Performance Summary

iQ-Check *Aspergillus* Method

Introduction

The iQ-Check *Aspergillus* PCR Detection Kit is a test based on gene amplification and detection by real-time PCR after cannabis samples are enriched in buffered peptone water (BPW) with chloramphenicol. Ready-to-use PCR reagents in the iQ-Check *Aspergillus* Kit contain oligonucleotides (primers and probes) highly specific for *A. flavus, A. fumigatus, A. niger,* and *A. terreus*. 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 *Aspergillus* method has been rigorously tested and validated by an internationally recognized validation agency (Table 1).

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

Validation	Certificate Number
AOAC	PTM 032104

Inclusivity/Exclusivity Testing

Inclusivity testing is performed to verify that the method can detect the four target *Aspergillus* strains while exclusivity studies test nontarget organisms, including other *Aspergillus* strains, to ensure there is no cross-reactivity. Exclusivity strains were enriched in tryptic soy broth for bacteria or Sabouraud broth for fungi and incubated for 24–48 hr at 37 ± 1°C for bacteria or 30 ± 1°C for fungi and were tested at high levels. A target of 10–100 colony forming units (CFU) of each *Aspergillus* inclusivity strain was cultured in BPW supplemented with 0.3 g/L of chloramphenicol for 48 ± 3 hr at 37 ± 2°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
53 target *Aspergillus* strains (14 *A. flavus*, 13 *A. fumigatus*, 13 *A. niger*, and 13 *A. terreus*)	53/53	100% inclusivity
36 non-*Aspergillus* and non–*A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*	2/36*	94.5% exclusivity

* *A. oryzae* (American Type Culture Collection [ATCC] 10124) and *A. parasiticus* (ATCC 15517) were detected as positive. Strains were deposited at ATCC as *A. flavus*.

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 *Aspergillus* strains and carrying out the validated enrichment, extraction, and detection protocols (Table 3).

The average LOD_{50} of the iQ-Check *Aspergillus* method was determined to be 0.6 (range: 0.4–1.1).

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

Matrix/Strain Pair	LOD_{50}, CFU/sample size (range)
Cannabis flower (10 g)/natural contamination	0.5 (0.4–0.9)
Cannabis concentrate, solvent based (5 g)/*A. flavus*	0.6 (0.4–1.1)
Cannabis concentrate, nonsolvent based (5 g)/*A. fumigatus*	0.6 (0.4–1.2)

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 *Aspergillus* 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 5. Matrices tested with the iQ-Check *Aspergillus* method.

Category	Matrices Tested
Cannabis plants and flowers	Cannabis flower; cannabis concentrate, solvent based; cannabis concentrate, nonsolvent based

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