



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

082102

The AOAC Research Institute hereby certifies the test kit known as:

PathoSEEK[®] 5-Color Aspergillus Multiplex Assay with SenSATIVAx[®] Extraction

manufactured by

Medicinal Genomics Corp.

100 Cummings Center, Suite 4051

Beverly, MA 01915 USA

This method has been evaluated in the AOAC[®] *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (August 10, 2021 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

August 10, 2021

Date

METHOD AUTHORS

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SUBMITTING COMPANYMedicinal Genomics Corp.
100 Cummings Center, Suite 4051
Beverly, MA 01915 USA**KIT NAME(S)**PathoSEEK® 5-Color Aspergillus Multiplex Assay with SenSATIVAx®
Extraction**CATALOG NUMBERS**

420147, 420148, 420001, 420004, 420201, 420330

INDEPENDENT LABORATORYCambium Analytica
1230 Woodmere Ave
Traverse City, MI 49686**AOAC EXPERTS AND PEER REVIEWERS**Yvonne Salfinger¹, Jim Agin², Wayne Ziemer³
¹Independent Consultant, Colorado, USA
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³USDA FERN (Retired), GA, USA**APPLICABILITY OF METHOD****Analytes – *Aspergillus species (A. flavus, A. fumigatus, A. niger & A. terreus)*****Matrixes – Dried cannabis flower (delta 9-tetrahydrocannabinol [THC] >0.3%, 10 g), THC-infused chocolate (25 g).****Performance claims - The PathoSEEK 5-Color Aspergillus Multiplex Assays with SenSATIVAx® Extraction Protocols met the requirements of the *Standard Method Performance Requirement (SMPR) for Detection of Aspergillus in Cannabis and Cannabis Products 2019.001 (2)* and AOAC Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (3).****REFERENCE GUIDELINES****AOAC International SMPR 2019.001, Standard Method Performance Requirements for Detection of *Aspergillus* in Cannabis and Cannabis Products. (2)
Official Methods of Analysis (2019) 21st Ed., Appendix J: AOAC INTERNATIONAL, Rockville, MD (3)****ORIGINAL CERTIFICATION DATE**

August 10, 2021

CERTIFICATION RENEWAL RECORD

New Approval 2021

METHOD MODIFICATION RECORD

NONE

SUMMARY OF MODIFICATION

NONE

Under this AOAC® Performance TestedSM License Number, 082102 this method is distributed by:

NONE

Under this AOAC® Performance TestedSM License Number, 082102 this method is distributed as:

NONE

PRINCIPLE OF THE METHOD (1)

The PathoSEEK® Microbial Safety Testing Platform utilizes a novel, contamination-free, PCR-based assay and provides an internal plant DNA control for every reaction. It is a simple two-step protocol (DNA extraction followed by RT-PCR analysis) which is flexible and automation compatible.

The PathoSEEK 5 – Color Aspergillus Multiplex microbial detection assays use a multiplexing strategy with an internal plant DNA reaction control to ensure accurate detection of 4 species of *Aspergillus* as well as cannabis DNA in every reaction. Unlike other techniques, this multiplexing strategy verifies the performance of the assay when detecting pathogens, resulting in the minimization of false negative results due to reaction set-up errors or failing experimental conditions.

The PathoSEEK process includes real-time quantitative PCR assays using a multiplex system of primers to detect potential pathogens within the plant, extract, or MIP (Marijuana Infused Product) sample. The forward and reverse primers have universal primer tails to enable potential Next Generation Sequencing of resulting products.

Two multiplex assays are available for use, dependent on the thermocycler used for analysis. The PathoSEEK 5 – Color Aspergillus Multiplex Assay for use on the AriaMx Real-Time PCR Thermocycler (Agilent) uses the ATTO 425 Fluorophore for detection of the *Aspergillus terreus*, while the PathoSEEK 5- Color Aspergillus Multiplex Assay for use on the CFX-96 (Bio-Rad) uses the Cy5.5 Fluorophore for the detection of *Aspergillus terreus*. Fluorophores for the three additional *Aspergillus* species are consistent between the two thermocyclers. Both PCR assays will be validated in the study.

SenSATIVAx is a proprietary DNA isolation process that uses magnetic particles to isolate and purify both plant and microbial DNA from a raw, homogenized plant sample. The SenSATIVAx approach is designed for ease of use and minimal requirement of laboratory equipment. Large centrifuges have been replaced with lightweight minicentrifuges, magnetic particles, and magnets. The use of magnetic particles affords 8 or 96-tip automation, enabling both minimal entry costs and high throughput applications. DNA can be isolated from a single sample or a large batch in under 1 hour. Hands-on time is less than 45 minutes.

