Final Research Report

Validation of Composite Sampling for Detection of *Escherichia coli* O157:H7 in Raw Beef Trim and Raw Ground Beef

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Prepared for:

Cattlemen's Beef Promotion and Research Board
9110 E. Nichols Ave., Suite 303
Centennial, CO 80112-3450

Submitted by:
Randall Huffman, Ph.D.
American Meat Institute Foundation
1150 Connecticut Ave, NW, 12th Floor
Washington DC, 20036

Research conducted and Final Report Prepared by:

Ann Marie McNamara, Sc.D.
Wendy A. McMahon, M.S.
Ann Schultz
Vicki Aleo
Michael Curiale, Ph.D.
Silliker, Inc., Research Center
160 Armory Drive
South Holland, IL 60473

ABSTRACT

The beef industry responded to recent FSIS regulatory requirements by designing sampling and testing protocols to detect *E. coli* O157:H7 in raw ground beef and raw beef trim. The effects of sample compositing sizes, shortened 8-hr incubation time, and choice of rapid test kit on protocol test results were unknown. This research was the first comparative study to determine the limits of sample compositing sizes on detecting low levels of O157 in ground beef and beef trims, with the use of new, rapid (8-hr) test kits. Sample compositing sizes were evaluated for reliable detection of *E. coli* O157:H7 using Neogen Reveal, Strategic Diagnostic's Rapid Check, BioControl's VIP, and Qualicon's ABAX test kits. Test kits were challenged with low numbers of *E. coli* O157:H7 in composite sample sizes commonly used in industry and ICMSF sampling plans: 75 g, 125 g and 375 g. Composite samples were compared against the ability of each kit to detect *E. coli* O157:H7 in a 25 g sample (X^2 analysis). Three lots of ground beef and beef trim were evaluated. Test kit enrichment broths were evaluated for the ability to detect *E. coli* O157:H7 after 8 hr, 12 hr, and 16 hr of incubation. Sensitivity of detection of *E. coli* O157:H7 in composited samples depended on the test kit used. Industry can use these results to choose a rapid test kit, incubation time, and composite size to reliably detect *E. coli* O157:H7 in beef products and to design testing protocols to meet desired Food Safety Objectives.

BACKGROUND

The beef industry has responded to recent USDA FSIS regulatory requirements by designing sampling and testing protocols to detect *E.coli* O157:H7 in raw ground beef and raw beef trim.[1] A survey of the industry showed that there is no standard protocol being employed across the industry to detect *E.coli* O157:H7.[2] Protocols vary in the complexity of the sampling procedures, the amount of meat composited for testing, and the test kit utilized. One raw beef trim sampling and testing protocol met the criteria of an example Food Safety Objective intended to produce safe beef products.[3]

Two highly variable factors in these industry sampling and testing protocols are the choice of a test method and the size of samples composited. Most popular of the detection methods are test kits using lateral flow chromatography (LFC), ELISA or automated Polymerase Chain Reaction (PCR) formats. The AOAC International website lists approximately 54 test kits available to the industry to detect *E.coli* O157:H7 in foods.[4]

Until recently, ELISA and automated PCR test kits required 20-24 hr incubation of enrichment broths to increase test kit sensitivity to levels which would detect low numbers of

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[1] USDA's Food Safety and Inspection Service, Federal Register Notice, "*E.coli* O157:H7 contamination of beef products" (October 7, 2002, vol. 67, No 194, pp 62325-62334).

[2] McNamara, A.M. (2003). A survey of Current Industry Protocols for the Detection of *E.coli* O157:H7 in Raw Ground Beef and Raw Beef Trim. Final Report to the National Cattlemen's Beef Association.

[3] ICMSF, Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. Kluwer Acad. Plenum Pub., 2002. pp 313-332.

[4] AOAC International. Rapid Microbiological Test Kits. www.AOAC.org/test kits/microbiology/htm.

E.coli O157:H7 present in a beef sample. Test kit manufacturers now claim that new, proprietary enrichment broth formulas permit the detection of low numbers of *E.coli* O157:H7 within 8 hr. To date, there has been no comparative evaluation of these new rapid test kit enrichment broths to prove their ability to detect low numbers of *E.coli* O157:H7 in beef products.

