Evaluation of New Technology to Detect *Escherichia* coli O157:H7 in Ground Beef and Spinach Using a Novel Food Security System

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ABSTRACT

Introduction
The *E. coli* O157:H7 LT Food Security System (FSS) is a PCR-based detection method that rapidly and specifically identifies *E. coli* O157:H7 in food. The entire procedure takes under 10 h to return positive or negative results with the PCR portion taking less than an hour. The method includes: a single 8 to 9 hour enrichment of samples, lysis of bacteria to release DNA, amplification and melting of target DNA in Idaho Technology's R.A.P.I.D.® LT instrument, internal amplification controls, and automatic interpretation of results by the system software. Samples may be tested individually, or pooled after enrichment and tested.

Purpose
The *E. coli* O157:H7 LT FSS was evaluated for sensitivity, specificity, ruggedness and reagent stability in an AOAC Performance Tested Methods℠ study.

Methods
Samples of ground beef and spinach (25 g) were inoculated at levels to provide recovery of ~1 CFU of *E. coli* O157:H7 per 25 g samples after equilibration. All enrichments were performed in Buffered Peptone Water (BPW) at 42°C and were incubated and tested after 8 h for individual samples and 9 h for pooled samples (50 mL from 1 inoculated to 50 mL from 4 uninoculated samples). Results were compared statistically to unpaired sample sets that were tested with the applicable reference method.

Specificity testing used an inclusivity panel of 60 *E. coli* O157:H7 isolates and an exclusivity panel of 45 non-*E. coli* O157:H7 strains including *E. coli* O157:non-H7 isolates. The ruggedness portion of the study evaluated the system's ability to withstand minor user variations in set-up. The stability evaluation looked at shelf life and lot-to-lot variation.

Results
The *E. coli* O157:H7 LT FSS is equivalent or better than the reference methods for ground beef and spinach. The system detected all 60 *E. coli* O157:H7 isolates and did not detect 45 closely related strains. The system is robust and reproducible as demonstrated by ruggedness and stability studies.

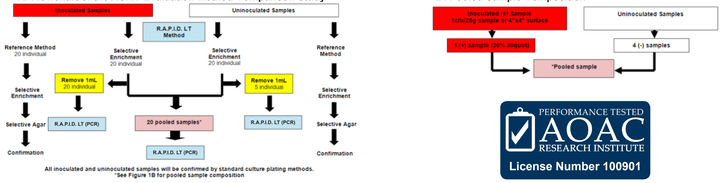
Significance
The *E. coli* O157:H7 LT FSS takes less than 10 h to obtain a result where the reference method can take 3 to 4 days.

- **Easy to use PCR instrument**
  - Freeze-dried reagents/no proprietary media
  - Designed for minimally trained users
- **Accurate and specific pathogen testing**
  - Double specificity (primer + probe)
  - Extensive scientific support & expertise
- **Timely results**
  - Shortened enrichment times
  - PCR in under an hour

ISSUE

Current methods can take 3–4 days to identify *E. coli* O157:H7 in food. The goal of Idaho Technology's *E. coli* O157:H7 FSS is to provide a sensitive and robust system that is faster than currently available detection systems.

Figure 1.



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METHODS

Method Comparison
The *E. coli* O157:H7 LT FSS was evaluated with two food types: raw ground beef and fresh spinach. Each food type was divided into two portions. One portion was not inoculated. The second portion was inoculated in a large batch to provide enough samples for testing by the *E. coli* O157:H7 LT FSS, Most Probable Number (MPN) analysis, and the reference method. Both inoculated and uninoculated batches were handled in the same manner. The inoculum concentrations were selected in order to result in approximately 1 CFU of *E. coli* O157:H7 per 25 g sample (fractional positive levels, 5–15 positives out of 20 replicates tested) after equilibration. Samples were inoculated with liquid culture and allowed to equilibrate at 4°C for 48–72 hours. Each matrix was inoculated with a different *E. coli* O157:H7 strain: *E. coli* O157:H7 (ATCC 43895) for raw ground beef and *E. coli* O157:H7 (ATCC 700599) for fresh spinach.