DISCUSSION OF THE VALIDATION STUDY (1)

The PathoSEEK 5-Color Aspergillus Multiplex Assays with SenSATIVAx Extraction Protocol successfully detected *Aspergillus* species from dried cannabis flower (>0.3% THC) and THC-infused chocolate. POD statistical analysis indicated no difference between the candidate presumptive and confirmed results for the matrixes evaluated. For THC-infused chocolate, 2 false positive results were obtained using the AriaMx instrument and 1 false positive result obtained using the CFX-96 instrument. Due to the 2-week equilibration time, the target organisms may have become nonviable, but the presence of the organisms DNA detected by the candidate method resulting in the false positive results. This is a limitation of spiking studies and may not be observed in real-world testing. In the independent laboratory study, 2 false positive results and 1 false negative result were observed. A low level of inoculum (0.48 CFU/test portion) was obtained in the independent laboratory study and is not indicative of contamination levels observed in real-world settings. This low level may have led to the discrepancies observed in the study.

In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified. Three exclusivity organisms (*A. oryzae* ATCC 10124, *A. parasiticus* ATCC 15517 and *A. pseudoterreus* ATCC 10020) were detected by the candidate method; however, these strains are recognized as being close neighbors to the target strains.

Table 5. Inclusivity List: *Aspergillus* species (1)

No.	Organism	Source ^a	Origin	Result	
				AriaMx ^b	CFX-96 ^b
1	<i>Aspergillus flavus</i>	ATCC 9643	Shoe sole, New Guinea	+	+
2	<i>Aspergillus flavus</i>	ATCC 26768	Dry cracower sausage, Poland	+	+
3	<i>Aspergillus flavus</i>	ATCC 16870	Butter, Japan	+	+
4	<i>Aspergillus flavus</i>	ATCC 16883	Cellophane. South Pacific	+	+
5	<i>Aspergillus flavus</i>	ATCC 11489	Not Available	+	+
6	<i>Aspergillus flavus</i>	ATCC 11497	Not Available	+	+
7	<i>Aspergillus flavus</i>	ATCC204304	Human sputum, Virginia	+	+
8	<i>Aspergillus flavus</i>	ATCC MYA-1004	Clinical Isolate, New York City, NY	+	+
9	<i>Aspergillus flavus</i>	ATCC MYA-200026	Peanut cotyledons, USA	+	+
10	<i>Aspergillus flavus</i>	ATCC 12693	Soil	+	+
11	<i>Aspergillus fumigatus</i>	ATCC 16903	Human chest cavity lining, Illinois	+	+
12	<i>Aspergillus fumigatus</i>	ATCC1022	Lung of chicken, Connecticut	+	+
13	<i>Aspergillus fumigatus</i>	ATCC 1028	Soil, Germany	+	+
14	<i>Aspergillus fumigatus</i>	ATCC 96918	Not Available	+	+
15	<i>Aspergillus fumigatus</i>	ATCC 13073	Human pulmonary lesion, Maryland	+	+
16	<i>Aspergillus fumigatus</i>	ATCC 26934	Human with Aspergillosis disease, Montana	+	+
17	<i>Aspergillus fumigatus</i>	ATCC 14110	Human sputum	+	+
18	<i>Aspergillus fumigatus</i>	ATCC 204305	Human sputum, Virginia	+	+
19	<i>Aspergillus fumigatus</i>	ATCC MYA-3626	California, USA	+	+
20	<i>Aspergillus fumigatus</i>	ATCC MYA-3627	Jacksonville, TN, USA	+	+
21	<i>Aspergillus niger</i>	ATCC 15475	Soil	+	+
22	<i>Aspergillus niger</i>	ATCC 16888	Not Available	+	+
23	<i>Aspergillus niger</i>	ATCC 9142	Not Available	+	+
24	<i>Aspergillus niger</i>	ATCC 9029	Not Available	+	+
25	<i>Aspergillus niger</i>	ATCC 10535	Painted pine board, Virginia	+	+
26	<i>Aspergillus niger</i>	ATCC 13496	Soil, Louisiana	+	+
27	<i>Aspergillus niger</i>	ATCC 1015	Not Available	+	+
28	<i>Aspergillus niger</i>	ATCC 6275	Leather	+	+
29	<i>Aspergillus niger</i>	ATCC 66295	Jerusalem artichoke tuber, Korea	+	+
30	<i>Aspergillus niger</i>	ATCC 201201	Farmland, Korea	+	+
31	<i>Aspergillus terreus</i>	ATCC 16793	Soil, Texas	+	+
32	<i>Aspergillus terreus</i>	ATCC 1012	Soil, Connecticut	+	+
33	<i>Aspergillus terreus</i>	ATCC 10690	Haversack, New Guinea	+	+
34	<i>Aspergillus terreus</i>	ATCC 16794	Not Available	+	+
35	<i>Aspergillus terreus</i>	ATCC 24839	Not Available	+	+
36	<i>Aspergillus terreus</i>	ATCC 52430	Decayed timber, India	+	+
37	<i>Aspergillus terreus</i>	ATCC 12238	Not Available	+	+
38	<i>Aspergillus terreus</i>	ATCC MYA-4897	Soil, Baghdad, Iran	+	+
39	<i>Aspergillus terreus</i>	ATCC MYA-4898	Not Available	+	+
40	<i>Aspergillus terreus</i>	ATCC 26604	Ankylosing spondylitis	+	+
41	<i>Aspergillus flavus</i>	ATCC 13697	Not Available	+	+
42	<i>Aspergillus flavus</i>	ATCC MYA-4921	Not Available	+	+
43	<i>Aspergillus flavus</i>	ATCC MYA-3631	Human biopsy, USA	+	+
44	<i>Aspergillus fumigatus</i>	ATCC MYA-4690	Not Available	+	+
45	<i>Aspergillus fumigatus</i>	ATCC MYA-4915	Human sputum, Shanghai, China	+	+
46	<i>Aspergillus fumigatus</i>	ATCC 16424	Human lung with aspergillosis, Pennsylvania	+	+
47	<i>Aspergillus niger</i>	ATCC 208815	Derived from ATCC 9029	+	+
48	<i>Aspergillus terreus</i>	ATCC 20542	Not Available	+	+
49	<i>Aspergillus terreus</i>	ATCC 20064	Not Available	+	+
50	<i>Aspergillus terreus</i>	ATCC 32587	Gamma mutant of NRRL 1960	+	+