Another change in the test kit industry is the adaptation of 20-24 hr, 96-well ELISA test kits to 8 hr LFC devices. LFC test kits have been widely replacing the 96-well ELISA in the industry due to their shortened incubation time and ease of handling/reading by relatively unskilled personnel.

The industry survey also showed that laboratory preparation of industry samples differs widely depending on the requirements of the protocol.[2] Highly variable is the amount of meat being composited for analysis. Survey results found that 25g, 75g, 225g or 375g of meat were common weights of meat being routinely composited for analysis. Early studies on the ability to composite samples and reliably detect *E.coli* O157:H7 in fresh red meat products concluded a 375g composite sample could be used.[5] However, these studies used primarily culture methods and were time-consuming. Subsequently, more rapid proprietary test kit methods were developed. Compositing studies of two of these methods have challenged these results and suggest no more than 125g can be composited and reliably detect *E.coli* O157:H7 in a LFC test kit and an automated PCR test.[6] Both of these test kits required 20-24 hr incubation of enrichment broths. Recently, improved enrichment broths have been developed for application with those kits, with even shorter incubation times (8-16 hrs). These methods have been widely implemented throughout the industry. However, no comparative evaluation of newer, rapid LFC or automated PCR test kits in an 8 hr incubation period format have been performed to determine the effects of sample composite size, enrichment broth formulation, and shortened incubation times on the ability of these kits to detect *E.coli* O157:H7 in raw ground beef or raw beef trim. Such an evaluation is imperative for the industry to understand the limitations of these test kits in designing sampling and testing protocols that meet both industry and regulatory expectations.

This research report performs a comparative evaluation of sample composite sizes for the reliable detection of *E.coli* O157:H7 by the new, rapid 8 hr LFC and automated PCR test kits. The four most widely sold 8 hr test kits were examined: Neogen Reveal 8 hr, Strategic Diagnostic's Rapid Check 8 hr, BioControl's VIP 8 hr, and Qualicon's ABAX 8 hr. Test kits were challenged to detect low numbers of *E.coli* O157:H7 in composite sample sizes commonly used in industry testing protocols and International Commission on Microbiological Specifications for Foods (ICMSF) sampling plans: 75g, 125g and 375g. These composite samples were compared against the ability of each test kit to detect *E.coli* O157:H7 in a 25g sample. Test kits were evaluated for the ability to detect *E.coli* O157:H7 after 8 hr, 12 hr, and 16 hr of incubation. Extended incubation times may provide the

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[5]Silliker, J.H., and Nickelson, R. (1995). Methods for Sampling and Compositing Fresh Red Meat Products for Analysis of Pathogenic and Indicator Bacteria. Final Report to The National Cattlemen's Beef Association (addendum of September 6, 1995). Englewood, CO: National Cattlemen's Beef Association.

[6] Curiale, M.S., W.A. Lepper, A. Schultz. 2002. Validation of the use of composite sampling for *Listeria Monocytogenes, Salmonella*, and *E.coli* O157:H7 in trims. Research project for National Cattlemen's Beef Association, unpublished.

increased sensitivity needed to reliably detect *E.coli* O157:H7 in larger composite sample sizes.

The results of this research are critical to the beef industry in order to design appropriate sampling and testing protocols to detect *E.coli* O157:H7 in raw beef products. Currently, the effects of sample compositing, shortened incubation time, and choice of rapid test kit on industry testing protocol results are unknown. This research was the first comparative study to determine the limits of sample composite sizes on detecting low levels of *E.coli* O157:H7 in new, rapid 8 hr test kit formats. The effect of increased enrichment incubation times was evaluated to compensate for loss of detection sensitivity in larger composite sample sizes. Results enable industry to correctly choose a new, rapid test kit, incubation time, and composite size to reliably detect *E.coli* O157:H7 in beef products and to design testing protocols to meet desired Food Safety Objectives.

