FOOD BIOLOGICAL CONTAMINANTS

Assurance® Enzyme Immunoassay Eight Hour Method for Detection of Enterohemorrhagic *Escherichia coli* O157:H7 in Raw and Cooked Beef (Modification of AOAC Official Method 996.10): Collaborative Study

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AOAC Official Method 996.10, Assurance® Enzyme Immunoassay (EIA) for *Escherichia coli* O157:H7 (EHEC), was modified to incorporate a new enrichment protocol using BioControl EHEC8™ medium for testing raw and cooked beef. Foods were tested by EIA and the U.S. Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) enrichment conditions and the FDA *Bacteriological Analytical Manual* (BAM) isolation and confirmation techniques. A total of 14 collaborators participated. Raw and cooked ground beef were inoculated with *E. coli* O157:H7 at 2 different levels: a high level where predominantly positive results were expected, and a low level where fractional recovery was anticipated. Collaborators tested 378 test portions and controls by both the 8 h EIA and the USDA/FSIS enrichment methods, for a total of 756 test portions. Of the 378 paired test portions, 75 were positive and 212 were negative by both methods. Thirteen test portions were presumptively positive by EIA and could not be confirmed culturally; 30 were negative by EIA, but confirmed positive by culture; and 65 were negative by the culture method, but confirmed positive by the EIA method. There was no statistical difference between results obtained with the Assurance EIA for EHEC 8 h method and the culture method for raw ground beef. The Assurance EIA had a significantly higher recovery for cooked beef.

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A multilaboratory collaborative study was conducted to demonstrate the equivalence of the Assurance® Enzyme Immunoassay (EIA) 8 h enrichment protocol for the detection of *Escherichia coli* O157:H7 (EHEC) to the U.S. Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) enrichment procedure (1) and the FDA *Bacteriological Analytical Manual* (BAM) isolation and confirmation techniques (2) for raw and cooked beef products. The Assurance EIA for EHEC is AOAC Official Method **996.10**, which consists of an overnight (18–28 h) enrichment protocol (3). This study describes a method modification that incorporates an 8 h enrichment protocol for use with raw and cooked beef test portions.

EHEC has recently been recognized as a pathogen, first linked with human disease in 1982 (4, 5). An extensive epidemiological study in 1995 reported a strong correlation between eating rare ground beef and EHEC infections (6). Many outbreaks have since been linked to the consumption of ground beef (7–11), including a large outbreak in Washington in 1993 (12–16). The presence of EHEC in ground beef and cattle herds (17–21) still presents a significant health risk. Most of the current methods require 20 or more hours of enrichment before test portions can be analyzed with a rapid test kit assay (3, 22–24).

A proprietary medium was developed to support the growth of *E. coli* O157:H7 to detectable levels in raw and cooked beef products in 8 h. A 25 g test portion is weighed into 225 mL BioControl EHEC8™ broth and incubated for 8 h at 42°C. A 4 mL aliquot is heat-inactivated at 100°C for 10 min, and cooled to room temperature; 0.1 mL each of test broth and positive controls is then added to the plate and incubated. After incubation with an antibody–enzyme conjugate specific for *E. coli* O157:H7, unbound conjugate is washed away. The substrate *p*-nitrophenylphosphate is added and positive test samples yield a colored product that is read with a plate reader at 405–410 nm. A reading ≥0.3 absorbance units

indicates a presumptive positive and must be confirmed by culture method.

Study Design

Raw and cooked beef were inoculated with EHEC at 2 levels: a high level where predominantly positive results were expected, and a low level where fractional recovery was anticipated. Each collaborator received 18 paired test portions (25 g/test portion) for a total of 36 test portions, of which 12 were uninoculated controls, 12 were inoculated at the low level, and 12 represented contamination at the high level. Test portions were delivered on dry ice via overnight mail and arrived during the week preceding testing.

