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.

<table>
<thead>
<tr>
<th>Validation</th>
<th>Certificate Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOAC</td>
<td>PTM 032104</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Strains Tested</th>
<th>Positives Detected</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>53 target Aspergillus strains (14 A. flavus, 13 A. fumigatus, 13 A. niger, and 13 A. terreus)</td>
<td>53/53</td>
<td>100% inclusivity</td>
</tr>
<tr>
<td>36 non-Aspergillus and non–A. flavus, A. fumigatus, A. niger, and A. terreus</td>
<td>2/36*</td>
<td>94.5% exclusivity</td>
</tr>
</tbody>
</table>

* 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 (LOD50) 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 LOD50 of the iQ-Check Aspergillus method was determined to be 0.6 (range: 0.4–1.1).

Table 3. LOD50 for the iQ-Check Aspergillus method.

<table>
<thead>
<tr>
<th>Matrix/Strain Pair</th>
<th>LOD50, CFU/sample size (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabis flower (10 g)/natural contamination</td>
<td>0.5 (0.4–0.9)</td>
</tr>
<tr>
<td>Cannabis concentrate, solvent based (5 g)/A. flavus</td>
<td>0.6 (0.4–1.1)</td>
</tr>
<tr>
<td>Cannabis concentrate, nonsolvent based (5 g)/A. fumigatus</td>
<td>0.6 (0.4–1.2)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Category</th>
<th>Matrices Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabis plants and flowers</td>
<td>Cannabis flower; cannabis concentrate, solvent based; cannabis concentrate, nonsolvent based</td>
</tr>
</tbody>
</table>
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