P2-153: Evaluation Of The MicroSEQ® *E. coli* O157:H7 Assay: Real-Time PCR Detection Method

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ABSTRACT

Introduction: *Escherichia coli* O157:H7 is a major food-borne pathogen and one of the main enterohemorrhagic *E. coli* serotypes. The MicroSEQ *Escherichia coli* O157:H7 method (AOAC PTM # 071001) uses PCR to amplify unique *Escherichia coli* O157:H7 specific DNA target sequences and TaqMan® probes that detect the amplified sequences. These probes contain a fluorescent dye and a quencher. When no target sequence is present, the fluorescence is quenched. Upon binding to a specific target sequence, the quencher is released and fluorescence can be detected. As target DNA is amplified, the fluorescent signal increases and this increase is detected by the instrument (real-time PCR). Sample DNA is extracted by either the PrepSEQ™ Rapid Spin Sample Preparation Kit which combines centrifugation with a spin column to clarify the sample and concentrate bacteria followed by heat lysis, or the PrepSEQ™ Nucleic Acid Extraction Kit which uses a Proteinase K lysis method followed by DNA purification by magnetic particles.

Purpose: The purpose of this internal evaluation was to evaluate ruggedness and inclusivity/exclusivity of the MicroSEQ method and compare to the USDA FSIS (meat) ISO 16654 (juice and spinach) reference methods for *Escherichia coli* O157:H7 as part of the AOAC Research Institute™ PTM validation process.

Methods: The method comparison analyzed 3 foods with a 16-h enrichment and a 6-h enrichment and 2 with a 16-h and 8-h using a 25-g test portion. The ground beef and beef trim were also validated as 375-g test portions with 16-h enrichment. Each matrix was spiked with a different strain of *Escherichia coli* O157:H7 at two levels (0.2-2 cfu/25g and 2-5 cfu/25g). For each test portion, DNA was extracted by both PrepSEQ™ procedures, analyzed by rtPCR and compared to the ISO 16654 method for spinach, orange juice and unpasteurized apple juice or the USDA FSIS method for raw ground beef and raw beef trim. The new method was also evaluated for inclusivity/exclusivity and ruggedness parameters.

Results: For this new assay, modified performance parameters in the ruggedness evaluation showed no significant differences. There were no significant differences between the new methods and their corresponding reference method as indicated by McNemar's chi-square analysis (> 3.84) for all 5 food types at varying incubation times and sample volumes. For inclusivity, all 50 strains of E. coli O157:H7 were detected as positive and for 29/30 exclusivity strains were negative and 1 E. coli O157 NM was positive.

Significance: This new method is a rapid, reliable alternative to the traditional method of detecting *Escherichia coli* O157:H7 in a variety of foods.

RESULTS

TABLE 1: MicroSEQ *E.coli* O157:H7 vs. USDA MLG 5.04 for Raw Ground Beef and Beef Trim

Inoc. Level	MPN/ 25g	MicroSEQ *E. coli* O157:H7		USDA MLG 5.04 Conf. +	X²* (MicroSEQ vs. USDA)
		NA Extraction Conf. +	Rapid Spin Conf. +		
RAW GROUND BEEF -6 HR ENRICHMENT (25 G)					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.52	11/20	11/20	15/20	1.71
2-10 cfu/25g	7.00	19/20	19/20	20/20	1.00
RAW GROUND BEEF -16 HR ENRICHMENT (25 G)					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.52	15/20	15/20	15/20	0.00
2-10 cfu/25g	7.00	20/20	20/20	20/20	0.00
RAW GROUND BEEF -16 HR ENRICHMENT (375 G)					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.58	3/20	3/20	1/20	1.08
2-10 cfu/25g	2.30	13/20	13/20	14/20	0.44
BEEF TRIM – 6 HR ENRICHMENT (25 G)					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	1.10	14/20	14/20	13/20	0.11
2-10 cfu/25g	27.5	20/20	20/20	20/20	0.00
BEEF TRIM – 16 HR ENRICHMENT (25 G)					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	1.10	12/20	12/20	13/20	0.44
2-10 cfu/25g	27.5	20/20	20/20	20/20	0.00
BEEF TRIM- 16 HR ENRICHMENT (375 G)					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	1.10	16/20	16/20	13/20	1.10
2-10 cfu/25g	27.5	20/20	20/20	20/20	0/00

