Evaluation of Culture- and PCR-Based Detection Methods for *Escherichia coli* O157:H7 in Inoculated Ground Beef[†]

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ABSTRACT

Currently, several beef processors employ test-and-hold systems for increased quality control of ground beef. In such programs, each lot of product must be tested and found negative for *Escherichia coli* O157:H7 prior to release of the product into commerce. Optimization of three testing attributes (detection time, specificity, and sensitivity) is critical to the success of such strategies. Because ground beef is a highly perishable product, the testing methodology used must be as rapid as possible. The test also must have a low false-positive result rate so product is not needlessly discarded. False-negative results cannot be tolerated because they would allow contaminated product to be released and potentially cause disease. In this study, two culture-based and three PCR-based methods for detecting *E. coli* O157:H7 in ground beef were compared for their abilities to meet the above criteria. Ground beef samples were individually spiked with five genetically distinct strains of *E. coli* O157: H7 at concentrations of 17 and 1.7 CFU/65 g and then subjected to the various testing methodologies. There was no difference ($P > 0.05$) in the abilities of the PCR-based methods to detect *E. coli* O157:H7 inoculated in ground beef at 1.7 CFU/65 g. The culture-based systems detected more positive samples than did the PCR-based systems, but the detection times (21 to 48 h) were at least 9 h longer than those for the PCR-based methods (7.5 to 12 h). Ground beef samples were also spiked with potentially cross-reactive strains. The PCR-based systems that employed an immunomagnetic separation step prior to detection produced fewer false-positive results.

Traditionally, detection of foodborne pathogens has involved sample collection, enrichment, and isolation of the target organism on selective and/or indicator media. Such culture-based approaches lack sensitivity and specificity and are time-consuming, taking from 48 to 96 h from sample collection to final results. The implementation of immunomagnetic separation (IMS) has greatly increased the sensitivity and specificity while decreasing the time of detection *(4, 24, 33)*. Recently, PCR techniques, both conventional and real-time platforms, have been adopted for routine detection of foodborne pathogens *(9, 20–22)*. The rapid PCR run times routinely reduce detection times by 24 h.

Escherichia coli O157:H7 is a foodborne pathogen that has been associated with meat-, produce-, and water-related disease outbreaks *(15, 17, 28)*. This pathogen, which is known for its low infective dose and its ability to cause severe disease and death, emerged as a foodborne threat in the 1980s and early 1990s *(27, 31)*. Because early outbreaks were associated with ground beef, the U.S. Department of Agriculture Food Safety and Inspection Service produced several regulations aimed at eliminating this pathogen from red meat *(32)*. At the same time, the public and private

sectors of the beef processing industry were working to design, validate, and implement several antimicrobial interventions for use in combating *E. coli* O157:H7 contamination *(7, 16, 26.)* Unfortunately, no single intervention or combination of interventions have yet been identified that will eliminate *E. coli* O157:H7 on beef, and sporadic beef-associated outbreaks have continued to occur.

The meat industry has recently employed test-and-hold systems to prevent release of product containing *E. coli* O157:H7 *(6)*. In such test-and-hold programs, each lot of product is tested for *E. coli* O157:H7, and if the results are negative the product can be released into commerce. Optimization of testing methods with respect to detection time, specificity, and sensitivity is critical to the success of such strategies. Because ground beef is a highly perishable product, the testing methodology used must be as rapid as possible. The test also must have a low rate of false-positive results so wholesome product is not needlessly discarded. False-negative results cannot be tolerated because they would allow contaminated product to be released, potentially causing disease and defeating the purpose of the program.

In this study, two culture-based and three PCR-based methods for detecting *E. coli* O157:H7 in ground beef were compared for (i) their sample throughput capacity and time requirements for sample processing and detection, (ii) their specificity of discriminating between *E. coli* O157:H7 and other members of the background flora, and (iii) their sensitivity in detecting *E. coli* O157:H7 inoculated in ground beef at low concentrations.