Collaborative Study

Test Portion Preparation

Strains of *E. coli* O157:H7, used as the source of inocula, were grown in Tryptic soy broth (TSB) for 18–24 h at 35°C. The raw and cooked beef test portions received inocula of stationary phase cells sufficient to achieve 2 seed levels. After artificial contamination, the beef (from each level) was thoroughly mixed and then divided into 25 g test portions. Test portions were randomized and stored frozen for a minimum of 1 week before use. Most probable number (MPN) procedures were conducted on the day of test initiation. To determine the *E. coli* O157:H7 level per gram of beef, test portions of 100, 10, 1, and 0.1 g were evaluated in triplicate by the USDA/FSIS enrichment procedure (1) followed by FDA BAM isolation and confirmation techniques (2).

Analysis

Collaborators were given 2 sets of identically labeled test portions. One set was tested by the culture method and the other by the test method. Collaborators were instructed to analyze the randomly paired test portions using the USDA/FSIS enrichment, modified EC medium + novobiocin (mEC + N), and by BioControl EHEC8 broth. After 8 h of incubation, 4 mL enriched EHEC8 broth was heat-inactivated at 100°C for 10 min. These test broths were subsequently analyzed on EHEC EIA.

Confirmation

EHEC isolation and confirmation were performed on all enriched test broths according to the FDA BAM culture method (2). All USDA/FSIS enrichment broths were plated on Tellurite-Cefixime Sorbitol MacConkey agar (TC-SMAC) after 6 h of incubation and at the end of the incubation period (total of 20–24 h incubation). For the test method, appropriate dilutions of the EHEC8 broths were plated onto TC-SMAC after 8 h incubation. All TC-SMAC plates were incubated overnight at 37°C. At least 10 suspect colonies from the TC-SMAC plates were selected for confirmation.

Statistical Analysis

A paired statistical analysis of the methods was performed for each level using the method of McNemar (25). A chi square value of greater >3.84 indicated a significant difference at the 5% probability level. Sensitivity, specificity, and percent agreement were also conducted according to the method of McClure (26).

AOAC Official Method 996.10
Escherichia coli O157:H7 in Selected Foods

Enzyme Immunoassay (EIA)
First Action 1996
Revised 2002

[Applicable to detection of *Escherichia coli* O157:H7 (EHEC) in dairy foods, meats, poultry products, fruits, nutmeats, seafood, pasta, and liquid eggs using an 18–28 h enrichment protocol. Applicable to detection of *E. coli* O157:H7 in raw and cooked beef using an 8 h enrichment protocol.]

Caution: EHEC are pathogenic bacteria. Symptoms of infection include bloody diarrhea and cramping, little or no fever, and hemolytic uremic syndrome. Sterilize contaminated equipment and media before disposal or reuse.

See Table **996.10** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

In Assurance® Enzyme Immunoassay (EIA), antibodies with high specificity for *E. coli* O157:H7 antigens are bound to microwell plates. Enriched test broths and positive controls are added to the plates. If *E. coli* O157:H7 antigens are present, they bind to the antibodies in the microwells, forming antigen–antibody complexes. Nonreactive material is washed away. Specific antibody conjugated to alkaline phosphatase is added and binds to these complexes, and, after incubation, unbound conjugate is washed away. The substrate *p*-nitrophenylphosphate is added, and absorbance of the resulting colored product is read spectrophotometrically at 405–410 nm.

B. Media and Reagents

(**a**) *Wash solution concentrate*.—2% Polyoxyethylene 20 sorbitan monolaurate (Tween 20) in water.

(**b**) *Liquid substrate*.—*p*-Nitrophenylphosphate solution, 4.33mM; 1.6 mg disodium salt/mL.

(**c**) *Positive control*.—Stabilized, inactivated *E. coli* O157:H7 antigen.

(**d**) *Conjugate solution*.—Specific antibodies to *E. coli* O157:H7 conjugated to alkaline phosphatase.

(**e**) *Stop solution*.—20% Ethylenediaminetetraacetic acid (EDTA) in water.

(**f**) *Antibody-coated microwells*.—Microwell strips, each well coated with *E. coli* O157:H7 antibody; 96-well holder, and plastic cover.