*Values >3.84 are significant at 5%

RESULTS

TABLE 2: MicroSEQ *E.coli* O157:H7 vs. ISO 16554(24 hr Enrichment) in Spinach, Apple Juice and Orange Juice

Inoc. Level	MPN/ 25g	MicroSEQ *E. coli* O157:H7		ISO 16554 Conf. +	X²* (MicroSEQ vs. ISO)
		NA Extraction Conf. +	Rapid Spin Conf. +		
APPLE JUICE- 8 HR ENRICHMENT					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.38	3/20	3/20	4/20	0.16
2-10 cfu/25g	1.10	9/20	9/20	10/20	0.90
APPLE JUICE- 16 HR ENRICHMENT					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.23	11/20	11/20	9/20	0.39
2-10 cfu/25g	11.5	20/20	20/20	20/20	0.00
ORANGE JUICE- 8 HR ENRICHMENT					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.23	3/20	3/20	3/20	0.00
2-10 cfu/25g	2.30	16/20	16/20	13/20	1.10
ORANGE JUICE- 16 HR ENRICHMENT					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.23	16/20	16/20	10/20	3.86
SPINACH- 6 HR ENRICHMENT					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.09	0/20	0/20	2/20	2.00
2-10 cfu/25g	2.30	12/20	12/20	10/20	0.39
SPINACH- 16 HR ENRICHMENT					
Control	< 0.075	0/5	0/5	0/5	-
0.2-2 cfu/25g	0.09	2/20	2/20	2/20	0.00
2-10 cfu/25g	2.30	10/20	10/20	10/20	0.00

DISCUSSION

Ruggedness Evaluation: The assay was evaluated for ruggedness by making minor modifications to the test kit parameters. The following parameters were evaluated using both sample prep methods for raw ground beef or and orange juice. Samples were confirmed according to their respective reference methods.

Raw Ground Beef	Orange Juice
Pre-enrichment Incubation Time (25 g) (5, 5.5, 6 h) & (14, 16 ,18 h)	Pre-enrichment incubation time (6,8,10h)
Pre-enrichment Incubation Time (375 g) (14, 16, 18 h)	
DNA Sample Volume (25, 30, 35 µl)	

False negative results were obtained at 5.5 h enrichment of raw ground beef and 6 h enrichment of orange juice. There were no significant differences between the results obtained by the test and reference methods at p≤0.05 for all additional ruggedness parameters evaluated.

Inclusivity/Exclusivity: For inclusivity, 51/51 strains of E. coli O157:H7 were detected as positive at both 6 and 16 h enrichment times. For exclusivity, 29/30 strains resulted in non-detection with one E. coli O157:NM strain resulting in a positive detection.

Method Comparison: Mantel-Haenszel chi-square analysis indicated no significant differences for this unpaired evaluation between the test and reference methods for all 5 foods tested, including sample prep methods, variance of sample size and enrichment times.

CONCLUSIONS

- The MicroSEQ *E.coli* O157:H7 assay (AOAC PTM # 071001) compared favorably with the ISO16554 and USDA MLG 5.04 reference methods
- The two sample prep methods, Automated PrepSEQ NA Extraction and the manual PrepSEQ Rapid Spin, provide added flexibility by offering two reliable options for the laboratory to reduce pre-enrichment time and extract high quality DNA from a variety of food types.
- Ease-of-use with a streamlined, lyophilized format and a simple, software guided 3-step process.
- Time to results is less than 3 hours after a one-step pre-enrichment and less than 9 hours total for raw ground beef, raw beef trim and spinach and less than 12 hours for apple and orange juice products.
- Results are highly specific due to real-time PCR and internal positive control and significantly eliminates risk of false-negatives or false-positives.
- The MicroSEQ *E. coli* O157:H7 real-time PCR assays are part of a complete food testing solution designed to make food detection as rapid and easy as possible.