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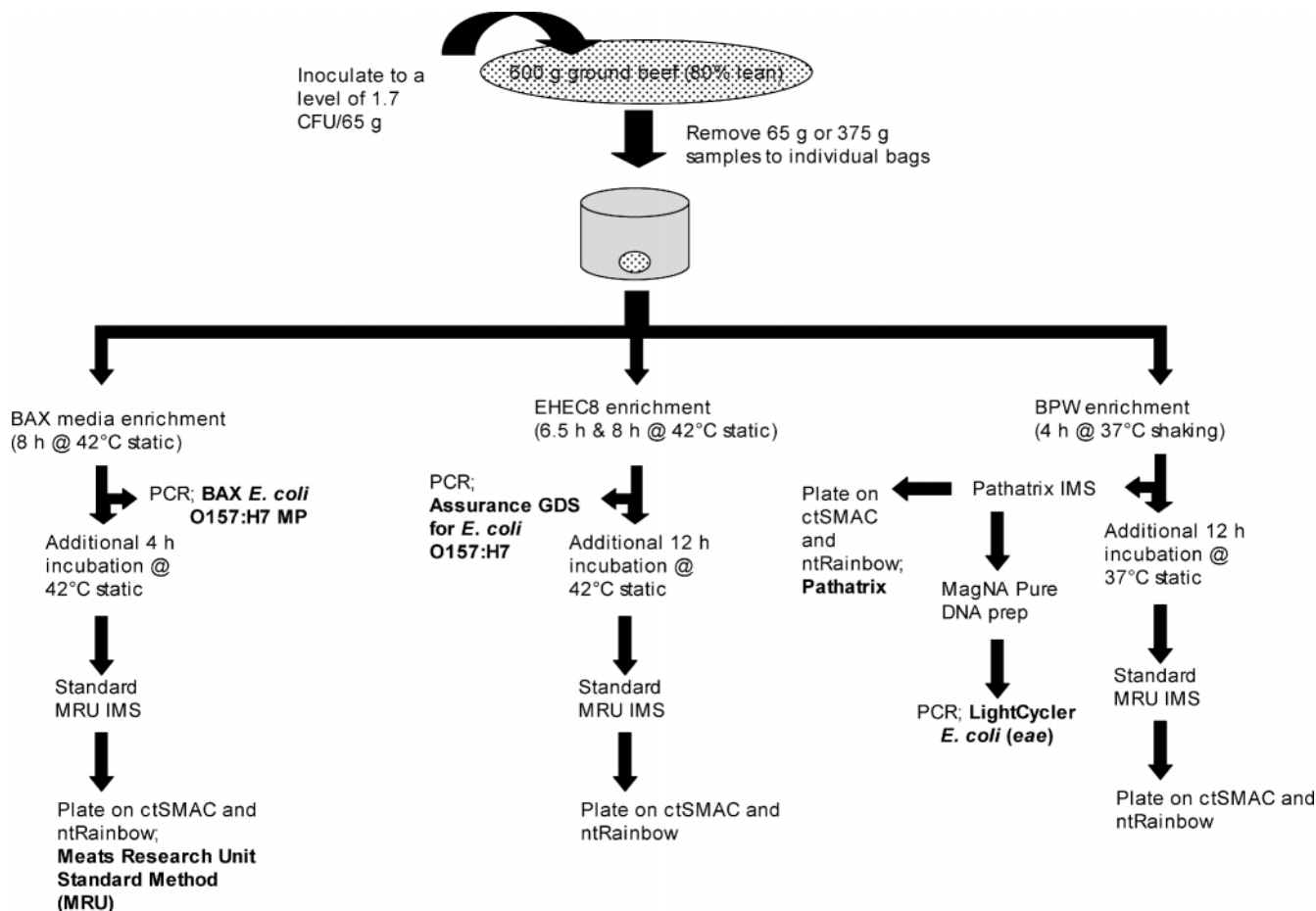
FIGURE 1. *Flowchart outlines the methods by which samples were inoculated, incubated, and processed for detection of* E. coli *O157: H7. Bold headings indicate the official methods tested. Pathways not ending in a bold heading were used in the data correction process to identify samples that had been inoculated.*

MATERIALS AND METHODS

***Escherichia coli* O157:H7 detection in ground beef.** Five methods for the detection of *E. coli* O157:H7 in ground beef were evaluated for their sensitivity and specificity (Fig. 1). Batch samples (600 g of 80% lean ground beef) were inoculated with individual strains of *E. coli* O157:H7 and then divided into portions for use with each method to minimize variation in samples or processing conditions. For the PCR-based methods, Roman L. Hruska U.S. Meat Animal Research Center (MARC) personnel were trained to perform the various tests by representatives from the specific company that designed each test kit. All testing was carried out at MARC using ground beef purchased from local retailers.

Sensitivity for *E. coli* O157:H7. Ground beef (80% lean) was spiked with approximately 17 or 1.7 CFU/65 g (equivalent to 100 or 10 CFU/375 g) for both PCR-based and culture-based procedures.

Strains. For the sensitivity trials, five strains of *E. coli* O157: H7 (55AC1, 114AC1, 131AC1, 237AC1, and 299AB3) from the MARC Meats Research Unit (MRU) culture collection were used for individual inoculation of samples. These strains are genetically different from one another and represent major subtypes within three relatedness clusters identified by pulsed-field gel electrophoresis (PFGE) analysis of *E. coli* O157:H7 isolates recovered from beef processing plants *(1, 3)*. Frozen stocks of the strains were prepared by inoculating tryptic soy broth (TSB; Difco, Becton

Dickinson, Sparks, Md.) and growing the cultures overnight at 37°C. The cultures were serially diluted in buffered peptone water (BPW; Difco, Becton Dickinson), sterile 50% (vol/vol) glycerol (Sigma, St. Louis, Mo.) was added to a final concentration of 15% (vol/vol), and cultures were then frozen at −70°C until use.

