Performance Summary

iQ-Check STEC VirX and SerO II Method

Introduction

The iQ-Check STEC VirX and SerO II PCR Detection Kits are tests based on gene amplification and detection by real-time PCR after food and environmental samples are enriched in buffered peptone water (BPW). Ready-to-use PCR reagents in the iQ-Check STEC VirX Kit contain oligonucleotides (primers and probes) highly specific for *stx1*, *stx2*, and *eae* virulence genes of Shiga toxin–producing *Escherichia coli* (STEC) while the iQ-Check STEC SerO II Kit identifies the serogroup of the top seven STEC strains, *E. coli* O157:H7, *E. coli* O26, *E. coli* O45, *E. coli* O103, *E. coli* O111, *E. coli* O121, and *E. coli* O145. A synthetic DNA internal control is included in the reaction mix. An internal control is critical in any reaction to monitor for inhibitors and allow for the validation of any negative result. The iQ-Check STEC VirX and SerO II method has been rigorously tested and validated by internationally recognized validation agencies (Table 1).

Table 1. Validations for the iQ-Check STEC VirX and SerO II method.

Validation	Certificate Number
AOAC	PTM 121203
USDA FSIS MLG	Chapter 5C.00

Inclusivity/Exclusivity Testing

Inclusivity testing is performed to verify that the method can detect STEC virulence genes and target serogroups while exclusivity studies test non-STEC organisms, including other *E. coli* strains, to ensure there is no cross-reactivity. Exclusivity strains were enriched in nonselective brain heart infusion broth for 18–24 hr at 35 ± 1°C and were tested at high levels. A target of 10–100 colony forming units (CFU) of each STEC inclusivity strain was cultured in BPW for 8 hr at 41.5 ± 1°C and diluted to a low level (~10^3) before testing. Results are shown in Table 2.

Table 2. Results of inclusivity/exclusivity testing.

Strains Tested	Positives Detected	Results
58 STEC	58/58	100% inclusivity
78 non-STEC	0/78	100% exclusivity

Limit of Detection

Limit of detection (LOD_{50}) is an estimation of the contamination level required to achieve positive detection in 50% of cases. This is measured by inoculating food matrices with STEC strains and carrying out the validated enrichment, extraction, and detection protocols (Table 3).

The average LOD_{50} of the iQ-Check STEC VirX and SerO II method was determined to be 0.7 (range: 0.4–1.2).

Table 3. LOD_{50} for the iQ-Check STEC VirX and SerO II method.

Matrix/Strain Pair	LOD_{50}, CFU/sample size (range)
Raw beef trim (375 g)/*E. coli* O26	0.6 (0.4–1.1)
Raw ground beef (375 g)/*E. coli* O103	0.7 (0.4–1.3)
Fresh spinach (375 g)/*E. coli* O145	0.5 (0.3–1.0)
Cannabis flower (10 g)/*E. coli* O157:H7	0.8 (0.4–1.3)

Method Comparison/Matrix Studies

Matrix testing is critical to demonstrating the performance of a method compared to the reference method with real-world food samples. The iQ-Check STEC VirX and SerO II method has been verified with external and internal testing on a wide variety of foods. No significant difference was found between the reference method and alternative method for all matrices tested (Table 4).

Table 4. Matrices tested with iQ-Check STEC VirX and SerO II method.

Category	Matrices Tested
Meat products	Raw ground beef, raw beef trim
Dairy products	Raw milk, raw milk cheese
Fresh produce	Spinach
Environmental samples	MicroTally swabs
Cannabis plants and flowers	Cannabis flower

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