

Applied Biosystems RapidFinder STEC Detection Workflow AOAC-RI PTM Validation: Inclusivity and Exclusivity

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Introduction

As part of the AOAC Research Institute Performance Tested MethodSM certification of the Applied BiosystemsTM RapidFinderTM STEC Detection workflow (comprised of the Applied BiosystemsTM RapidFinderTM STEC Screening Assay and Applied BiosystemsTM RapidFinderTM STEC Confirmation Assay), an inclusivity and exclusivity study was conducted by Q Laboratories Inc., Cincinnati, Ohio, USA, as an independent laboratory study. The following is a summary of that part of the study.

Methodology

Choice of strains

Fifty isolates of pathogenic *Escherichia coli* known to express the *stx*₁ or *stx*₂ genes coding for Shiga-like toxin 1 and 2 proteins and the *eae* gene coding for intimin protein (inclusivity isolates) and thirty *E. coli* strains known not to express the *stx* and *eae* genes (exclusivity strains) were analyzed. During the inclusivity study conducted on the RapidFinder STEC Screening Assay, isolates of *E. coli* O55 and *E. coli* O163 were included in the panel as they are both known to be positive for *stx* and *eae* genes.

Culture enrichment

One colony of each inclusivity isolate was inoculated into a tube containing Tryptone Soya Broth and incubated for 10±1 hours at 42±1 °C. The cultures were then decimally diluted to approximately 10⁴ CFU/ml before performing both the Applied BiosystemsTM PrepSEQTM Nucleic Acid Extraction sample preparation workflow and Applied BiosystemsTM PrepSEQTM Rapid Spin sample preparation workflow. The DNA prepared by each of the PrepSEQ sample preparation workflows was then analyzed with both the RapidFinder STEC Screening and Detection Assays.

Each exclusivity isolate was inoculated into Brain Heart Infusion (BHI) Broth and incubated for 22±2 h at 37±2 °C. Prepared cultures were diluted in Buffered Peptone Water (ISO) to obtain approximately 10⁵ CFU/ml. The cultures were then processed using both the PrepSEQ Nucleic Acid Extraction sample preparation and PrepSEQ Rapid Spin sample preparation workflows and the resulting prepared DNA analyzed with the RapidFinder STEC Screening and Detection Assays.

Method

Thirty microlitres of DNA extract prepared using the PrepSEQ Nucleic Acid Extraction or PrepSEQ Rapid Spin sample preparation workflows were added to each of the required number of RapidFinder STEC Screening and Confirmation Assay PCR tubes and the tubes vortexed at high speed for 5-10 seconds to dissolve the PCR pellets and mix the contents of the tube thoroughly, before centrifuging the tubes at 1000-2000 x g for approximately 30 seconds. PCR tubes were checked to ensure that pellets had dissolved and were then analyzed on the Applied Biosystems™ 7500 Fast PCR Instrument with Applied Biosystems™ RapidFinder™ Express Software.

Results

Inclusivity and exclusivity results are summarised in Tables 1 and 2. All inclusivity isolates tested with the RapidFinder Screening and RapidFinder Confirmation Assays returned positive results with RapidFinder Express Software and all of the exclusivity isolates gave negative results with both of the PCR kits with RapidFinder Express Software.

Table 1a: AOAC-RI PTM study: Inclusivity results for the RapidFinder STEC Screening Assay

Isolate	Serotype	Source & Identification Number	Origin	Screening Assay 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	NCTC™ 13125	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	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

Isolate	Serotype	Source & Identification Number	Origin	Screening Assay Result
<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

Table 1b: AOAC-RI PTM study: Inclusivity results for the RapidFinder STEC Confirmation Assay

Isolate	Serotype	Source & Identification Number	Origin	Confirmation Assay Result
<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	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	Human feces	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

Isolate	Serotype	Source & Identification Number	Origin	Confirmation Assay Result
<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

Table 2a: AOAC-RI PTM study: Exclusivity results for the RapidFinder STEC Screening Assay

Isolate	Source & Identification Number	Origin	Screening 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

Isolate	Source & Identification Number	Origin	Screening Assay Result
<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® 9290™	Pork liver	Negative
<i>Escherichia coli</i> O146	NCTC™ 10677	Not available	Negative
<i>Shigella sonnei</i>	ATCC® 9270™	Human feces	Negative
<i>Escherichia fergusonii</i>	ATCC® 35469™	Human feces	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

Table 2b: AOAC-RI PTM study: Exclusivity results for the RapidFinder STEC Confirmation Assay

Isolate	Source & Identification Number	Origin	Confirmation 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> O146	ATCC® 8739™	Feces	Negative
<i>Kluyvera intermedia</i>	ATCC® 33110™	Surface water	Negative

Isolate	Source & Identification Number	Origin	Confirmation Assay Result
<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® 9290™	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™	Human feces	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

Conclusions

The inclusivity and exclusivity study conducted as part of this AOAC-RI PTM validation study demonstrated that the RapidFinder STEC Detection workflow, comprised of the RapidFinder STEC Screening Assay and RapidFinder STEC Confirmation Assay showed 100% inclusivity and 100% exclusivity with the isolates analyzed.

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