For each matrix, 25 samples (20 inoculated and 5 uninoculated) were prepared, enriched, and evaluated according to the reference method (USDA MLG Chapter 5.04 for raw ground beef (1); FDA BAM Eighth edition, revision A, Chapter 4a for fresh spinach (2)). For each matrix, 40 samples (20 inoculated, 5 uninoculated, and 15 uninoculated for pooling) were prepared and enriched in BPW. The samples were incubated for 8 hours at 42°C.

Twenty-five inoculated and 5 uninoculated FSS samples were tested individually, returned to the incubator for an additional hour at 42°C and then pooled and tested (see figure 1A and 1B). Each pooled sample was prepared by combining one 50 mL aliquot of an inoculated sample with 50 mL aliquots from each of four uninoculated samples. This created a 250 mL wet composite or pooled sample (see Figure 1B). All positive and negative samples were confirmed by appropriate reference methods. Twenty-five inoculated and 5 uninoculated reference method samples were tested individually according to the appropriate reference method. Pooled samples were not evaluated using the reference method.

Most Probable Number (MPN) quantification was conducted on the day that analysis of test samples was initiated. The MPN is calculated according to the methods in Appendix 2 of the FDA BAM Eighth edition, Revision A. Specifically, a 3-tube MPN with 100g, 10g, and 1g was performed. The samples were set up and confirmed as described according to the USDA method or FDA method as appropriate to food type.

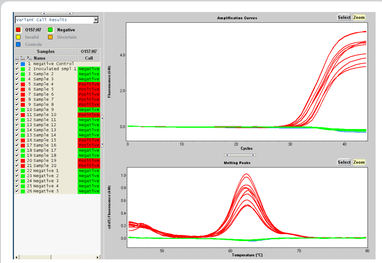
Specificity
A total of 60 strains of *E. coli* O157:H7 were evaluated by the *E. coli* O157:H7 LT FSS. Thirty seven of these strains were isolated from food-related sources. Organisms were initially cultured in BHI overnight. Approximately 10–50 CFU were added to 250 mL BPW and incubated at 42 ± 2°C for 8 hours. Samples were processed according to the method protocol for *E. coli* O157:H7 detection. In addition a total of 45 non-*E. coli* and *E. coli* non-O157:H7 strains were evaluated, including closely related taxa. Organisms were initially cultured in BHI overnight, from these overnight cultures, 1 mL of undiluted culture (approximately 10^9 CFU/mL) was added to 250 mL BHI and enriched for 22–24 hours at 37 ± 2°C. Samples were then tested with the sample processing and DNA amplification portions of the *E. coli* O157:H7 LT FSS.

Ruggedness and Reagent Variation
Two different *E. coli* O157:H7 strains (ATCC 43895 and ATCC 43888) and one non-O157 *E. coli* organism (*E. coli* O55:H7) were tested from pure culture. For each organism, five samples were prepared and evaluated for each ruggedness parameter. The strains were grown for 16 hours in BHI at 42 ± 2°C. Approximately 10–50 CFU of each *E. coli* O157:H7 and 1 mL of undiluted *E. coli* O55:H7 was added to 250 mL portions of BPW. Samples were enriched 8 hours at 42 ± 2°C, after incubation the samples were individually processed according to the *E. coli* O157:H7 LT FSS protocol.

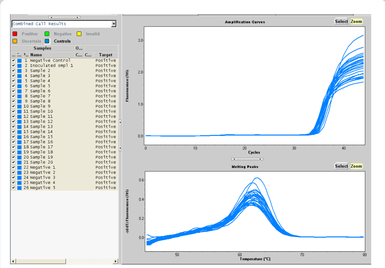
Ruggedness Parameters Tested
- **Storage time of sample prior to analysis.** Enriched samples were tested immediately and after storage times (at 4°C) of 2, 4, and 24 hours.
- **Volume of sample added to the small bead tube.** Varied volume of cultured sample added to the bead tube. Sample volumes of 2.5 µL, 5 µL (correct volume), and 10 µL were evaluated.
- **Storage time of lysed sample in the bead tube prior to PCR analysis**. Samples were tested immediately and after storage times (at 4°C) of 2, 4, and 24 hours.
- **Reagent preparation time.** Processed sample was added to reagent vials along with reconstitution buffer and run on the instrument immediately or after sitting at room temperature for 1, 2, or 4 hours.
- **Final enrichment time.** Samples were tested after 8 hours of enrichment and again after 24 hours of enrichment