MATERIALS AND METHODS

Test Organisms

Three *E. coli* O157:H7 isolates were used in the study. The reference numbers of *E. coli* O157:H7 were SLR1431 (ATCC 35150), SLR1486 (Salami Outbreak) and SLR1883 (Ground beef isolate, Penn State). Each isolate was maintained at -70°C in 15% glycerol for long-term storage. Working stock cultures were kept on Trypticase Soy Agar (TSA; BBL, Becton Dickinson and Co., Cockeysville, MD). Cultures for inoculation were grown in Trypticase Soy Broth (TSB; BBL, Becton Dickinson and Co., Cockeysville, MD) at 35°C for 18-24 hr. Numbers of colony forming units were determined on TSA incubated at 35°C for 24 +/- 2 hr. Broth cultures were stored at 4°C prior to product inoculation. Dilutions were prepared with Butterfield's phosphate buffer (0.3 mM, pH 7.2).

Preparation of Samples

Three production lots of ground beef were purchased from local grocers for inoculation and testing. Three production lots of trim were obtained directly from anonymous suppliers.

Portions of ground beef and trim were used for inoculation. The target inoculation level was 1 cell per enrichment. Cells per enrichment were kept constant throughout the composite sample sizes.

Ground Beef: Each inoculated portion consisted of 1,600g. After inoculation, the sample was mixed thoroughly and 48 samples of 25g were prepared. Samples were stored for 36 hr at 4°C to adapt the inoculum to the product. The 25g samples were combined with 0g, 50g, 100g, or 350g of non-inoculated meat just prior to analysis to form test samples for each of four methods at each of four sample sizes: 25g, 75g, 125g, and 375g. Sample sizes were evaluated in triplicate, testing three separate instances of each of the four composite sizes. Separate portions were inoculated for each of the three isolates.

Trim: Forty-eight, 25g pieces of trim were surface-inoculated. After inoculation, the samples were stored for 36 hr at 4°C to adapt the inoculum to the product. These were combined with 0g, 50g, 100g, or 350g of non-inoculated meat just prior to analysis to form test samples for each of four methods at each of four sample sizes: 25g, 75g, 125g, and 375g.

Sample sizes were evaluated in triplicate, testing three separate instances of each of the four composite sizes. Separate portions were inoculated for each of the three isolates.

Inoculated product was used for determination of the *E. coli* O157:H7 inoculation level by Most Probable Number (MPN) analysis. Non-inoculated portions of each product were prepared as negative controls. Three uninoculated, 25g samples for each beef product lot (3) and for each test method (4) and for each *E. coli* O157:H7 strain (3) were tested for a total of 108 negative control samples each for ground beef and trim.

Microbial Analyses

Samples were analyzed for *E. coli* O157:H7 by four enrichment methods: Neogen's Reveal 8 hr, BioControl's VIP 8 hr, Strategic Diagnostic's RapidChek 8 hr, and Qualicon's automated BAX PCR 8 hr. The pre-enrichments for the test methods were modified to accommodate the 75g, 125g, and 375g sample sizes. The added broth volumes were 675 ml, 1125 mL, and 3375 mL, respectively. These modifications maintained the 1:9 sample to broth ratio specified by each test method. All methods were analyzed according to the manufacturer's package insert.

Enrichment broths were incubated and tested at three time points: 8 hr, 12 hr, and 16 hr. Extended incubation times were evaluated to test the hypothesis that increased incubation times may increase the sensitivity of the test kits in detecting low levels of *E. coli* O157:H7 in larger composite size samples.

***E. coli* O15:H7 Methods**

1. Neogen Reveal's 8 hr

Each sample was combined with 9 volumes of Reveal enrichment broth and homogenized (Stomacher® 400 or 3500, Tekman Co., Cincinnati, Ohio) for 2 min. Following incubation at 8 hr, 12 hr and 16 hr at 42 ± 1 °C, enrichments were tested using Neogen's Reveal assay. Manufacturer's instructions for the test method were followed. Suspect results from assay were considered confirmatory when uninoculated control sample results were negative.

