Detection of *E. coli* O157 in Beef Trim Field Trial Comparison Study for Crystal Diagnostics: AutoXpress vs. Commercial Real Time PCR

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1. Introduction & Purpose

Crystal Diagnostics (CDx) AutoXpress (AX) *E. coli* O157 (E assay) and a commercials real time PCR platform were used to analyze 325 g raw beef trim samples. A total of 144 samples, in three sets of 48 samples each, were setup as 325 g test portions and process controls. Samples were enriched in Modified Tryptic Soy Broth with 8mg/L Novobiocin and Acid Digest of Casein (mTSB+n) (Neogen, Lansing, MI, USA). 144 samples were analyzed according to the CDx testing protocol on the AX instrument and 144 samples were analyzed on a commercial real time PCR platform utilizing the platform assay for *E. coli* O157:H7. Each analysis batch included one media negative, one media positive, and six matrix spiked controls containing ATCC 35150 *E. coli* O157:H7.

2. Materials Methods

Sample Preparation and Cell Culture

Cubed raw beef trim was received at refrigerated temperature from CDx. Trim samples were stored at refrigerated temperature until inoculation and enrichment preparation. Matrix spike preparation was as follows: *E. coli* O157:H7 ATCC 35150 was grown overnight at 37C in brain-heart infusion broth. Subsequent serial dilutions were performed, and spike levels were confirmed day of use by plating using plate count agar. Spike level data can be found in the attached raw data spreadsheet.

Enrichment and Quality Control

Beef trim samples (325 g) were removed from refrigeration and transferred to 10×15 WHIRL-PAK™ filter bags (Nasco, Fort Atkinson, WI, USA). Individual trim samples were spiked with low level *E. coli* O157:H7 and hand massaged. All trim samples were allowed to equilibrate a minimum of one hour at room temperature. Samples were enriched with 1000 mL of mTSB+n, prewarmed at 44°C. Media negative and positive controls were prepared for quality control purposes per laboratory procedure. All samples were placed in incubation at 42°C for 9.5 hours.

Preparation for Detection

After 9.5hrs of incubation, the samples were gently mixed by hand with circular motion and let set for five minutes. A 25mL serological pipette was used to aspirate 42mL from the filtered side of each sample bag into a 50mL conical tube, pulled from the center of the filtered liquid. The 50mL conical tubes were allowed to set for 10 minutes to settle. Testing preparation and analysis performed as instructed in the *Test Protocol for AX Detection of E. coli 0157 in Beef Trim* procedure provided by CDx and by commercial PCR kit insert instructions.

Confirmation

Non-matching sample results between the two pathogen platforms were sent to ALS Marshfield Wisconsin for cultural confirmation.

3. Results

The study included 144 total samples. CDx AX correctly identified 18/18 spiked trim samples. The commercial real time PCR assay correctly identified 18/18 spiked trim samples and identified three additional presumptive positive samples from the non-spiked sample group. The three additional samples were negative for *E. coli* O157:H7 by cultural confirmation. All quality controls were successful.

4. Conclusions

The CDx AX demonstrated successful performance by detection of spiked samples with no false positives identified by analysis. The comparison PCR platform successfully detected the spiked samples but did have three false positives, based on negative cultural confirmation: 3/144 (2.1%).