



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
032104

The AOAC Research Institute hereby certifies the method known as

iQ-Check *Aspergillus* and iQ-Design *Aspergillus* Real-Time PCR Kits

Corporate Location

**Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547 USA**

Manufacturing Location

**Bio-Rad Laboratories
925 Alfred Nobel Drive
Hercules, CA 94547 USA**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date	November 20, 2025
Expiration Date	December 31, 2026

METHOD NAMEiQ-Check *Aspergillus*
iQ-Design *Aspergillus***CATALOG NUMBERS**

12010806, 12015336, 12015260, 12015337, 12018492

ORIGINAL CERTIFICATION DATE

March 19, 2021

PRINCIPLE OF THE METHOD

The iQ-Check *Aspergillus* Kit is a multiplex test based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific for *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus*, as well as DNA polymerase and nucleotides. The iQ-Design Assay *Aspergillus* Speciation Solution products are ready-to-use individual primer-probe mix reagents that are for use with Bio-Rad's iQ Multiplex Powermix amplification reagent. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instruments, such as the CFX96 Touch Deep Well or the CFX Opus Deepwell systems and CFX Manager Software, Industrial Diagnostic Edition.

CERTIFIED CLAIM STATEMENT: The method including the iQ-Check *Aspergillus* Kit and the iQ-Design Assay products is certified for the detection of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus* within the scope of Tables 1 and 2 and the modifications in Table 3.

Certified method includes:

1. Real-Time PCR Systems:
 - a. CFX96 Touch Deepwell, CFX Maestro Software, IDE v4.0
 - b. CFX Opus Deepwell, CFX Maestro Software, IDE v4.0
2. Optional Free DNA Removal Solution step

Table 1. Method Performance Claims

Matrix	Test Portion	Enrichment Conditions				SMPR ^b	Claim ^c
		Broth ^a	Volume	Temperature	Time		
Cannabis (>0.3%THC) flower	10 g	BPW+c	90 mL	37 ± 1°C	45-51 h	2019.001	NSDD
Cannabis (>0.3%THC) concentrates	5 g	BPW+c+T7	45 mL	37 ± 1°C	45-51 h	2019.001	NSDD
Cannabis (>0.3%THC) infused gummies	25 g	BPW+c	225 mL	37 ± 1°C	45-51 h	2019.001	NSDD
Cannabis (>0.3%THC) infused chocolate	25 g	BPW+c	225 mL	37 ± 1°C	45-51 h	2019.001	NSDD

^a BPW+c = Buffered Peptone Water + 0.3 g/L of chloramphenicol; BPW+c+T7 = Buffered Peptone Water + 0.3 g/L of chloramphenicol + 1% v/v Tergitol 7.

^b SMPR = *Standard Method Performance Requirements* 2019.001 for Detection of *Aspergillus* in Cannabis and Cannabis Products.

^c NSDD = No statistical difference detected using SLV study design from OMA Appendix J (2012). The SLV qualitative method comparison study design from OMA Appendix J (2012) is not intended to demonstrate statistical equivalence. Expert opinion is that the method is appropriate for its intended use. For cannabis matrices, comparison is only between presumptive and confirmed candidate method results.

Table 2. Method Selectivity

Target	Broth ^a	Temperature	Inclusivity Strains		Exclusivity Strains	
			No. Tested	No. Positive	No. Tested	No. Positive
<i>Aspergillus</i>	BPW+c	37 ± 1°C	53 ^b	53	36 ^c	2 ^d

^a BPW+c = Buffered Peptone Water + 0.3 g/L of chloramphenicol.

^b Comprising 14 strains *A. flavus*, 13 strains *A. fumigatus*, 11 strains *A. niger* and 14 strains *A. terreus*.

^c Comprised of 20 strains of *Aspergillus* species (non-*flavus*, *fumigatus*, *niger*, *terreus*) and 16 additional yeast and mold species.

^d *A. oryzae* (ATCC 10124) and *A. parasiticus* (ATCC 15517) tested positive in FAM channel. At the time of the study, the ATCC website indicated both strains were deposited as *Aspergillus flavus*.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	March 2021	Original certification (No longer applicable due to the addition of iQ-Design in April 2022).	Certification Report
2	April 2021	Level 1 Modification: Software updated from Version 3 to Version 4 allowing compatibility with Windows 10.	No report required
3	April 2022	Level 2 Modification: Addition of iQ-Design <i>Aspergillus</i> Speciation Solution.	Modification Report 1
4	January 2023	Level 2 Modification: Addition of CFX Opus Deepwell, with CFX Manager Software, Industrial Diagnostic Edition version 3.1 using Free DNA Removal Solution and Fast APF protocols.	Modification Report 2
5	August 2023	Level 2 Modification: Evaluation of the iQ-Design Assays for <i>A. fumigatus</i> and <i>A. terreus</i> solutions for identification and new Application Protocol File (T014) for <i>Aspergillus</i> compatible to CFX Manager Software, Industrial Diagnostic Edition, version 3.1. Matrix Extension to add cannabis infused gummies (25 g) and cannabis infused chocolate (25 g).	Modification Report 3
6	November 2024	Level 1 Modification: Editorial changes including software update to CFX Maestro Software, IDE v4.0.	No report required