2. Strategic Diagnostic's RapidChek 8 hr

Each sample was combined with 9 volumes of RapidChek enrichment broth and homogenized (Stomacher 400 or 3500, Tekman Co., Cincinnati, Ohio) for 2 min. Manufacturer's instructions for the test method were followed. Following incubation at 8 hr, 12 hr and 16 hr at 42 ± 1 °C, enrichments were tested using SDI's RapidChek assay. Suspect results from assay were considered confirmatory when uninoculated control sample results were negative.

3. Qualicon's Automated BAX Procedure – 8 hr

Each test sample was combined with 9 volumes of BAX System media and homogenized for 2 min. Manufacturer's instructions for the test method were followed. Following incubation at 8 hr, 12 hr and 16 hr at 42 ± 1 °C, enrichments were tested using Qualicon's

Automated BAX system. Enrichments from suspect samples from the assay were considered confirmatory when uninoculated control sample results were negative.

4. BioControl's VIP– 8 hr

Each test sample was combined with 9 volumes of BioControl's EHEC media and homogenized (Stomacher® 400 or 3500, Tekman Co., Cincinnati, Ohio) for 2 min. Manufacturer's instructions for the test method were followed. Following incubation at 8 hr, 12 hr and 16 hr. at 42 ± 1 °C, enrichments were tested using BioControl's VIP assay. Enrichments from suspect samples from the assay were considered confirmatory when uninoculated control sample results were negative.

Control Samples

Enrichments for uninoculated control samples testing positive for *E. coli* O157:H7 by any test kit were confirmed to detect the presence of *E. coli* O157:H7 or cross-reacting bacteria by culturing onto CTSMAC agar. In addition, inoculated samples for the same analyses testing positive were confirmed for the presence of *E. coli* O157:H7. Samples with typical colonies were confirmed serologically.

Most Probable Number Analysis

Determination of the *E. coli* O157:H7 contamination level were performed by a 4 dilution, 3 tube Most Probable Number (MPN) procedure using USDA, FSIS method modified EC broth with novobiocin[7]. Enrichments were tested using Neogen's Reveal assay.

Most Probable Number Calculation and Statistical Analysis

A 4 dilution, 3 tube Most Probable Number (MPN) table[7] was used to determine MPN index values. Microsoft Excel functions were used to calculate X^2 test statistics. The Yates correction factor for small numbers of samples was used in the calculation. Differences were considered significant at the $p<0.05$ level.

Data Analysis

For the purpose of evaluating the **efficacy of compositing** for each method, the results for the 25g sample size enriched for 8 hr were treated as the expected results. The composite results at the 75g, 125g, and 375g sample sizes were compared to the 25g, 8 hr results using the X^2 test. The compositing scheme was interpreted as equivalent to the non-compositing result if the probability (p value) of the X^2 was greater than or equal to 0.05. The compositing scheme was interpreted as different from the non-compositing result if the probability (p value) of the X^2 was less than 0.05.

[7] Garthright, W.E. 1995. Most probable number from serial dilutions. Food and Drug Administration, Bacteriological Analytical Manual, AOAC International, Gaithersburg, MD. Appendix 2.

RESULTS AND DISCUSSION

Test kits evaluated were chosen by the sponsors to represent the most commonly used 8 hr test kits by their members. All test kits have either AOAC Research Institute (ABAX, Rapid Chek) or Official Methods of Analysis (Reveal, VIP) approval. The intent of this study was not to repeat the AOAC validations but to validate the effectiveness of compositing various sample sizes for the detection of *E. coli* O157:H7 compared to the approved AOAC sample size result for 25g samples at 8 hr of incubation. The compositing scheme was interpreted as equivalent to the non-compositing result if the probability (p value) of the X^2 test was greater than or equal to 0.05. The compositing scheme was interpreted as different from the non-compositing result if the probability (p value) of the X^2 was less than 0.05.

Ground Beef Compositing

Compositing was less effective for the recovery of *E. coli* O157:H7 in raw ground beef compared to raw beef trim. Although the reason for this discrepancy was not evaluated in this study, higher numbers of background flora typically found in ground beef may out-compete the low numbers of *E. coli* O157:H7 present and interfere with its detection.

