Project Title: Sample Size and Matrix Effect on the Ability of Rapid Methods to Detect *E. coli* O157:H7

Principle Investigator(s): Joseph M. Bosilevac[1], John W. Schmidt[1], Norasak Kalchayan[1] Tommy L. Wheeler[1], and Mohammad Koohmaraie[2]

Institution(s): [1]Meat Safety and Quality Research Unit, U.S. Meat Animal Research Center [2]IEH Laboratories & Consulting Group

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Background

E. coli O157:H7 is a significant health risk and is associated with cattle and beef products. To reduce the presence of this organism, processors have implemented a series of interventions to decontaminate carcasses, cuts and trim. The last line of defense in the control measures is test-and-hold, that is testing products for *E. coli* O157:H7 before releasing into commerce. In November of 2009 over 40 members representing all sectors involved in and affected by test-and-hold practices participated in a meeting to discuss issues that surround testing for pathogens. While a whole series of issues were discussed, it became clear that there were specific areas that affect everyone involved and that required closer examination. First, it was noted that testing schemes and test methods were constantly evolving areas and that as better ways of testing came about, there was a tendency to implement these changes across the industry while not recognizing potential problems that may result later. Changes that reduced the volume of enrichment media in order to concentrate the *E. coli*, or increased the size of the sample from 25 g up to 325 g to provide greater sensitivity have been widely implemented. These changes were made in with the intentions of improved results. However, if the change merely documented by a literature citation, rather than a complete validation this became a significant problem during Food Safety Audits when inspectors are carefully scrutinizing tests and methods in use. Second, it was noted that the rapid detection tests were being used on a variety of matrices that were not specifically mentioned in protocol and validation documents. Third, screening tests must often be shipped to a third party lab for confirmation. There has been concern that during shipment *E. coli* O157:H7 if present does not survive or is lost into an increased background of other bacteria present. The results presented address these two topics and are intended as a step in answering the questions raised at that meeting.

The objectives of this study were to:

1. Determine if a validated rapid test requires revalidation for use at a different sample size or in a different volume of enrichment media.

2. Determine if a validated test requires revalidation for use in a different matrix.

3. Identify the necessary conditions required to ensure stability of an enrichment during inter-lab shipping.

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Methods

Each objective was addressed in a separate experiment.

Experiment 1. Three sample sizes of 80% lean beef trim (25g, 150g and 325g) were inoculated with 1 to 2 CFU *E. coli* O157:H7 and enriched in 1, 3 and 9 volumes of tryptic soy broth (TSB) for 8, 12 and 16 h at 42C. This series of samples, volumes and times was repeated using modified TSB with casamino acids and novobiocin (mTSBcn). The effects of the varying sizes, volumes and times were determined on the detection of the inoculum by culture isolation, antigen detection using lateral flow devise (LFD) and molecular DNA detection (PCR). The LFD method was VIP Gold EHEC O157 (BioControl), and the PCR method was the BAX-MP (Qualicon) used according the package inserts. Culture isolation was performed according to established protocols.

Experiment 2. The effects of using different sample matrixes on the detection of 1-2 CFU *E. coli* O157:H7 were examined at two different size, volume and time conditions: 25g in 225mL TSB incubated 8 h, and 325g in 1L mTSBcn incubated 16 h. The different sample types were boneless beef trim (<50% lean and >95% lean), ground beef (73%, 85% and 93%), beef hearts, kidneys, cheek meat, liver, and sponge samples (collected from 500cm^2 hide and 4000cm^2 carcass). The detection methods were the same as used in Experiment 1, culture isolation, LFD and PCR.

Experiment 3. During each replicate performed in Experiments 1 and 2 using beef trim or ground beef, enrichments positive by LFD and culture were identified (n=42). Then, two 10-mL portions of each were placed in 15mL conical tubes and stored on crushed ice in a refrigerator or at 10C for 24 h to simulate proper and abusive shipping conditions. After 24 h, samples were subjected to *E. coli* O157:H7 detection by culture isolation and PCR.