Inoculation. For a final concentration of 17 or 1.7 *E. coli* O157:H7 CFU/65 g of ground beef, an appropriate volume was removed from a freshly thawed glycerol stock containing approximately 10^3 CFU/ml of the particular *E. coli* O157:H7 strain and added to 45 ml of BPW. The stock cultures were then plated (50 μl) in duplicate onto tryptic soy agar (Difco, Becton Dickinson) plates for enumeration of the inoculum. Samples (600 g) of refrigerated ground beef obtained from various local retailers were placed into 3,500-ml nonfilter stomacher bags (BA 6042, Seward, Worthington, UK). Inoculated BPW (45 ml) was then added to the bags, which were thoroughly hand massaged and then stomached at 190 rpm for 30 s. After the 600 g of ground beef was batch inoculated, 65- or 375-g samples were removed and processed according to the various method protocols.

Detection methods. At the time of this evaluation, all of the PCR-based methods tested here were premarket tests in their final stages of validation before entering the retail market.

LightCycler *E. coli* (*eae*) Detection Kit. Probes, primers, and method for this kit were designed by Marshfield Clinic Laboratories, Food Safety Services (Marshfield, Wis.; marketed by Roche Applied Science, Indianapolis, Ind.). This method was

evaluated using 375 g of ground beef (as specified by the manufacturer). We also ran the tests with 65-g samples to allow comparison with other procedures that specified 65-g samples.

For the 65-g test, 70 g of spiked ground beef (65 g of ground beef and 5 ml of BPW from inoculum) was removed from the batch inoculation bag and placed in a 1,650-ml filter stomacher bag (B01318, Nasco Whirl-Pak, Fort Atkinson, Wis.), and 275 ml of BPW (preheated to 37°C) was added to the bag. The sample was stomached at 230 rpm for 30 s and incubated at 37°C with shaking at 125 rpm for 4 h. After 4 h, ca. 250 ml from each sample was poured off through the filter into a new 1,650-ml filter stomacher bag. The new sample bags were placed in Matrix/Pathatrix warming pots (Matrix Microsciences, Golden, Colo.), which had been preheated to 37°C. The Pathatrix apparatus was inserted into the bag on the opposite side of the filter from that where the sample was poured. Fifty microliters of Matrix anti-O157 immunomagnetic beads was added to the connector tubing, and the samples were recirculated for 1 h at 37°C. The beads were then collected, washed, and resuspended in 100 μl of BPW. DNA was extracted from the 100-μl bead-cell suspension using the MagNA Pure LC Instrument (Roche Applied Science). Three microliters of the extracted DNA was used in the PCR performed on the LightCycler Instrument (Roche Applied Science). Following bead collection, the sample bags were incubated overnight at 37°C. The following morning, all sample bags were processed by IMS and plating according to standard MRU IMS procedures.

For the 375-g test, 403 g of spiked ground beef (375 g of ground beef and 28 ml of BPW from inoculum) was removed from the bag and placed in a 3,500-ml filter stomacher bag (123 010, Bagpage 3500, Interscience, Weymouth, Mass.), and 1 liter of BPW (preheated to 37°C) was added to the bag. The sample was stomached at 190 rpm for 30 s and incubated at 37°C with shaking at 125 rpm for 4 h. After 4 h, two ca. 250-ml portions from each sample were poured off through the filter into two 1,650-ml filter stomacher bags. The new sample bags were placed in Matrix/Pathatrix warming pots (preheated to 37°C). The Pathatrix apparatus was inserted into each bag on the opposite side of the filter from that into which the sample was poured. Fifty microliters of Matrix anti-O157 immunomagnetic beads was added, and the samples were recirculated for 1 h at 37°C. The beads were washed with BPW and then processed according to the LightCycler E. coli (eae) Detection Kit procedures.

When this evaluation was performed the software for automated determination of positive or negative results was not available for this kit; therefore, the results were interpreted by the operator based on the assay protocol. Samples with positive amplification signals and melting curves with peaks of approximately 63°C were considered positive.

Assurance GDS for *E. coli* O157:H7, 6.5- and 8-h methods. For the Assurance GDS test (BioControl Systems, Inc., Seattle, Wash.), 70 g of spiked ground beef (65 g ground beef and 5 ml BPW from inoculum) was removed from the batch inoculation bag and placed in a 1,650-ml filter stomacher bag (B01318, Nasco Whirl-Pak). EHEC8 medium (27.6 g; BioControl Systems) and 290 ml of sterile water (preheated to 42°C) were added to the stomacher bag, the sample was stomached at 230 rpm for 30 s, and the remaining 290 ml of sterile water (preheated to 42°C) was added for a final volume of 580 ml of medium. The bag was hand massaged and incubated at 42°C for 6.5 h, and then 1 ml from each sample was removed to a deep-well block (each well containing 20 μl of sample preparation reagent). The sample bags were returned to incubation at 42°C. The deep-well block was sealed and processed according to the Assurance GDS for *E. coli*

O157:H7 protocol as written. Sample preparation reagent (20 μl) was mixed with each 1-ml sample in the deep-well block, and the block was vortexed at 900 rpm for 5 min. Samples were then processed by IMS using the PickPen eight-channel magnetic particle separation device. Recovered immunobeads were resuspended in 25 μl of resuspension buffer (BioControl Systems). Twenty microliters of the resuspension mixture was added to 5 μl of the polymerase buffer solution (BioControl Systems) and loaded onto a Rotor-Gene 3000 real-time PCR system (Corbett Research, Sydney, Australia).