The ground beef inoculation level ranged from 0.18 to 1.88 cells per enrichment. All non-inoculated ground beef samples tested negative for *E. coli* O157:H7. Negative results were independent of sample size and test method. Inoculation sets that produced only positive results or only negative results were excluded from analysis so as not to bias the statistical analysis.

The total number of inoculated ground beef samples from usable inoculation sets for each method equaled 180 samples. Each sample was assayed at three time points for a total of 540 assays. There was no statistical difference between ABAX (234 positive assays), Reveal (222), and RapidChek (194) in detecting positive samples ($p>0.05$). VIP (149) detected significantly fewer positive samples than the other 3 methods ($p<0.05$).

Although AOAC approvals for these test kits are based on 25g samples enriched for 8 hr, this study showed that each method had an optimal enrichment time for detecting low levels of *E. coli* O157:H7 in 25g samples of ground beef. The best enrichment time for ABAX was 8 hr or 12 hr, 8 hr for Reveal, 12 hr or 16 hr for RapidChek, and 16 hr for VIP (data not shown). At the optimal time (8 hr for ABAX and Reveal; 16 hr for Rapid Chek and VIP), the tests were equivalent in their ability to detect *E. coli* O157:H7 in 25g samples ($p>0.05$).

Compositing 75g and 125g ground beef samples at both 8 hr or 12 hr of incubation was possible using ABAX (Tables 1 and 5) when compositing results were compared to ABAX 8 hr, 25g results ($p>0.05$). Compositing 75g samples was possible using RapidChek (Tables 2 and 5) when longer incubation times of 12 hr or 16 hr were employed. Compositing was not possible using Reveal since the Reveal 8 hr, 25g result detected more positive samples than at any other sample size or incubation time (Table 3). Table 4 shows that several VIP composite size results were statistically similar to the VIP 8 hr, 25g result. VIP detected fewer positive samples than any other method and the number of positive results only approximated the test results of the other three methods when the 75g composite was incubated for 16 hr. Therefore, although compositing is statistically valid according to the study parameters, further studies to

determine why lower detection levels occurred with this method must be performed before compositing can be evaluated (Table 5).

Trim Compositing

Compositing was more effective for the recovery of *E. coli* O157:H7 in raw beef trim compared to compositing results in raw ground beef. Compositing may work better in trim due to the lower numbers of background flora and the nature of the *E. coli* O157:H7 contamination which is located on the surface, rather than the interior, of the meat.

The trim inoculation level ranged from 0.12 to 1.93 cells per enrichment. All non-inoculated trim samples tested negative for *E. coli* O157:H7. Negative results were independent of sample size and test method. Inoculation sets that produced only positive results or only negative results were excluded from analysis so as not to bias the statistical analysis.

The total number of inoculated trim samples per method from usable inoculation sets equaled 144 samples. Each sample was assayed at three time points for a total of 432 assays. There was no statistical difference between ABAX (252 positive assays), Reveal (251), and RapidChek (238) in detecting positive samples ($p>0.05$). VIP (208) detected significantly fewer positive samples than the other 3 methods ($p<0.05$).

This study showed that each method had an optimal enrichment time for detecting low levels of *E. coli* O157:H7 in 25g samples of trim. The best enrichment time for 25g samples using ABAX or Reveal was 12 hr; 8 hr, 12 hr, or 16 hr for RapidChek; and 12 hr or 16 hr for VIP (data not shown). At the optimal time (12 hr for ABAX and Reveal; 8 hr for RapidChek; and 12 hr for VIP), the tests were equivalent in their ability to detect *E. coli* O157:H7 in 25g samples ($p>0.05$).

Compositing 75g samples at any incubation time and 125g or 375g samples at longer incubation times of 12 hr or 16 hr was possible using ABAX (Tables 1 and 5) when compositing results were compared to ABAX 8 hr, 25g results ($p>0.05$). Compositing 125g or 375g samples at 8 hr incubation using ABAX was not possible ($p<0.05$). An earlier Silliker study which evaluated the effect of compositing on 125g samples incubated for 24 hr and tested by ABAX showed that compositing 125g samples was possible with 24 hr incubation. Therefore, results requiring 12 hr or longer incubation times to obtain valid compositing results for 125g composite samples is not unexpected. Personal communications with other researchers have also suggested that they have determined that longer incubation times than 8 hr are required for ABAX detection with larger sample sizes. Although reasons for the longer incubation times required was not a part of this study, perhaps the longer incubation times were necessary to grow *E. coli* O157:H7 to high enough levels to generate enough PCR amplicons to be detected in the larger volumes of media.