Findings

Changing sample size from 25 g to 150 g to 325 g effects detection of *E. coli* O157:H7 by rapid methods but not culture isolation. Reducing the ratio of media used also effects the detection of *E. coli* O157:H7 by the rapid methods but not culture isolation. The best rates of detection were usually observed at those of the AOAC validation (25 g samples enriched in 225 ml medium). Increasing the time of incubation to 12 and 16 h did not help rapid tests detect *E. coli* O157:H7 in the larger sample sizes or reduced volumes. Using a modified media (mTSBcn) helped the rapid methods detect *E. coli* O157:H7 with increased sample size and reduced volumes, but only at 12 and 16 h.

The rapid methods were equally effective at detecting *E. coli* O157:H7 in all sample types examined except those containing liver. Measurements of pH showed samples containing liver had lower pH than other samples. A phosphate buffered TSB and mTSBcn were used and this remedied the poor detection of *E. coli* O157:H7 in samples containing liver.

When samples that were LFD positive were held 24 h and tested by PCR and culture, only samples that had been maintained on ice were still positive although a small number were not detected.

In summary, altering sample size, media, volume, and time requires revalidation of rapid detection methods. Rapid detection methods can be used on numerous sample types, except those that might alter the pH of the enrichment media during incubation. The detection of positive samples is reduced following 24 h storage or transport, but not significantly if the storage is on ice.

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Implications

These studies have addressed a series of highly relevant issues that affect all sectors involved with the testing for *E. coli* O157 in the beef industry. The systematic approach used here has shown the effect of the sample size and matrix on the ability of the rapid tests to detect *E. coli* O157:H7. These results identify situations when a revalidation is needed and when it is not. These results can also be used by in-house laboratories of beef processors, ground beef manufacturers and others, such as third party laboratories, as supporting documents for their testing programs.

Table 1. Percentage of study samples positive for *E. coli* O157:H7 following inoculation with 1 to 2 CFU and enriched in tryptic soy broth.

Sample size[a]	Enrichment volume[b]	8 hour enrichment[c]			12 hour enrichment[d]			16 hour enrichment[e]		
		LFD[f]	PCR[g]	Culture	LFD	PCR	Culture	LFD	PCR	Culture
25 g	25 ml	73A[h]	86AB	91A	92[i]A	67ABC	83A	100[i]A	83AB	83A
25 g	75 ml	77A	91AB	100A	100A	100A	100A	100A	92A	100A
25 g	225 ml	86A	100A	100A	83AB	67ABC	83A	92[i]AB	83AB	83A
150 g	150 ml	36BC	68BC	82A	92[i]A	33CD	83A	92[i]AB	33C	83A
150 g	450 ml	36BC	86AB	95A	42BC	50BCD	100A	50BC	58ABC	100A
150 g	1.35 L	59AB	95A	100A	67ABC	92AB	100A	58BC	92A	100A
325 g	325 ml	18C	45C	91A	33CD	17D	100A	33CD	33%	92A
325 g	1 L	14C	50C	82A	50BC	58BCD	100A	50BC	50ABC	100A
325 g	2.9 L	23C	91AB	100A	0D	17D	92A	0D	42BC	83A

[a]Samples were boneless beef trim of 85% lean, sampled in mock-N60 fashion.
[b]Enrichment media was TSB.
[c]For 8 h enrichment n=22, the 8 h samples were run independently of the 12 and 16 h samples and confirmed to be culture positive after 12 h
[d]For 12 h enrichment n=12, 12 h samples were incubated an additional 4 h after sampling to be used as the 16 h samples.
[e]For 16 h enrichment n=12.
[f]The representative LFD (lateral flow devise) used in these studies was the VIP Gold EHEC-O157 test. All LFDs run on deactivated boiled enrichment, regardless of incubation time.
[g]The representative PCR detection used was the BAX-MP assay.
[h]Values (%) within a column followed by the same letter are not different (P > 0.05).
[i]Some LFD percentage positives are greater than culture due to the presence of *E. coli* O157 non-H7 present in background bacteria.

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