Performance Summary

iQ-Check *Enterobacteriaceae* Method

Introduction

The iQ-Check *Enterobacteriaceae* PCR Detection Kit is a test based on gene amplification and detection by real-time PCR after food and environmental samples are enriched in buffered peptone water (BPW) with or without PIF Supplement. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) highly specific for *Enterobacteriaceae*. A synthetic DNA internal control is included in the reaction mix. An internal control is critical in any reaction to monitor for inhibitors and allows for the validation of any negative result. The use of the iQ-Check Free DNA Removal Solution is recommended. The iQ-Check *Enterobacteriaceae* method has been rigorously tested and validated by an internationally recognized validation agency (Table 1).

Table 1. Validation for the iQ-Check *Enterobacteriaceae* method.

Validation	Certificate Number
AOAC	PTM 082003

Inclusivity/Exclusivity Testing

Inclusivity testing is performed to verify that the method can detect *Enterobacteriaceae* while exclusivity studies test non-*Enterobacteriaceae* strains to ensure there is no cross-reactivity. Exclusivity strains were enriched in nonselective broth for 24 hr at 37 ± 1°C and were tested at high levels. A single colony of each *Enterobacteriaceae* inclusivity strain was cultured in BPW and in BPW with PIF Supplement for 8–20 hr at 37 ± 1°C and diluted to a low level (~10^3) before testing. Results are shown in Table 2.

The following genera of *Enterobacteriaceae* were tested in the inclusivity study:

Citrobacter
Cronobacter
Edwardsiella
Enterobacter
Escherichia
Franconibacter
Hafnia
Klebsiella
Kluyvera
Morganella
Pantoea
Proteus
Rahnella
Raoultella
Salmonella
Serratia
Shigella
Yersinia

Table 2. Results of inclusivity/exclusivity testing.

Strains Tested	Positives Detected	Results
51 *Enterobacteriaceae*	51/51	100% inclusivity
30 non-*Enterobacteriaceae*	0/30	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 *Enterobacteriaceae* strains and carrying out the validated enrichment, extraction, and detection protocols (Table 3).

The average LOD_{50} of the iQ-Check *Enterobacteriaceae* method was determined to be 0.7 (range: 0.6–0.9).

Table 3. LOD_{50} for the iQ-Check *Enterobacteriaceae* method.

Matrix/Strain Pair	Enrichment	LOD_{50}, CFU/sample size (range)
Milk powder (10 g)/*S.* Anatum	BPW	0.7 (0.4–1.3)
Milk powder (10 g)/*S.* Anatum	BPW + PIF	0.8 (0.4–1.4)
Powdered infant formula (10 g)/*E. coli*	BPW	0.6 (0.4–0.9)
Powdered infant formula (10 g)/*E. coli*	BPW + PIF	1.2 (0.7–2.2)
Powdered infant formula (375 g)/*E. coli*	BPW + PIF	0.5 (0.3–0.9)
Powdered infant formula with probiotics (10 g)/*C. sakazakii*	BPW	0.8 (0.5–1.4)
Powdered infant formula with probiotics (10 g)/*C. sakazakii*	BPW + PIF	0.6 (0.3–1.1)
Powdered infant formula with probiotics (375 g)/*C. sakazakii*	BPW + PIF	0.4 (0.2–0.7)

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 *Enterobacteriaceae* 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 the iQ-Check *Enterobacteriaceae* method.

Category	Matrices Tested
Dairy products	Milk powder
Infant formula and cereals	Powdered infant formula, powdered infant formula with probiotics
Environmental samples	Stainless steel, broths (HiCap, D/E neutralizing), material (cellulose, polyurethane)

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