For both Reveal (Tables 2 and 5) and RapidChek (Tables 3 and 5), one could composite any size composite sample (75g, 125g or 375g) at any incubation time ($p>0.05$).

Table 4 shows that several VIP composite size results were statistically similar to the VIP 8 hr, 25g result (75g, 125g, or 375g at 8 hr and 375g at either 12 hr or 16 hr, $p>0.05$). Although compositing is statistically valid according to this study's parameters, VIP detected

fewer positive samples than any other method. Compositing is therefore not suggested for this method (Table 6) until further studies can ascertain the reasons for the detection of lower numbers of positive samples. One insight to improve test performance might be gleaned from the data presented in Table 4. The number of positive results only approximated the test results of the other three methods when the 75g, 125g and 375g composite samples were incubated for 12 hr or 16h. This data suggests that detection of low levels of *E. coli* O157:H7 cells by VIP may be improved by longer incubation times than 8 hr.

In conclusion, this study showed that the ability to detect low levels of *E. coli* O157:H7 in raw ground beef and raw beef trim composite samples using four 8 hr test kits varied with the product type being composited, the test kit, and the incubation time. There was no statistical difference between ABAX, Reveal, and RapidChek in detecting overall numbers of inoculated ground beef or trim samples ($p>0.05$). VIP detected significantly fewer positive samples than the other three methods for both ground beef and trim ($p<0.05$). The reason for this lower detection by VIP was not part of this study, but should be further investigated before compositing scheme recommendations can be made.

For the purpose of evaluating the efficacy of compositing for each method, the AOAC approved 25g, 8 hr result for each method was considered as the expected result. The compositing results at the 75g, 125g, and 375g sample sizes were compared to the 25g result using a X^2 test. The compositing scheme was interpreted as equal to the 25g, 8 hr result if the probability (p value) of the X^2 test was greater than or equal to 0.05. The compositing scheme was interpreted as different from the non-compositing result if the probability (p value) of the X^2 was less than 0.05.

Compositing is possible for *E. coli* O157:H7 testing in raw ground beef ($p>0.05$) using ABAX at 75g and 125g sample sizes at either 8 hr or 12 hr of incubation, for RapidChek at the 75g sample size at 12 hr or 16 hr of incubation, and for VIP at the 75g sample size for 16 hr incubation. Compositing was not possible using Reveal since the method detected more positive samples at the 8 hr, 25g result than at any other sample size or incubation time.

Compositing is possible for *E. coli* O157:H7 testing in raw beef trim ($p>0.05$) using ABAX at a 75g sample size at any incubation time and at 125g or 375g sample sizes at either 12 hr or 16 hr of incubation; for RapidChek and Reveal, compositing was possible at any sample size at all incubation times. Compositing was statistically possible using VIP, however, since VIP detected significantly fewer positive samples than the other three methods, this discrepancy should be further studied before compositing schemes can be evaluated.

Table 1. Validation of compositing ground beef and trim samples for *E. coli* O157:H7 analysis using ABAX.

			Incubation Time (hr)					
Product	**Analytical Unit**	**Total Samples**	**8**		**12**		**16**	
			No. Pos.	**p**	**No. Pos.**	**p**	**No. Pos.**	**p**
Ground Beef	25	45	26	---	---	---	---	---
	75	45	23	0.37	22	0.23	21	<0.05*
	125	45	22	0.23	20	0.07	16	<0.05*
	375	45	18	<0.05*	15	<0.05*	17	<0.05*
Trim	25	36	23	---	---	---	---	---
	75	36	23	1.00	22	0.73	22	0.73
	125	36	17	<0.05*	22	0.73	21	0.49
	375	36	14	<0.05*	19	0.17	20	0.30