After 8 h of incubation, 1-ml aliquots were removed from all samples that did not give a positive result in the Assurance GDS assay after 6.5 h. These aliquots were processed for *E. coli* O157:H7 detection in the same manner as the 6.5-h sample aliquots. Following removal of the sample aliquots, all samples were returned to incubation at 42°C. The following morning, all samples that gave negative results for the Assurance GDS assay were processed by IMS and plating according to standard MRU IMS procedures.

BAX System PCR assay for screening *E. coli* O157:H7, MP method. For the BAX *E. coli* O157:H7 MP method (DuPont Qualicon, Wilmington, Del.), 70 g of spiked ground beef (65 g ground beef and 5 ml BPW from inoculum) were removed from the batch inoculation bag and placed in a 1,650-ml filter stomacher bag (B01318, Nasco Whirl-Pak), and 580 ml of BAX medium (preheated to 42°C) was added to the bag. The sample was stomached at 230 rpm for 30 s and incubated at 42°C for 8 h. After 8 h, 5 ml was removed from each sample enrichment and lysed according to the BAX protocol. After lysis, 50 μl of the cell lysate was added to the lyophilized PCR reagent pellet and loaded into the BAX System cycler/detector instrument. The results were obtained with the BAX system T1.70 software package.

MRU standard method. After the aliquots for the BAX *E. coli* O157:H7 MP test were removed, the enrichments were returned to incubation at 42°C for an additional 4 h (total of 12 h at 42°C) and then held at 4°C overnight. The following morning, all sample bags were processed by IMS and plating according to standard MRU IMS procedures. IMS was performed to recover *E. coli* O157:H7 based on a previously described method (2). One milliliter of enrichment was added to 20 μl of anti-O157 immunomagnetic beads (Dynal, Lake Success, N.Y.) in 1.5-ml microtubes and rotated for 30 min. The beads were held in place by a magnet while the supernatant was removed. The beads were then washed three consecutive times with 1 ml of phosphate-buffered saline plus 0.1% Tween 20 (PBS-Tween; Sigma) and resuspended in 100 μl of PBS-Tween. After IMS, *E. coli* O157:H7 beads were spread directly onto (i) ntRainbow medium, which is Rainbow agar (Biolog, Inc., Hayward, Calif.) supplemented with novobiocin (20 mg/liter; Sigma) and potassium tellurite (0.8 mg/liter; Sigma), and (ii) ctSMAC, which is sorbitol MacConkey agar (Becton Dickinson) supplemented with cefixime (0.05 mg/liter; Dynal) and potassium tellurite (2.5 mg/liter; Dynal).

Pathatrix. The Pathatrix culture method (Matrix Microsciences) was tested in conjunction with the LightCycler *E. coli* (eae) PCR-based method and therefore the protocol used differed from that provided by the manufacturer. Four hundred three grams of spiked ground beef (375 g of ground beef and 28 g of BPW from inoculum) was removed from the batch inoculation bag and placed in a 3,500-ml filter stomacher bag (123 010; Bagpage 3500, Interscience), and 1 liter of BPW (preheated to 37°C) was added to the bag. The sample was stomached at 190 rpm for 30 s and incubated at 37°C with shaking at 125 rpm for 4 h. After

TABLE 1. *Sensitivity of various tests for* E. coli *O157:H7 inoculated at 17 CFU/65 g (100 CFU/375 g)*

Detection test[a]	Sample size (g)	Medium volume (ml)	No. of samples	No. (%) of positive samples[b]
Assurance GDS 6.5 h	65	585	57	57 (100)
LightCycler *E. coli* (*eae*)	375	1,000	57	57 (100)
Pathatrix	375	1,000	57	57 (100)
MRU	65	585	57	57 (100)

[a] The BAX *E. coli* O157:H7 MP test not included in these experiments because it was not available at the time of testing.

[b] Percentage was determined by dividing the number of positive samples from the indicated test by the number of samples tested.

4 h, ca. 250 ml was poured off through the filter into a 1,650-ml filter stomacher bag, and the new sample bags were placed in Matrix/Pathatrix warming pots (preheated to 37°C). The Pathatrix apparatus was inserted into each bag on the opposite side of the filter from where the sample was poured, and 50 μl of Matrix anti-O157 immunomagnetic beads was added to the connector tubing. The samples were recirculated for 1 h at 37°C, and then the beads were washed and plated directly to ctSMAC and nt-Rainbow agar plates.