Table 996.10. Interlaboratory study results for detection of *E. coli* O157:H7 in raw and cooked beef by EHEC EIA and culture methods

Food type	Level	MPN/g	No. of labs	No. of test portions	Test broths positive: EIA Pres.[a]	Test broths positive: EIA Conf.[a]	Test broths positive: Culture	Chi square[b]	Sensitivity rate, %[c]: EIA	Sensitivity rate, %[c]: Culture	Incidence of false negatives among total positive samples, %[d]: EIA	Incidence of false negatives among total positive samples, %[d]: Culture	Specificity rate, %[e]: EIA	Incidence of false positives among total negative samples, %[f]: EIA	Agreement between EIA and culture methods, %[g]
Raw ground beef	Low	0.007	10	60	27	28	16	2.2	80	46	20.0	54.0	100	0	52
	High	0.147	10	60	52	55	53	0.0	96	93	4.0	7.0	100	0	85
	Uninoculated	NA[h]	10	60	1	0	0	—	—	—	—	—	—	—	—
Cooked beef	Low	0.004	11	66	26	25	11	4.3	81	36	19.0	64.0	100	0	59
	High	0.014	11	66	39	37	21	6.8	86	49	14.0	51.0	100	0	57
	Uninoculated	NA[h]	11	66	6	0	0	—	—	—	—	—	—	—	—

[a] Pres. = presumptive positive data, Conf. = culturally confirmed data.

[b] Chi square, as defined by McNemar is $(|a - b| - 1)^2 / (a + b)$ where a = test portions positive by EIA and negative by culture, and b = test portions negative by EIA and positive by culture. A chi square value >3.84 indicates significance at $p < 0.05$.

[c] Sensitivity rate is defined as total number of confirmed positive test portions by EIA divided by total number of confirmed positive test portions by both the EIA and culture methods.

[d] Incidence of false negatives is 100 – sensitivity rate. Low number of total confirmed positives will result in high false negative data.

[e] Specificity rate is defined as total number of analyzed negative test portions by EIA divided by the total number of confirmed negative test portions by both the EIA and culture methods.

[f] Incidence of false positives is 100 – specificity rate.

[g] Rate reflects number of confirmed determinations that were equivalent between EIA and culture.

[h] NA = not applicable. This previously screened food lot was verified to be negative for EHEC.

(**g**) *For 8 h enrichment.—8 h EHEC enrichment medium (BioControl EHEC8™).*—Prewarm 225 mL sterile deionized water at 42°C overnight. On the day of use, aseptically transfer 10.6 g BioControl EHEC8 powder or one packet of premeasured single test dose medium into the sterile water. Mix gently to dissolve powder completely.

(**h**) *For 18–28 h enrichment.—Modified Trypticase (tryptic) soy broth (TSB) with novobiocin (mTSB + N).*—Mix 30.0 g TSB (dehydrated), 1.5 g bile salts No. 3, and 1.5 g anhydrous dipotassium phosphate in 1 L water. Sterilize by autoclaving at 121°C for 15 min. Prepare novobiocin solution by dissolving 100 mg novobiocin in 1 mL H_2O. Sterilize with 0.2 μm filter. On day medium is used, add 0.2 mL novobiocin solution to 1 L mTSB.

Items (**a**)–(**f**) are available as Assurance EHEC Enzyme Immunoassay (EIA) test kit from BioControl Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005.

C. Apparatus

(**a**) *Incubators.*—Maintaining 36 ± 1°C and 42 ± 0.5°C.

(**b**) *Micropipets.*—Accurately dispensing 0.1 and 4.0 mL.

(**c**) *Microplate washer.*—For washing microwell strips. Plastic squeeze bottle can be used.

(**d**) *Microplate reader.*—Photometer with 405–410 nm filter, capable of reading microwell plates. May include optional printer.

(**e**) *Vortex mixer.*—For mixing test broth tubes.

(**f**) *Water bath.*—Maintaining 100°C. Alternatively, flowing steam autoclave set at 100°C or dry heat block may be used.

(**g**) *Top loading balance.*—For weighing test portions. Measuring up to 1000 g, sensitivity of ± 0.1 g.

(**h**) *Stomacher.*—IUL Instruments masticator or equivalent for macerating test portions.