* $p<0.05$ = results statistically different from 25-gram result

Table 2. Validation of compositing ground beef and trim samples for *E. coli* O157:H7 analysis using RapidChek

			Incubation Time (hr)					
Product	**Analytical Unit**	**Total Samples**	**8**		**12**		**16**	
			No. Pos	**p**	**No. Pos.**	**p**	**No. Pos.**	**p**
Ground Beef	25	45	23	---	---	---	---	---
	75	45	16	<0.05*	24	0.77	24	0.77
	125	45	11	<0.05*	12	<0.05*	12	<0.05*
	375	45	4	<0.05*	9	<0.05*	9	<0.05*
Trim	25	36	19	---	---	---	---	---
	75	36	24	0.10	24	0.10	24	0.10
	125	36	21	0.50	20	0.74	20	0.74
	375	36	16	0.32	16	0.32	16	0.32

*p<0.05 = results statistically different from 25-gram result

Table 3. Validation of compositing of ground beef and trim samples for *E. coli* O157:H7 analysis using Reveal

			Incubation Time (hr)					
Product	**Analytical Unit**	**Total Samples**	**8**		**12**		**16**	
			No. Pos	**p**	**No. Pos.**	**p**	**No. Pos.**	**p**
Ground Beef	25	45	29	---	---	---	---	---
	75	45	18	<0.05*	18	<0.05*	15	<0.05*
	125	45	16	<0.05*	18	<0.05*	17	<0.05*
	375	45	14	<0.05*	12	<0.05*	13	<0.05*
Trim	25	36	21	---	---	---	---	---
	75	36	20	0.74	19	0.50	20	0.74
	125	36	24	0.31	23	0.50	24	0.31
	375	36	18	0.31	18	0.31	18	0.31

*P<0.05 = results statistically different from 25-gram result

Table 4. Validation of compositing of ground beef and trim samples for *E. coli* O157:H7 analysis using VIP

			Incubation Time (hr)					
Product	**Analytical Unit**	**Total Samples**	**8**		**12**		**16**	
			No. Pos	**p**	**No. Pos.**	**p**	**No. Pos.**	**p**
Ground Beef	25	45	13	---	---	---	---	---
	75	45	9	0.19	12	0.74	24	<0.05*
	125	45	7	<0.05*	10	0.32	13	1.00
	375	45	2	<0.05*	5	<0.05*	10	0.32
Trim	25	36	13	---	---	---	---	---
	75	36	13	1.00	19	<0.05*	24	<0.05*
	125	36	15	0.49	21	<0.05*	20	<0.05*
	375	36	10	0.30	18	0.08	16	0.30

*P<0.05 = results statistically different from 25-gram result

Table 5. Enrichment conditions for ground beef compositing for the detection of *E. coli* O157:H7 using 4 rapid test kits

Composite Size	75g			125g			375g		
Incubation Time (hr)	**8**	**12**	**16**	**8**	**12**	**16**	**8**	**12**	**16**
ABAX	+[a]	+	-[b]	+	+	-	-	-	-
RapidChek	-	+	+	-	-	-	-	-	-
Reveal	-	-	-	-	-	-	-	-	-
VIP	-[c]	-	-	-[c]	-[c]	-	-	-[c]	-[c]

[a] Results statistically similar to 25-gram result: compositing possible.
[b] Results statistically different from 25-gram result: compositing not suggested.
[c]Compositing valid statistically, however low detection rates need investigation.

Table 6. Enrichment conditions for trim compositing for the detection of *E. coli* O157:H7 using 4 rapid test kits

Composite Size	75g			125g			375g		
Incubation Time (hr)	**8**	**12**	**16**	**8**	**12**	**16**	**8**	**12**	**16**
ABAX	+[a]	+	+	-[b]	+	+	-	+	+
RapidChek	+	+	+	+	+	+	+	+	+
Reveal	+	+	+	+	+	+	+	+	+
VIP	-[c]	-	-	-[c]	-	-	-[c]	-[c]	-[c]

[a] Results statistically similar to 25-gram result: compositing possible.
[b] Results statistically different from 25-gram result: compositing not suggested.
[c]Compositing valid statistically, however low detection rates need investigation.