Specificity for *E. coli* O157:H7. A subset of the MRU strain collection was screened in pure culture using only the detection portion (no IMS) of the PCR-based detection systems to identify those strains that would be recognized as *E. coli* O157:H7. For this experiment, pure cultures were grown overnight in TSB and then diluted in BPW to 10^4 CFU/ml. A portion of the diluted samples was used for detection.

Once the cross-reacting strains were identified, frozen stocks of those strains were made as described for *E. coli* O157:H7. Detection experiments using the complete processes and spiked ground beef samples were conducted to determine whether the growth media or the immunomagnetic separation phase would prevent the false-positive detection of these strains. The experiments were performed using methods similar to those described for O157:H7. For these experiments, the inoculum was added at 17 CFU/65 g (100 CFU/375 g).

Statistics. The various test results were compared in two-by-two contingency tables using the chi-square test from SAS statistical software version 6.12 (SAS Institute, Inc., Cary, N.C.).

RESULTS

Sensitivity for *E. coli* O157:H7. Prior to inoculation experiments, pure cultures were tested, and all commercial tests evaluated in this project correctly identified the five genetically distinct *E. coli* O157:H7 strains used in this study.

The first set of experiments was performed to evaluate the ability of the tests to detect *E. coli* O157:H7 that had been inoculated into ground beef at 17 CFU/65 g (100 CFU/375 g). The tests evaluated in these experiments were the LightCycler *E. coli* (*eae*), Assurance GDS 6.5 h, MRU, and Pathatrix. The BAX *E. coli* O157:H7 MP was not available at the time these experiments were conducted, so it was not evaluated at this inoculation concentration. All of the methods evaluated detected 100% of the samples inoculated with *E. coli* O157:H7 at the 17 CFU/65 g (100 CFU/375 g) concentration (Table 1).

The lower limits of detection were evaluated using ground beef inoculated at 1.7 CFU/65 g (10 CFU/375g). The LightCycler *E. coli* (*eae*) test was the only one for

which a particular sample size, 375 g, was specified by the manufacturer. The other methods allowed the user to determine the amount of ground beef used in a sample. Sixty-five grams was chosen as the sample size to be evaluated because it is an amount commonly used in the beef processing industry for product testing. Because 65 g was not the specified amount for the LightCycler *E. coli* (*eae*) test, this test was performed with both 65- and 375-g samples. This approach allowed direct comparisons to be made regarding the sensitivity of all methods under identical conditions of inoculation (65-g samples) while also performing the tests according to the manufacturer's directions (375-g samples).

Three PCR-based tests were evaluated at this low concentration of inoculum. The Assurance GDS test was evaluated with both 6.5- and 8-h enrichment times. The Assurance GDS 6.5-h method identified 56 (41%) of 136 spiked samples as positive for *E. coli* O157:H7 (Table 2). After 8 h of incubation, the number of samples determined to be positive rose to 77 (57%) of 136. The BAX *E. coli* O157:H7 MP test was able to detect *E. coli* O157:H7 in 76 (57%) of 134 spiked samples. For the LightCycler *E. coli* (*eae*) evaluated using 65-g samples, 82 (65%) of 126 spiked samples were considered positive for *E. coli* O157:H7. There was no significant difference ($P > 0.05$) between the BAX *E. coli* O157:H7 MP, Assurance GDS 8 h, or LightCycler *E. coli* (*eae*) tests in the ability to detect *E. coli* O157:H7 inoculated at 1.7 CFU/65 g using 65-g samples. When the LightCycler *E. coli* (*eae*) test was evaluated on the larger 375-g samples inoculated at the same concentration, almost all of the samples (56 of 57, 98%) were identified as positive for *E. coli* O157:H7.

Two culture-based tests were also evaluated at this inoculation concentration. With the Pathatrix method, 56 (98%) of 57 spiked 375-g samples were positive for *E. coli* O157:H7, whereas with the MRU method, *E. coli* O157:H7 was isolated from 105 (78%) of 134 spiked 65-g samples.

Specificity for *E. coli* O157:H7. Because isolation and confirmation of the target bacteria is not a primary part of PCR-based testing, the specificity of these tests is very important. The specificity was evaluated in two parts. First, only the detection portion of each method was used to screen pure cultures of 68 non-O157:H7 *E. coli* strains. This group of strains contained both nontoxigenic and Shiga toxin–producing *E. coli* (Table 3).