(**i**) *pH meter*

D. General Instructions

Store at 2–8°C when not in use. Let reagents equilibrate to room temperature before use. Include 2 positive control and 1 blank test wells with each run of test samples. Use separate pipets for each test sample and reagent to avoid cross-contamination. Kit reagents and components must be used as an integrated unit and may not be mixed with components from other manufacturing batches or sources. Use dedicated trough or glassware for each reagent to avoid cross-contamination. Do not use reagents after expiration date. Do not reuse microwells.

E. Preparation of Enriched Broth

(**a**) *Enrichment.*—(*1*) *8 h enrichment.*—Raw and cooked beef only: Aseptically weigh 25.0 g test portion into 225 mL prewarmed (42°C) BioControl EHEC8, **B**(**g**) and masticate 2 min. Incubate for 8 h at 42°C.

(*2*) *18–28 h enrichment.*—Food products: Aseptically weigh 25.0 g test portion into 225 mL mTSB + N, **B**(**h**). If larger test portion sizes are analyzed, proportionately increase volume of mTSB + N to maintain 1:9 dilution ratio. Mix well. Incubate overnight (18–28 h) at 35–37°C. For viscous materials (i.e., powdered dairy products) add 2.25 mL steamed (15 min) Triton X-100 per 225 mL mTSB + N at time of test portion addition and prior to incubation.

(**b**) *Inactivation.—For either 8 h enrichment or 18–28 h enrichment.*—Gently mix enriched test broth and let food particles settle. Transfer 4 mL enriched broth for 8 h protocol or 1 mL for 18–28 h protocol to test tube. Inactivate broth for 10 min at 100°C. Cool inactivated broths to room temperature before testing on EIA. Inactivated broths can be stored up to 4 days at 2–8°C. Store remaining broths, which have not been inactivated, at 2–8°C for confirmation of presumptive positives.

F. Enzyme Immunoassay Procedure

(*1*) Prepare wash solutions by adding 1.0 mL wash solution concentrate, **B**(**a**), to 100 mL water. Label container. This volume is sufficient to wash 40 wells. Wash solution is stable for 30 days when stored at 2–8°C.

(*2*) Install 405–410 nm filter in microwell plate reader.

(*3*) Fit required number of microwells, **B**(**f**), into holder. Reseal unused microwells. In addition to test wells, allow 3 extra wells for 2 positive controls and 1 blank. Carefully record positions of positive controls, blank, and tests in holder.

(*4*) Equilibrate enrichment broth to 25–37°C prior to assay. Do not mix inactivated test broths, **E**(**b**). Use a new pipet tip for each test. Pipet 100 μL aliquot of liquid from each test broth into microwell. Do not transfer food particles. Vortex the positive control, **B**(**c**), and pipet 100 μL aliquot into each positive control well. *Leave blank well empty.*

(*5*) Cover microplate with plastic cover provided and incubate 30 min at 35–37°C. Do not stack anything on top of microwell holder during incubation. Do not agitate plate during any incubation step.

(*6*) After incubation, wash each well 3 times using either procedure (**a**) or (**b**) below:

(**a**) *Washing procedure.*—Completely remove contents of well with microwell washer. Immediately fill wells completely with 250 μL wash solution, **F**(*1*). *Note*: Effective washing is critical to obtain accurate data. Avoid overfilling wells to prevent antigen carryover to adjacent nonreactive wells. Avoid underfilling wells to prevent ineffective washing.

(**b**) *Alternative washing procedure.*—Remove contents of well by inverting and vigorously tapping plate. Completely fill each well with wash solution, **F**(*1*), using clean wash bottle. Repeat twice for total of 3 aspiration/wash cycles per step.

(*7*) Immediately after removal of the last wash, mix conjugate solution, **B**(**d**), by gently inverting the bottle several times. Add 100 μL conjugate solution to each well, including control and blank wells. Cover plate with plastic cover and incubate 30 min at 35–37°C.

(*8*) After incubation, wash each well 3 times. Refer to washing procedure instructions, **F**(*6*).

(*9*) Immediately after removal of the last wash, add 100 μL substrate, **B**(**b**), to each well, including control and blank wells. Cover plate with plastic cover and incubate 30 min at 35–37°C. After incubation, *do not wash wells*. Pro-

ceed directly to **G**. If reading will be delayed, add 50 µL stop solution, **B**(**e**), to each well. Read within 1 h.