^aATCC – American Type Culture Collection, Manassas, VA; ^b(+) = positive, (-) = negative

Table 6. Exclusivity List (1)

No.	Organism	Source ^a	Origin	Result	
				SenSATIVax Flower AriaMx ^b	CFX-96 ^b
1	<i>Acinetobacter baumannii</i>	ATCC 19606	Urine	-	-
2	<i>Alternaria alternata</i>	ATCC 6663	Not Available	-	-
3	<i>Aspergillus aculeatus</i>	ATCC 24147	Not Available	-	-
4	<i>Aspergillus brasiliensis</i>	ATCC 9642	Wireless radio equipment, New South Wales, Australia	-	-
5	<i>Aspergillus casingii</i>	ATCC 42693	Dried chilies, New Guinea	-	-
6	<i>Aspergillus carbonarius</i>	ATCC MYA-4641	Grape berry, Brindis, Apulia, Italy	-	-
7	<i>Aspergillus clavatus</i>	ATCC 1007	Not Available	-	-
8	<i>Aspergillus deflectus</i>	ATCC 62502	Wheat, China	-	-
9	<i>Aspergillus fijiensis</i>	ATCC 20611	Not Available	-	-
10	<i>Aspergillus niveo-glaucus</i>	ATCC 10075	Not Available	-	-
11	<i>Aspergillus japonicus</i>	ATCC 16873	Soil, Panama	-	-
12	<i>Aspergillus nidulans</i>	ATCC 38163	Not Available	-	-
13	<i>Aspergillus oryzae</i>	ATCC 10124	Not Available	+ ^c	+
14	<i>Aspergillus parasiticus</i> Speare	ATCC 15517	Not Available	+ ^c	+
15	<i>Aspergillus pseudoterreus</i>	ATCC 10020	Not Available	+ ^d	+
16	<i>Aspergillus tamarii</i>	ATCC 1005	tomato	-	-
17	<i>Aspergillus tubingensis</i>	ATCC 1004	Not Available	-	-
18	<i>Aspergillus tubingensis</i>	ATCC 10550	Not Available	-	-
19	<i>Aspergillus ustus</i>	ATCC 1041	Culture containment, USA	-	-
20	<i>Aspergillus versicolor</i>	ATCC 11730	Cellophane gas mask, India	-	-
21	<i>Botrytis cinerea</i> Persoon	ATCC 11542	Azalea flowers, Washington, D.C.	-	-
22	<i>Candida albicans</i>	ATCC 10231	Man with bronchomycosis	-	-
23	<i>Cryptococcus laurentii</i>	ATCC 18803	Palm wine, malaffou, Congo	-	-
24	<i>Cryptococcus neoformans</i>	ATCC 208821	Patient with Hodgkin's disease, New York	-	-
25	<i>Fusarium proliferatum</i>	ATCC 76097	Raw cotton, North Carolina	-	-
26	<i>Fusarium oxysporum</i>	ATCC 62506	Celery, <i>Apium graveolens</i> var. <i>dulce</i> , California, USA	-	-
27	<i>Fusarium solani</i>	ATCC 52628	Cardamom fruit pod, <i>Elettaria cardamomum</i> , Guatemala	-	-
28	<i>Mucor circinelloides</i>	ATCC 38592	N/A	-	-
29	<i>Mucor hiemalis</i>	ATCC 28935	Soil in spruce forest, Germany	-	-
30	<i>Penicillium chrysogenum</i>	ATCC 18476	Cheese?, USSR	-	-
31	<i>Penicillium rubens</i>	ATCC 11709	Selected from Wis. 48-701, after N-mustard exposure	-	-
32	<i>Penicillium venetum</i>	ATCC 16025	<i>Hyacinthus</i> sp. Bulb, England	-	-
33	<i>Pseudomonas aeruginosa</i>	ATCC 35554	Not Available	-	-
34	<i>Rhizopus stolonifer</i>	ATCC 14037	Not Available	-	-
35	<i>Yarrowia lipolytica</i>	ATCC 20390	Nonsporulating diploid	-	-