Two O55:H7 strains cross-reacted in all three PCR-

TABLE 2. *Sensitivity of various tests for* E. coli *O157:H7 inoculated at 1.7 CFU/65 g (10 CFU/375 g)*

Detection test	Sample size (g)	Medium volume (ml)	No. positive samples/ no. of samples tested (%)[a]	No. positive samples/no. of samples confirmed inoculated (%)[a,b]
Assurance GDS				
6.5 h	65	585	56/136 (41) A	56/105 (53) A
8 h	65	585	77/136 (57) B	77/105 (73) B
BAX *E. coli* O157:H7 MP	65	585	76/134 (57) B	76/116 (66) B
LightCycler *E. coli* (*eae*)	65	275	82/126 (65) B	82/107 (77) B
	375	1,000	56/57 (98) D	56/57 (98) C
Pathatrix	375	1,000	56/57 (98) D	56/57 (98) C
MRU	65	585	105/134 (78) C	105/116 (91) C

[a] Prevalences in the same column that do not share a common letter are significantly different ($P < 0.05$).
[b] Number of positive samples as determined by indicated test/number of positive samples by PCR and culture methods.

based tests. Three O145:NM strains cross-reacted with both the LightCycler *E. coli* (*eae*) and the Assurance GDS tests. Three of four O103:H2 strains cross-reacted in the LightCycler *E. coli* (*eae*) test. None of the other strains tested, including seven *E. coli* O157 serotypes other than H7, produced positive results in any of the tests evaluated.

For the second phase of the specificity testing, a subset of those strains that were identified as cross-reactors from the first phase were inoculated into ground beef and processed through the complete testing protocol. This step was used to determine whether the growth media or the IMS procedures particular to a given method would prevent non-O157:H7 bacteria from generating positive signals. The O55:H7 samples did not produce positive signals in the two methods employing IMS, the LightCycler *E. coli* (*eae*) and the Assurance GDS, but did produce positive signals in the BAX *E. coli* O157:H7 MP (Table 4). Both the BAX *E. coli* O157:H7 MP and the Assurance GDS tests produced positive results for one of four samples spiked with O145:NM. The two samples producing positive results were not from the same inoculated sample. None of the O103:H2-spiked

samples produced positive results in any of the tests. Eight nonspiked samples were also processed. One of these samples generated a positive signal in the BAX *E. coli* O157:H7 MP assay.

Time of detection. Approximate minimum detection times for the PCR-based methods were 7.5, 9, and 12 h for the LightCycler *E. coli* (*eae*), Assurance GDS (8-h enrichment), and BAX *E. coli* O157:H7 MP methods, respectively. These times are minimum because they only account for the times of the individual processing steps and not sample handling between steps. The Assurance GDS and BAX *E. coli* O157:H7 MP methods involve minimal sample handling, whereas the LightCycler *E. coli* (*eae*) method requires considerably more handling. During the trial period preceding this evaluation, a second filtration step was found to be needed prior to the IMS procedure for both the LightCycler *E. coli* (*eae*) and Pathatrix methods to ensure adequate recovery of the beads following IMS. This deviation from the manufacturer's instructions was incorporated during this evaluation.

TABLE 3. *Specificity of detection only (no IMS) of various* E. coli *serotypes for three PCR-based tests*

E. coli serotype	STEC[a]	No. of samples	No. of positive samples by:		
			LightCycler *E. coli* (*eae*)	Assurance GDS	BAX *E. coli* O157:H7 MP
O103:H2	Yes	4	3	0	0
O111:H8	Yes	1	0	0	0
O145:NM	Yes	3	3	3	0
O111:NM	Yes	3	0	0	0
O157 other than H7	No	7	0	0	0
O26:H11	Yes	8	0	0	0
O55:H7	No	2	2	2	2
Orough:H11	Yes	1	0	0	0
Orough:H2	Yes	1	1	0	0
O8:H19	No	1	0	0	0
O20:H17	No	1	0	0	0
O118:H20	No	1	0	0	0
O49:Hunt	No	1	0	0	0
ECOR strains 1–34	No	34	0	0	0

[a] STEC, Shiga toxin–producing *E. coli*.

TABLE 4. *Specificity of complete PCR-based process (with IMS for those that apply) for* E. coli *serotypes*[a]

			No. of positive samples by:		
Target inoculum	*E. coli* serotype	No. of samples	LightCycler *E. coli* (*eae*)	Assurance GDS	BAX *E. coli* O157:H7 MP
17 CFU/65 g	O103:H2	4	0	0	0
	O145:NM	4	0	1[b]	1[c]
	O55:H7	4	0	0	4[c]
Nonspiked		8	0	0	1[c]

[a] Sixty-five–gram samples were used with all tests.

[b] Positive by Assurance GDS at 6.5 and 8 h but not by IMS and plating from the same enrichment.

[c] Positive by BAX *E. coli* O157:H7 MP but not by MRU IMS and plating from the same enrichments. The positive BAX *E. coli* O157: H7 MP result from the O145:NM-spiked sample probably is not due to the O145:NM strain (from results on the pure culture, Table 3) but rather is a spurious positive signal or cross-reactivity with an unknown member of the bacterial flora associated with that particular sample. This was not the same sample that produced a positive result in the Assurance GDS test.

Time of detection is also a function of sample capacity. The LightCycler *E. coli* (*eae*) method is limited to five samples per instrument during the IMS step and 32 samples per instrument for the DNA extraction and detection steps. The Assurance GDS method can easily handle 96 samples at the IMS and DNA extraction steps, with the detection instrument accepting either 36 or 72 samples depending on instrument model. The BAX *E. coli* O157:H7 MP method does not have an IMS step and can accommodate up to 96 samples per detection instrument. The culture-based methods add at least 9 h to the time of detection. The Pathatrix method is limited to five samples per instrument at the IMS step.