G. Reading

Read control and test well absorbances (A) at 405–410 nm. For valid results, the microwell plate reader must be calibrated against the blank well before test and control wells are read. Standardize reader by reading the blank well and adjusting absorbance to zero. Read the absorbance of each well, starting with the 2 positive controls. *Note*: When reader is standardized to blank well, certain test wells may read <0. This is not uncommon and indicates a negative result.

H. Interpretation of Test Results

(*1*) *8 h enrichment*.—*Positive control value*.—The positive control absorbance readings should be >0.8 A units. Absorbance readings below this value may indicate problems with the washing procedure. Contact BioControl Technical Services for more information.

Positive results.—Test wells with absorbance readings ≥0.3 are considered presumptive positive and need to be confirmed. Test wells with absorbance readings <0.3 are negative.

(*2*) *18–28 h enrichment*.—*Positive control value*.—The positive control absorbance readings should be >0.8 A units. Absorbance readings below this value may indicate problems with the washing procedure. Contact BioControl Technical Services for more information.

Positive results.—Calculate average value of the 2 positive control absorbance readings (in A units) and multiply by 0.25 to establish the cutoff value:

$$\frac{(PC1+PC2)}{2}\times 0.25 = \text{cutoff value}$$

where PC = positive control absorbance reading (in A units). Repeat positive controls for each test run. Any values above this cutoff value are considered presumptive positive and need to be confirmed. Test wells with absorbance readings lower than the cutoff are negative.

Note: Microwell plate reader linear range is variable depending on manufacturer's specifications. If PC is reported as "over" or a numerical value >2.5, use 2.5 A for calculation purposes.

Confirmation of positive EIA test portions.—Presumptive positive tests must be confirmed using culture methods as described in the current edition of *Bacteriological Analytical Manual*. Isolate from previously enriched broths.

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Results

Fourteen collaborators participated in the study. Each group analyzed 18 test portions by Assurance EIA for EHEC and 18 test portions by the culture method. Eleven collaborators analyzed both raw and cooked ground beef, one analyzed raw ground beef only, and 2 analyzed cooked ground beef only (Table 1). At the end of the study, valid data were submitted for 378 test portions comprising 252 inoculated test portions and 126 uninoculated test portions.

Table 1. Collaborator participation for EHEC EIA 8 h enrichment by food type[a]

Laboratory	Raw ground beef	Cooked beef
1	Y	Y
2	Y[b]	Y
3	Y[b]	N
4	Y	Y
5	Y	Y[c]
6	Y	Y
7	Y	Y
8	N	Y
9	Y	Y
10	Y	Y
11	N	Y[b]
12	Y	Y
13	Y	Y
14	Y	Y
Total[d]	12	13

[a] Y = collaborator analyzed this food type; N = collaborator did not analyze this food type.

[b] Laboratory did not follow study instructions/did not complete analysis. Results were not included in the statistical analysis for the designated food types.

[c] Uninoculated control samples were confirmed as *E. coli* O157:H7. Results were not included in the statistical analysis for the designated food types.

[d] Total number of laboratories participating in the analysis of this food type.

Of the 378 test portions, 75 were positive and 212 were negative by both EIA and culture methods. Additionally, 13 test portions were presumptively positive by EIA, but could not be confirmed culturally. A total of 30 test broths was negative by EIA, but confirmed positive by the culture method; 65 were negative by the culture method, but confirmed positive by the EIA method; and 4 were negative by EIA and culture method, but confirmed positive when EIA enrichment broths were subcultured to selective agar. These 4 test broths were included as false negatives for both EIA and culture methods.

Tables 2 and 3 present individual collaborator results. Results are analyzed by food type in the following sections. Table **996.10** summarizes interlaboratory study results as well as sensitivity and specificity data for each food type and inoculation level.

Raw Ground Beef

Twelve collaborators agreed to analyze raw ground beef. Collaborators 2 and 3 did not follow study instructions. Data from these collaborators were not included in the analysis.