Reagent Variation
Three different lots of Idaho Technology *E. coli* O157:H7 LT freeze-dried PCR reagents were evaluated (one to six months old). Stability and lot-to-lot variation were evaluated simultaneously. Five samples were evaluated using each lot.

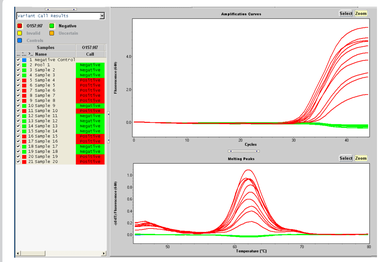
Figure 2: Raw Ground Beef Testing Results



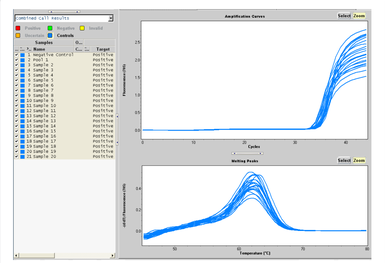
A: Individual raw ground beef samples target channel (640). Negative control (position 1), 10/20 fractionally spiked positive samples confirmed by culture (positions 2–21) and 5 uninoculated (positions 22–26).



B: Individual raw ground beef samples AC channel (705), internal positive control (included in reagent vial).

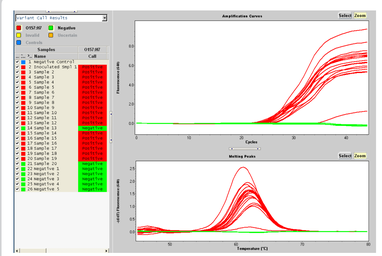


C: Pooled raw ground beef samples: 50 mL aliquot of inoculated beef sample combined with 50 mL aliquots from each of 4 uninoculated samples (figure 2A). Negative control in position 1.

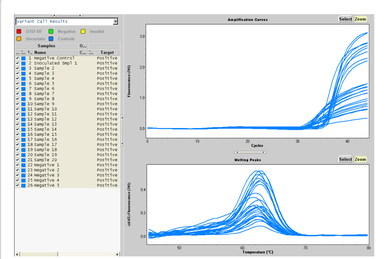


D: Pooled raw ground beef samples AC channel (705), internal positive control (included in each reagent vial).

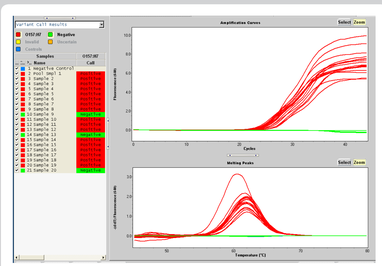
Figure 3: Fresh Spinach Testing Results



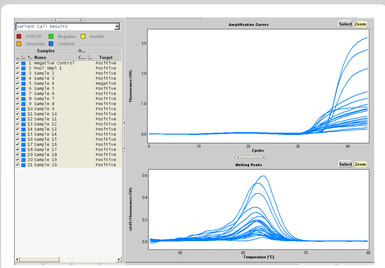
A: Individual fresh spinach samples target channel (640). Negative control (position 1), 18/20 fractionally spiked positive samples confirmed by culture (positions 2–21) and 5 uninoculated samples (positions 22–26).



B: Individual fresh spinach samples AC channel (705), internal positive control (included in reagent vial).



C: Pooled fresh spinach samples: 50 mL aliquot of inoculated spinach sample combined with 50 mL aliquots from each of 4 uninoculated samples (figure 2A). Negative control in position 1.