DISCUSSION

Pathogen testing has long been used in food production environments as a means of process and quality control, tracking of contamination sources, and monitoring regulatory compliance. The methods used have been evolving as more sensitive and rapid techniques have become available. Currently, several beef processors are employing test-and-hold strategies as a further measure to ensure that contaminated product is not released into commerce (6). Such a system requires rigorous testing to fulfill its objectives. The testing methods (i) must be rapid, because ground beef is a highly perishable product, (ii) must have low numbers of false-positive results to ensure wholesome product is not needlessly discarded, and (iii) must have no false-negative results, which would defeat the purpose of such a system. The most important of these issues is that of false-negative results.

False-negative results can come in one of two forms. First, a test may not detect a certain subtype of *E. coli* O157:H7. A wide range of genetic variability has been identified among *E. coli* O157:H7 strains (1, 12, 18, 19). Therefore, genetics-based tests may focus on a target that is not present in all O157:H7 strains. Five *E. coli* O157:H7 strains were used in the sensitivity experiments in the present study. These strains previously were determined to be genetically different from one another based on results of PFGE (1). The strains were isolated from samples collected at various commercial beef processing plants and represent major subtypes within three relatedness clusters derived from the PFGE typing of a total of 343 *E. coli* O157:H7 strains (1, 10). All five strains were accurately detected by each of the tests evaluated in this study.

The second type of false-negative result is not as well defined and comes from testing methods in which the sensitivity is simply insufficient for the application in which the method is used. *E. coli* O157:H7 has a low infectious dose; a dose as low as 100 organisms has been considered sufficient to cause disease (30, 31). Therefore, the various testing methods must be able to detect less than 100 organisms per sample. In this study, tests were evaluated for their ability to detect *E. coli* O157:H7 at concentrations of 17 and 1.7 CFU/65 g, where a typical ingested dose would be less than the 100 CFU infective dose. These concentrations of inoculum are equivalent to 30 CFU and 3 CFU, respectively, in 0.25 lb (0.11 kg) of ground beef. The results indicate that at 17 CFU/65 g all of the methods tested were able to detect *E. coli* O157:H7 100% of the time.

The sensitivity experiments described here were done with spiked samples. When evaluating methods with short enrichment times, the nature of the inoculum becomes very important. Samples should be spiked with an inoculum that exactly reproduces the bacterial growth characteristics, especially the lag phase, that would be found in naturally contaminated samples. We presume that using an inoculum that was frozen at −70°C from a stationary phase culture is a good representative; however, even the best representative may differ from a natural contaminant in some aspects.

The tests were also evaluated at a lower concentration of inoculum, 1.7 CFU/65 g. Using a 65-g sample, the PCR-based methods were similarly sensitive. Both the Assurance GDS 8 h and the BAX *E. coli* O157:H7 MP tests correctly detected O157:H7 in 57% of the samples, whereas the percentage of correct LightCycler *E. coli* (*eae*) test results was slightly higher at 65%. The efficiency of these tests could be a result of the effective volume that is used in the detection portion of the method. For the BAX *E. coli* O157: H7 MP method, which does not use an IMS step, only 1.25 μl of the enrichment is actually placed into the PCR tube. Both the Assurance GDS and LightCycler *E. coli* (*eae*)

methods employ an IMS step that concentrates the target cells and presents a larger effective volume of enrichment sample for detection. The Assurance GDS system performs IMS from 1 ml of enrichment sample, whereas the LightCycler *E. coli* (*eae*) method uses 250 ml, leading to effective sample volumes of 0.8 and 83 ml, respectively.

Of the three PCR-based detection systems, the LightCycler *E. coli* (*eae*) was the only method that did not have an automated system for determination of positive or negative results; determination was left to the operator. This approach cannot be entirely objective and may have introduced bias, either positive or negative, into the results. Therefore, the results reported here for this test could be higher or lower than they would have been with automated determination.

The MRU method was also evaluated with an inoculum of 1.7 CFU/65 g using 65-g samples. *E. coli* O157:H7 was detected in 78% of the samples. The higher detection rate for this method most likely can be attributed to the longer enrichment time.

When 375-g samples were tested, both the LightCycler *E. coli* (*eae*) and the Pathatrix methods correctly identified 98% of the samples as positive. The larger sample has more pathogen cells at a given inoculum concentration, leading to a better chance that O157:H7 will be detected.