^aATCC – American Type Culture Collection, Manassas, VA; ^b(+) = positive, (-) = negative; ^c Cross reacts with *A. flavus*; ^d Cross reacts with *A. terreus*

Table 7: PathoSEEK 5 – Color Aspergillus Multiplex Assays with SenSATIVAx Extraction Presumptive vs Confirmed Results (Paired) – POD Results (1)

Matrix and Inoculum	PCR Thermocycler	MPN _a / Test Portion	N ^b	x ^c	Presumptive		x	Confirmed		dPOD _{cp} ^f	95% CI ^g
					POD _{cp} ^d	95% CI		POD _{cc} ^e	95% CI		
Dried cannabis flower (>0.3% THC) 10g (<i>Aspergillus niger</i> ATCC 16888)	AriaMX	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.02 (0.57, 1.74)	20	11	0.55	0.34, 0.75	11	0.55	0.34, 0.75	0.00	-0.13, 0.13
		>10 (8.00, >30.0)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	CFX-96	NA	5	0	0.00	-0.47, 0.47	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.02 (0.57, 1.74)	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.75	-0.05	-0.21, 0.11
		>10 (8.00, >30.0)	5	5	0.00	-0.47, 0.47	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
THC-Infused Chocolate 25g (<i>Aspergillus fumigatus</i> ATCC MYA-4609)	AriaMX	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		2.85 (1.64, 4.96)	20	14	0.70	0.48, 0.86	12	0.60	0.39, 0.78	0.10	-0.08, 0.28
		>10 (8.00, >30.0)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	CFX-96	NA	5	0	0.00	-0.47, 0.47	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		2.85 (1.64, 4.96)	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.11, 0.21
		>10 (8.00, >30.0)	5	5	0.00	-0.47, 0.47	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Dried cannabis flower (>0.3% THC) 10g (<i>Aspergillus niger</i> ATCC 16888) - Independent Laboratory	AriaMX	NA	5	0	0.00	-0.47, 0.47	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.48 (0.24, 0.80)	20	6	0.30	0.14, 0.52	5	0.25	0.11, 0.47	0.05	-0.16, 0.26
		>10 (8.00, >30.0)	5	5	1.00	-0.47, 0.47	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	CFX-96	NA	5	0	0.00	-0.47, 0.47	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.48 (0.24, 0.80)	20	6	0.30	0.14, 0.52	5	0.25	0.11, 0.47	0.05	-0.16, 0.26
		>10 (8.00, >30.0)	5	5	1.00	-0.47, 0.47	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_{cp} = Candidate method presumptive positive outcomes divided by the total number of trials.

^ePOD_{cc} = Candidate method confirmed positive outcomes divided by the total number of trials.

^fdPOD_{cp} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

REFERENCES CITED

- McKernan, K. and Helbert, Y., Validation of the PathoSEEK® 5-Color Aspergillus Multiplex Assays with SenSATIVAx® Extraction for the Detection of *Aspergillus* in Dried Cannabis Flower and THC-infused Chocolate, AOAC® Performance TestedSM certification number 082102.
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