Food Safety

Detection of Shiga Toxin-Producing *Escherichia coli* in Flour-Based Foods

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Introduction

Shiga toxin-producing *Escherichia coli* (STEC) outbreaks are typically associated with raw meat, raw vegetables, and unpasteurized milk. In recent years, flour has been described as a vehicle for the transmission of STEC[1]. In 2018, a multi-state outbreak of STEC, originated from contaminated flour and resulted in three (14.3%) hospitalizations out of 21 reported cases[2]. A study conducted to assess the survivability of STEC isolates in flour found that two out of ten samples (20%) tested positive for the *stx* gene after two years' storage[1]. Screening of flour products for the presence of STEC may be used as an effective risk mitigation strategy in food safety.

This study verified and statistically compared the Thermo Scientific™ SureTect™ Escherichia coli O157:H7 and STEC Screening and Identification PCR Assays (molecular workflow) with a culture media method for the detection of STEC isolates in flour-based foods.

Methods

The verification study method has been outlined in Figure 1.

Sample preparation

25 g samples artificially contaminated with diverse STEC isolates.

Storage

Study 1: Samples stored at ambient temperature (avoiding light) for 7 days.

Study 2: Samples enriched immediately after artificial contamination.

Enrichment

Samples diluted in 225 mL of Thermo Scientific™ Buffered Peptone Water (BPW) with α-amylase solution (10 mL to 1 L of media) and incubated at 37ºC for 18 hours.

Workflow

Culture media: 10 µL enrichment plated onto Tryptone Bile X-Glucuronide Medium (TBX) and Chromogenic Coliform Agar (CCA).

PCR: Following the product instructions, lysis and qPCR was conducted using the Escherichia coli O157:H7 and STEC screening PCR assay.

Data analysis

The number of positives observed on each method was statistically analysed using Chi-squared analysis.

Figure 1. Verification study method

Twenty flour-based products (including flour, bread and cake mixes etc.) and 20 STEC isolates representing the top seven serotypes were tested in a paired study.

Samples were enriched at 37ºC for 18 hours with the addition of α-amylase, in accordance with ISO 6887–4:2017[3] for high-starch matrices.

Study one tested ten flour-based products. STEC isolates were artificially contaminated at a low level (1.2 – 9 CFU/sample) before ambient storage for one week prior to testing .

A second study was conducted without the storage step, due to high levels of die-off observed during the first study (figure 1).

Results

Presence of non-STEC strains was observed in these matrices; ten (50%) uncontaminated samples showed typical *E. coli* colonies on either TBX or CCA but were negative when tested with PCR for *stx* genes. Different colony morphologies were picked and tested directly with both the screening and identification PCR assay and were confirmed not to be STEC isolates.

Twenty-two (55%) of the artificially contaminated samples tested positive for STEC with the PCR workflow. All positive samples were confirmed as STEC when presumptive positive *E. coli* colonies were picked from CCA (figure 2).

A simplified PCR confirmation method was utilized to confirm the presence of STEC. The confirmation test was able to confirm that 24 (60%) of the artificially contaminated samples were positive, with seven (22.58%) presumptive positives proven not to be STEC isolates.

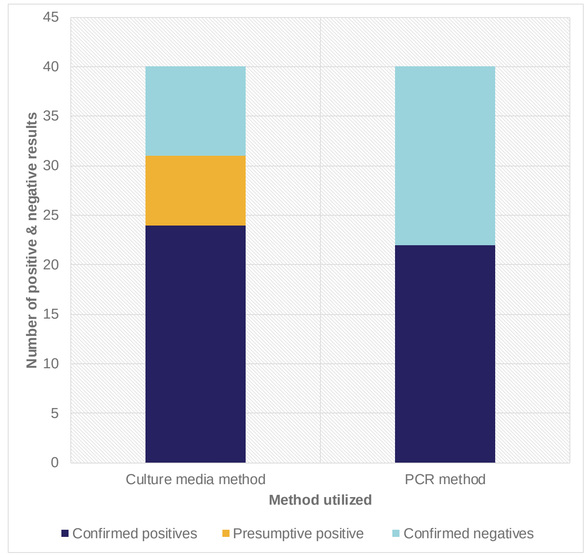

Figure 2. Distribution of positive and negative results for the PCR and culture media methods.

The PCR workflow successfully identified 23 (57.5%) of the artificially contaminated samples, with the culture media method identifying 25 (62.5%) samples. A Chi-squared test of independence concluded that there was no significant difference between the two methods, X2 (1, n=120) = 0.14, p=0.05 (Table 1).

Table 1. Chi-squared analysis to compare PCR workflow with culture media workflow; Σ positive data points for the culture media method adjusted to account for the number of false positives observed on this method.

Intervention	Outcome	Observed (O)	Expected (E)	O - E	$(O-E)^2$	$(O-E)^2/E$
PCR method	Positive	23	24.00	-1.00	1.00	0.04
	Negative	37	36.00	1.00	1.00	0.03
Culture media method	Positive	25	24.00	1.00	1.00	0.04
	Negative	35	36.00	-1.00	1.00	0.03

Chi-squared (x^2)	0.14	Degrees of freedom (df)	1	Significance (p value)	0.05	Critical value	3.841

Conclusions

- Traditional plating methods cannot detect toxin and virulence genes.
- Confirmation methods match PCR workflow results.
- Presumptive results in ≤ 18 hours using the PCR method.
- The PCR method is a reliable alternative to the culture media method.

References

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