For inocula at low concentrations (1.7 CFU/65 g), it is difficult to ensure that all samples have been inoculated; therefore we have included a data correction factor, taking into account only those samples that could be confirmed as inoculated. For each method type, a second detection method was employed on the same enrichment sample to determine how many of the samples that were not positive by the original detection method were inoculated and were simply missed for one reason or another. For example, 82 (65%) of the 126 LightCycler *E. coli* (*eae*) 65-g samples were positive by PCR assay. The 44 negative samples were further incubated overnight and then processed by IMS and plated (Fig. 1). Of those 44 samples, 25 were positive by culture detection. Thus, 107 of the samples were positive by either PCR assay or culture, indicating that at least 107 samples were inoculated and giving 82 of 107 or 77% (Table 2) for the corrected percentage of positive samples. The Assurance GDS enrichments were handled in a similar fashion. For the BAX *E. coli* O157:H7 MP enrichments, samples were removed for PCR assay at 8 h, and the enrichment cultures were incubated another 4 h at 42°C and held at 4°C overnight. These enrichment cultures were then used for the MRU procedure. By analyzing each sample by two different methods, it was possible to determine an absolute minimum number of samples that actually received cells in the inoculation procedure. Although there should be no difference in the distribution of truly inoculated samples between the individual methods, this approach provides a minimum (corrected data) and maximum (total) number of inoculated samples for evaluation.

Only 105 of the 136 Assurance GDS samples could be confirmed as inoculated, leading to corrected positive results of 53 and 73% for the 6.5- and 8-h incubations, respectively. Not all samples could be confirmed as inoculated for the BAX *E. coli* O157:H7 MP and MRU enrichments, giving corrected positive results of 66 and 91%, respectively. Because only 107 of the LightCycler *E. coli* (*eae*) 65-g sample enrichments were confirmed as inoculated, the percentage of positive results was corrected to 77%. Inoculation of all of the 375-g sample enrichments was confirmed, so no correction was needed.

These corrected data should be interpreted with some caution because the final result is heavily influenced by the ability of the secondary test to detect *E. coli* O157:H7. If the secondary test is substantially less sensitive at detecting O157:H7, the corresponding primary test will have a higher corrected sensitivity than it would have if all of the secondary tests had equal sensitivity.

Specificity for *E. coli* O157:H7. Several PCR-based detection methods for *E. coli* O157:H7 have been developed previously that targeted genes for the Shiga toxins or the O157 antigen (8, 13, 14, 23, 25). However, these targets are not specific to *E. coli* O157:H7, and tests using such targets have high rates of false-positive results (5, 11). In this evaluation, all three tests identified the target sequences in the *E. coli* O55:H7 strains. This result is not surprising because serotype O55:H7 is believed to be the precursor and closest genetic relative to the O157:H7 (11, 29). The DNA sequence targets for the Assurance GDS and LightCycler *E. coli* (*eae*) tests were found in one and two other serotypes, respectively. Both of these *E. coli* serotypes produce Shiga toxin, and their genomes may be highly homologous with that of serotype O157:H7.

Just because a strain possesses the DNA target for the Assurance GDS or LightCycler *E. coli* (*eae*) tests does not mean that these tests will produce false-positive results. Prior to detection, both of these methods employ an IMS step, which targets the O157 antigen. In theory, this step would prevent all strains except those expressing the O157 antigen from being present in the final detection reaction. In reality, there can be some nonspecific binding that allows non-O157 strains to pass through, as occurred in 1 of 12 runs for the Assurance GDS test but did not occur at all for the LightCycler *E. coli* (*eae*) tests. Because the BAX *E. coli* O157:H7 MP method does not employ an IMS step, there is no process to prevent cross-reacting strains from producing false-positive results. The BAX *E. coli* O157:H7 MP test also was the only one of the five methods that gave a positive result for a nonspiked negative control. Eight such samples were included for each test. The cause of this false-positive result is unknown.

The last factor of the analysis is time of detection, i.e., the time it takes for a sample to be run and the number of samples that can be processed at a given time. Both of the culture-based methods, MRU and Pathatrix, are considered lengthy because of the long incubation period needed for the agar plates. The PCR-based methods have similar times, especially if sample-handling times are included. The Assurance GDS and BAX *E. coli* O157:H7 MP involve very little sample handing, but that is not the case for the LightCycler *E. coli* (*eae*) method, which requires substantial sample manipulation prior to and after the IMS step.

Sample throughput is a concern for beef processors that have multilot sampling protocols. The LightCycler *E. coli* (*eae*) and Pathatrix methods are limited to five samples per machine at the IMS step, so several units are required for appreciable throughput. The LightCycler *E. coli* (*eae*) method also is limited to 32 samples per machine for the DNA prep and detection steps. The sample capacity of the Assurance GDS and BAX *E. coli* O157:H7 MP methods are only limited at the detection step. The Assurance GDS system can handle 36 or 72 samples, depending on the sample rotor used, and the BAX *E. coli* O157:H7 system accepts 96 samples.

In weighing all three factors, the PCR-based systems all had comparable performance. There are advantages for each system, but nothing that firmly placed one system over the others. The culture-based systems were more sensitive, but the detection times (21 to 48 h) were at least 9 h longer than those of the PCR-based methods (7.5 to 12 h).

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