Table 2. Analysis of raw ground beef by individual collaborators

	High-level portions						Low-level portions						Uninoculated portions					
Lab	2	6	7	10	14	17	3	4	9	12	13	16	1	5	8	11	15	18
EHEC EIA method[a]																		
1	+/+	+/+	+/+	–/+	+/+	+/+	+/+	+/+	+/+	+/+	–/–	+/+	–/–	–/–	–/–	–/–	–/–	–/–
4	+/+	–/–	+/+	+/+	+/+	+/+	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	+/–	+/–
5	+/+	–/–	–/–	–/–	+/+	+/+	–/–	–/–	–/–	+/–	+/+	–/–	+/–	–/–	–/–	–/–	–/–	–/–
6	+/+	+/+	+/+	+/+	+/+	+/+	+/+	–/–	–/–	–/–	–/+	+/+	–/–	–/–	–/–	–/–	–/–	–/–
7	+/+	+/+	+/+	+/+	+/+	+/+	–/+	+/+	–/–	+/+	+/+	–/–	–/–	–/–	–/–	–/–	–/–	–/–
9	+/+	+/+	+/+	+/+	+/+	–/–	–/–	–/–	+/+	+/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
10	+/+	–/+	–/+	+/+	+/+	+/+	+/+	–/–	–/–	+/+	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
12	+/+	+/+	+/+	+/+	+/+	+/+	–/–	–/–	–/–	–/–	–/–	+/+	–/–	–/–	–/–	–/–	–/–	–/–
13	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	–/–	+/+	+/+	–/–	–/–	–/–	–/–	–/–	–/–
14	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	–/+	–/–	–/–	–/–	–/–	–/–	–/–
Culture method[b]																		
1	+	+	+	+	+	+	–	–	+	–	+	–	–	–	–	–	–	–
4	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
5	+	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–
6	–	+	+	+	+	+	–	–	–	–	+	+	–	–	–	–	–	–
7	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
9	+	+	–	+	+	+	–	–	+	+	–	–	–	–	–	–	–	–
10	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–
12	+	+	+	–	+	+	+	+	–	–	–	+	–	–	–	–	–	–
13	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–
14	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–

[a] + = EHEC was detected in sample; – = EHEC was not detected in sample. (/) = First entry is presumptive result / second entry is confirmed result.

[b] + = EHEC was detected in sample; – = EHEC was not detected in sample.

Ten collaborators followed study instructions and appeared to have valid data as indicated by their summary of data worksheets (Table 2). Fractional recovery was observed for this food type.

Test portions inoculated at the low level contained 0.007 colony-forming units (CFU)/g. Seven test portions were confirmed positive and 25 were negative by both methods. Nineteen test portions were negative by culture, but confirmed positive by EIA, and 10 were confirmed positive by culture, but negative by EIA. There was one unconfirmed positive test portion by EIA. One test portion was negative by the culture method and by EIA, but was confirmed positive when subcultured from the EIA enrichment broth. This test portion was reported as a false negative for both the EIA and culture methods. Chi square analysis for the low inoculation level was 2.2, indicating that the methods are equivalent. There was no statistically significant difference between the EIA and culture methods in this test set.

Test portions inoculated at the high level contained 0.147 CFU/g. Forty-eight test portions were confirmed positive and 3 were negative by both methods. Four test portions were negative by culture, but confirmed positive by EIA, and 5 were confirmed positive by culture, but negative by EIA. Chi square analysis for the high inoculation level was 0.0, indicating that the methods are equivalent. There was no statistically significant difference between the EIA method and culture method in this test set.

Cooked Beef

Thirteen collaborators agreed to analyze cooked beef. Collaborator 5 reported uninoculated controls as confirmed positive for EHEC. Collaborator 11 did not complete the study. Data from these collaborators were not included in the analysis. Eleven collaborators followed study instructions and appeared to have valid data as indicated by data summary worksheets (Table 3). Fractional recovery was observed for this food type.