D: Pooled raw fresh spinach samples AC channel (705), internal positive control (included in each reagent vial).

Table I. Method Comparison Results

Matrix	Inoculating organism	Test Portion	MPN/25g[a]	No. test portions for each method	Reference Method Positive[b]	Test Kit Presumptive Positive[c]	Test Kit Confirmed Positive[c]	X^{2e}	Test Kit Performance False Negative[f]	Test Kit Performance False Positive[g]
Raw ground beef	*E. coli* O157:H7 (ATCC 43895)	Individual	0.09	20	2	10	10	7.42	0	0
		Pooled	0.018	20	2	10	10	7.42	0	0
		Control	0	5	0	0	0	-	-	-
Fresh spinach	*E. coli* O157:H7 (ATCC 700599)	Individual	1.075	20	15	18	18	1.52	0	0
		Pooled	0.215	20	15	17	18	0.61	6%	0
		Control	0	5	0	0	0	-	-	-
				Total	17	28 Individual 27 Pooled	28	-	1.8%	0

[a]Most Probable Number: Colony forming units in a 25 g samples. Pooled MPN calculated by dividing individual MPN by five.
[b]Number of positive samples as determined by the applicable reference culture method (same samples listed in pooled for comparison).
[c]Number of positives given by the test method.
[d]Number of enrichments confirmed positive from the test method.
[e]Mantel-Haenszel chi square for comparison to reference samples.
[f]Number of test kit presumptive negatives confirmed positive divided by the total number of confirmed positives.
[g]Number of test kit presumptive positives confirmed negative divided by the total number of confirmed negatives.

Table II. Exclusivity Summary

Organisms Not Detected		
Alcaligenes faecalis	*Bacillus cereus*	*Candida albicans*
Citrobacter freundii	*Edwardsiella tarda*	*Enterobacter aerogenes*
Klebsiella pneumoniae	*Lactobacillus fermentum*	*Microbacterium testaceum*
Micrococcus luteus	*Morganella morganii*	*Proteus hauseri*
Pseudomonas aeruginosa	*Shigella flexneri*	*Shigella dysenteriae*
Staphylococcus aureus	*Streptococcus pneumoniae*	*Enterococcus faecalis*
Lactobacillus delbrueckii subp. *lactis*	*Salmonella enteritidis*	*Staphylococcus xylosus*
Hafnia alvei	*Escherichia blattae*	*Escherichia fergusonii*
Escherichia hermannii	*Escherichia vulneris*	*Escherichia coli* –O:H48
Escherichia coli O?:H8	*Escherichia coli* O45:H8	*Escherichia coli* O55:H7
Escherichia coli O98:H8	*Escherichia coli* O117:H27	*Escherichia coli* O128:H16
Escherichia coli O145:H25	*Escherichia coli* O145:NM	*Escherichia coli* O157:H8
Escherichia coli O157:H42	*Escherichia coli* O157:H12	*Escherichia coli* O157:other than H7
Escherichia coli O121:H19	*Escherichia coli* O111:H8	*Escherichia coli* O26:H11
Escherichia coli O103:H2	*Escherichia coli* O113:H21	

