid Spin	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.69 (0.40, 1.14)	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.33, 0.24)
			1.97 (0.91, 4.27)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cX = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hPrepSEQ Rapid Spin = Enrichments analyzed using the PrepSEQ Rapid Spin sample preparation kit followed by PCR analysis with the RapidFinder STEC Detection Workflow

ⁱPrepSEQ Nucleic Acid Extraction = Enrichments analyzed using the PrepSEQ Nucleic Acid Extraction sample preparation kit followed by PCR analysis with the RapidFinder STEC Detection Workflow

^jNot applicable

Table 9: RapidFinder STEC PCR Confirmation Assay Presumptive PCR vs RapidFinder STEC Detection Workflow Confirmation Method Confirmed POD Results (1)

Matrix	Strain	Enrichment Time Point/sample preparation method	MPN ^a /test portion	N ^b	Confirmation Kit presumptive result			Confirmation result (CC)			dPOD _{CP} ^f	95% CI ^g
					X ^c	POD _{CP} ^d	95% CI	X ^c	POD _{CC} ^e	95% CI		
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	18 h PrepSEQ Rapid Spin ^h	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.55 (0.29, 0.93)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
			2.58 (1.15, 5.78)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	10 h PrepSEQ Nucleic Acid Extraction ⁱ	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.61 (0.33, 1.04)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
			3.70 (1.521, 9.20)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	18 h PrepSEQ Nucleic Acid Extraction	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.61 (0.33, 1.04)	20	10	0.50	(0.30, 0.70)	7	0.35	(0.18, 0.57)	0.15	(-0.15, 0.41)
			3.70 (1.521, 9.20)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw beef trim	<i>E. coli</i> O103 MSU TW08101	10 h PrepSEQ Nucleic Acid Extraction	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.69 (0.40, 1.14)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
			1.97 (0.91, 4.27)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw beef trim	<i>E. coli</i> O103 MSU TW08101	18 h PrepSEQ Rapid Spin	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.69 (0.40, 1.14)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
			1.97 (0.91, 4.27)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cX = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Confirmation method positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hPrepSEQ Rapid Spin = Enrichments analyzed using the PrepSEQ Rapid Spin sample preparation kit followed by PCR analysis with the RapidFinder STEC Detection Workflow

ⁱPrepSEQ Nucleic Acid Extraction = Enrichments analyzed using the PrepSEQ Nucleic Acid Extraction sample preparation kit followed by PCR analysis with the RapidFinder STEC Detection Workflow

^jNot applicable

Table 10: POD Analysis of Results of RapidFinder STEC Confirmation Assay Confirmed vs USDA Reference Method (1)

Matrix	Strain	Enrichment Time Point/sample preparation method	MPN ^a /test portion	N ^b	Candidate method			USDA FSIS MLG 5.09 or 5B.05 Reference Method			dPOD _c ^f	95% CI ^g
					X ^c	POD _c ^d	95% CI	X ^c	POD _R ^e	95% CI		
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	18 h PrepSEQ Rapid Spin ^h	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.55 (0.29, 0.93)	20	9	0.45	(0.26, 0.66)	8	0.40	(0.22, 0.61)	0.05	(-0.24, 0.33)
			2.58 (1.15, 5.78)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	10 h PrepSEQ Nucleic Acid Extraction ⁱ	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.61 (0.33, 1.04)	20	7	0.35	(0.18, 0.57)	9	0.45	(0.26, 0.66)	-0.10	(-0.37, 0.19)
			3.70 (1.521, 9.20)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (73% lean)	<i>E. coli</i> O157:H7 ATCC 43895	18 h PrepSEQ Nucleic Acid Extraction	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.61 (0.33, 1.04)	20	7	0.35	(0.18, 0.57)	9	0.45	(0.26, 0.66)	-0.10	(-0.37, 0.19)
			3.70 (1.521, 9.20)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw beef trim	<i>E. coli</i> O103 MSU TW08101	10 h PrepSEQ Nucleic Acid Extraction	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.69 (0.40, 1.14)	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.33, 0.24)
			1.97 (0.91, 4.27)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw beef trim	<i>E. coli</i> O103 MSU TW08101	18 h PrepSEQ Rapid Spin	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
			0.69 (0.40, 1.14)	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.33, 0.24)
			1.97 (0.91, 4.27)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cX = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hPrepSEQ Rapid Spin = Enrichments analyzed using the PrepSEQ Rapid Spin sample preparation kit followed by PCR analysis with the RapidFinder STEC Detection Workflow

ⁱPrepSEQ Nucleic Acid Extraction = Enrichments analyzed using the PrepSEQ Nucleic Acid Extraction sample preparation kit followed by PCR analysis with the RapidFinder STEC Detection Workflow

^jNot applicable

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.

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.

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