Test portions inoculated at the low level contained 0.004 CFU/g. Five test portions were confirmed positive and 35 were negative by both methods. Twenty test portions were

Table 3. Analysis of cooked beef by individual collaborators

	High-level portions						Low-level portions						Uninoculated portions					
Lab	4	8	10	12	15	18	2	3	6	9	14	17	1	5	7	11	13	16
EHEC EIA method[a]																		
1	+/+	+/+	+/+	+/+	–/–	+/+	–/–	–/–	+/+	+/+	+/+	+/+	–/–	–/–	–/–	–/–	–/–	–/–
2	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
4	–/–	–/–	–/–	–/–	+/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
6	+/–	+/+	+/+	+/+	+/+	–/–	–/–	+/–	+/–	–/–	–/+	+/–	+/–	–/–	+/–	–/–	+/–	–/–
7	–/–	–/–	–/–	–/–	–/+	–/–	–/–	–/–	–/–	–/–	–/–	–/+	–/–	–/–	–/–	–/–	–/–	–/–
8	+/+	+/–	+/+	+/+	–/–	–/–	+/+	+/+	–/–	+/+	–/–	+/+	–/–	+/–	–/–	–/–	–/–	+/–
9	–/–	+/+	+/+	+/+	–/–	–/–	–/–	–/–	–/–	–/–	+/+	–/–	–/–	–/–	–/–	+/–	–/–	–/–
10	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	–/–	–/–	+/+	+/+	–/–	–/–	–/–	–/–	–/–	–/–
12	+/+	+/+	+/+	+/+	–/–	+/+	+/+	–/–	–/–	–/–	+/+	–/–	–/–	–/–	–/–	–/–	–/–	–/–
13	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	–/–	+/+	–/–	–/–	–/–	–/–	–/–	–/–
14	–/–	+/+	–/–	+/+	+/+	+/+	–/–	+/+	+/+	–/–	–/–	+/+	–/–	–/–	–/–	–/–	–/–	–/–
Culture method[b]																		
1	–	–	+	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
4	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
6	–	–	+	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	–	+	+	+	–	+	–	–	+	–	–	+	–	–	–	–	–	–
9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	–	–	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–
12	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–
13	+	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
14	–	+	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–

[a] + = EHEC was detected in sample; – = EHEC was not detected in sample. (/) = First entry is presumptive result / second entry is confirmed result.

[b] + = EHEC was detected in sample; – = EHEC was not detected in sample.

negative by culture, but confirmed positive by EIA, and 8 were confirmed positive by culture, but negative by EIA. There were 3 unconfirmed positive test portions by EIA. Two test portions were negative by the culture method and by EIA, but were confirmed positive when subcultured from the EIA enrichment broth. These test portions were reported as false negatives for both the EIA and culture methods. Chi square analysis for the low inoculation level was 4.3, indicating a statistically significant difference between the EIA and culture methods. The EIA method recovered a significantly higher number of confirmed positive test portions than the culture method.

Test portions inoculated at the high level contained 0.014 CFU/g. Fifteen test portions were confirmed positive and 23 were negative by both methods. Twenty-two test portions were negative by culture, but confirmed positive by EIA, and 7 were confirmed positive by culture, but negative by EIA. There were 2 unconfirmed positive test portions by EIA. One test portion was negative by the culture method and by EIA, but confirmed positive when subcultured from the EIA enrichment broth. This test portion was reported as a false negative for both the EIA and culture methods. Chi square analysis for the high inoculation level was 6.8, indicating a statistically significant difference between the EIA and culture methods. The EIA method recovered a significantly higher number of confirmed positive test portions than the culture method.

Discussion

The Assurance EIA for EHEC is currently AOAC Official Method **996.10**, consisting of an overnight (18–28 h) enrichment protocol. In this method modification, an alternative enrichment protocol that uses the BioControl EHEC8 broth was equivalent to, or better than, the culture method in raw and cooked beef.

Conclusions

The data indicate no statistical difference between results obtained with the Assurance EIA for EHEC 8 h method and the culture method except for cooked beef, where the EIA had a significantly higher recovery.

Recommendations

It is recommended that the method applicability statement for Assurance EIA for the detection of EHEC be modified to include an 8 h enrichment procedure for raw and cooked beef products and be adopted as revised First Action.

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