Table III. Ruggedness Results

Organism	Parameter I Storage time of enriched samples	Parameter II Sample volume added to bead tube	Parameter III Storage time in bead tubes after lysis	Parameter IV Reagent preparation time	Parameter V Enrichment time
E. coli O157:H7 (ATCC 43895)	0 h: 5/5 positive	2.5 µL: 5/5 positive	0 h: 5/5 positive	0 h: 5/5 positive	8 h: 5/5 positive
	2 h: 5/5 positive	5 µL: 5/5 positive	2 h: 5/5 positive	1 h: 5/5 positive	24 h: 5/5 positive
	4 h: 5/5 positive	10 µL: 5/5 positive	4 h: 5/5 positive	2 h: 5/5 positive	
	24 h: 5/5 positive		24 h: 5/5 positive	4 h: 5/5 positive	
E. coli O157:H7 (ATCC 43888)	0 h: 5/5 positive	2.5 µL: 5/5 positive	0 h: 5/5 positive	0 h: 5/5 positive	8 h: 5/5 positive
	2 h: 5/5 positive	5 µL: 5/5 positive	2 h: 5/5 positive	1 h: 5/5 positive	24 h: 5/5 positive
	4 h: 5/5 positive	10 µL: 5/5 positive	4 h: 5/5 positive	2 h: 5/5 positive	
	24 h: 5/5 positive		24 h: 5/5 positive	4 h: 5/5 positive	
E. coli O55:H7	0 h: 5/5 negative	2.5 µL: 5/5 negative	0 h: 5/5 negative	0 h: 5/5 negative	16 h: 5/5 negative
	2 h: 5/5 negative	5 µL: 5/5 negative	2 h: 5/5 negative	1 h: 5/5 negative	24 h: 5/5 negative
	4 h: 5/5 negative	10 µL: 5/5 negative	4 h: 5/5 negative	2 h: 5/5 negative	
	24 h: 5/5 negative		24 h: 5/5 negative	4 h: 5/5 negative	

Table IV. Shelf life and Lot-to-Lot Results

Organism	Lot 1: 129008 Expires 15-APR-09 (Age: 6 months)	Lot 2: 167409 Expires 29-JUL-09 (Age: 3 months)	Lot 3: 177309 Expires 03-SEP-09 (Age: 1 month)
E. coli O157:H7 (ATCC 43895)	5/5 positive	5/5 positive	5/5 positive
E. coli O157:H7 (ATCC 43888)	5/5 positive	5/5 positive	5/5 positive
E. coli O55:H7	5/5 negative	5/5 negative	5/5 negative

RESULTS

Method Comparison
Sponsor lab results were evaluated using the Mantel-Haenszel Chi square (χ^2) calculation for unpaired samples, where a χ^2 value of less than 3.84 is considered statistically equivalent. Sponsor lab results for raw ground beef indicate that the *E. coli* O157:H7 protocol is statistically different, with greater sensitivity than the reference method, for individual and pooled samples. Sponsor lab results for fresh spinach indicate that the *E. coli* O157:H7 protocol is statistically equivalent to the reference method for individual and pooled samples, the pooled samples had 1 false negative sample with a false negative rate of 6%. Method comparison results are summarized in Table I.

Specificity
Of the 60 *E. coli* O157:H7 tested,
- All 60 strains were detected in BPW.
- Two strains detected are O157:NM, which are genetically H7.

None of the 45 non-E.coli O157:H7 strains tested were detected by the *E. coli* O157:H7 LT FSS. Exclusivity results are summarized in Table II.

Ruggedness and Reagent Variation
None of the parameters tested led to an unexpected result; results are summarized in Table III. All of the reagent lots performed equivalently; results are summarized in Table IV.

CONCLUSION

The data indicates that the *E. coli* O157:H7 LT FSS is equivalent to or better than current reference methods used to detect low levels of *E. coli* O157:H7 in food with individual and pooled samples.

The *E. coli* O157:H7 LT FSS represents a significant improvement over standard methods in a number of ways:

- The *E. coli* O157:H7 LT FSS is significantly faster, providing results in as little as 9 hours after sampling as opposed to 72 for the USDA and FDA methods. The R.A.P.I.D. LT can perform real-time PCR and provide automated results in less than 1 hour, after enrichment and sample processing.
- Results are easier to interpret than standard methods because the software gives a "positive" or "negative" answer.
- The *E. coli* O157:H7 LT FSS is easy to use, with single enrichment and minimal sample handling, including freeze-dried PCR reagents.

REFERENCES

(1) United States Department of Agriculture/Food Safety and Inspection Service Microbiology Laboratory Guidebook http://www.fsis.usda.gov/PDF/MLG_5_04.pdf
(2) United States Food and Drug Administration, FDA Bacteriological Analytical Manual http://www.cfsan.fda.gov/~ebam/bam-4a.html