CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

072105

The AOAC Research Institute hereby certifies the method known as:

PathogenDx Quant[X] Fungal One Step

manufactured by

PathogenDx, Inc.
9375 E. Shea Blvd. Ste. 100
Scottsdale, AZ 85260 USA

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	December 10, 2023
Expiration Date	December 31, 2024

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SUBMITTING COMPANY
PathogenDx, Inc.
9375 E. Shea Blvd. Ste. 100
Scottsdale, AZ 85260 USA

METHOD NAME
PathogenDx Quant^{X} Fungal One Step

CATALOG NUMBER
QF-003

INDEPENDENT LABORATORY
Steadfast Analytical
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SV Laboratories
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Cincinnati, OH 45204

APPLICABILITY OF METHOD
Analytes – Viable Yeasts and Molds.

Matrixes – Dried cannabis flower (delta 9-tetrahydrocannabionl >0.3%, 10g).

Performance claims
ORIGINAL VALIDATION – The study data were unable to find a statistically detectable difference from zero between the PathogenDx Quant^{X} Fungal One Step method and traditional plating on Dichloran Rose Bengal Chloramphenicol (DRBC) agar plates, due to insufficient sample size.
MODIFICATION SEPTEMBER 2023 – The study data were unable to find a statistically detectable difference from zero between the PathogenDx Quant^{X} Fungal One Step (manual or automated sample preparation) and the traditional cultural procedures outlined in AOAC *Standard Method Performance Requirements* (SMPR) 2021.009 (3).

REFERENCE METHOD

ERV Matrix Extension for dried cannabis flower compared to Dichloran Rose Bengal Chloramphenicol agar.

***Standard Methods of Analysis (SMPRs®) for Viable Yeast and Mold Count enumeration in Cannabis and Cannabis Products (AOAC SMPR 2021.009)* (3)**

ORIGINAL CERTIFICATION APPROVAL DATE
July 30, 2021

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **February 2022 Level 1**
2. **October 2022 Level 1**
3. **September 2023 Level 2**

SUMMARY OF MODIFICATION
1. **Editorial changes.**
2. **Editorial changes to add caution statement added.**
3. **Addition of automated sample preparation, Octa™ AutoPrep Station and extend the range of method to include regulatory level at 100,000 cfu/g.**

Under this AOAC *Performance Tested Methods℠* License Number, 072105 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The PathogenDx Quant^{X} Fungal One Step assay is a quantitative test to detect the amount of Total Yeast/Mold (TYM) in dried cannabis flower specimens. The test involves extraction of nucleic acids from samples followed by Labeling PCR performed in a single reaction well. The PCR amplification product is then hybridized to a DNA microarray. Quantitation of fungal pathogens is determined by the conversion of fluorescence value of specific spots in the microarray when visualized in a plate reader to the corresponding colony forming unit (CFU). Quantitative results (presence/absence) are obtained at action limits established by cannabis regulatory agencies.

DISCUSSION OF THE VALIDATION STUDY (1)

In the matrix study, the Quant^{X} Fungal One Step assay successfully detected the target analyte from dried cannabis flower samples at the tested thresholds of detection. The POD statistical analysis in Table 10 indicated that the candidate method performance was not statistically different than that of the reference method for all contamination levels evaluated. Out of the 100 data points evaluated, 7 discrepant results were observed across mutiple dilutions evaluated (4 false positive and 3 false negative results). The Quant^{X} method has a high level of sensitivity and would be expected to detect DNA from low concentrations of the target organism that may not be able to be culturally confirmed. The delay in sample testing from original preparation of the materials until analysis may have resulted in the loss of viability of some species of organisms, making it harder to recover the organism on culture plates but still allowing the Quant^{X} method to detect the presence of the organisms. Testing in the matrix study was also performed at contamination levels as close to the dilution thresholds as possible. In the instances where no detection of the target occurred by the Quant^{X} method, but the organisms were recovered on agar plates may have resulted in concentrations right at the threshold and the distribution of the organism being slightly different in the aliquots used for the candidate method and those used for the reference method.

At the higher threshold, both the candidate and cultural confirmation method failed to detect the target analyte at the medium high and high levels of contamination. At these levels, a POD of 1.00 was expected. Testing for the validation study was delayed, and it is believed that the viability of the organisms may have been reduced at these levels resulting in less than optimal detection. Since this was observed with both the candidate method and culture confirmation it was not deemed to be an issue.

In the inclusivity and exclusivity study, the Quant^{X} method demonstrated a high level of specificity in detecting the 50 inclusive organisms and no detection of the 30 exclusive organisms (Table 8 and 9).

Table 8: Results for Inclusivity of the Quant^{X} Method (1)

No.	Organism	Source	Origin	Quant^{X} Result
1	*Alternaria alternata*	ATCC[a] 66981	*Arachis hypogaea*	Positive
2	*Arthrinium* species (aureum)	ATCC 56042	Not Available	Positive
3	*Aspergillus aculeatus*	ATCC 56925	Grape	Positive
4	*Aspergillus brasiliensis*	ATCC 16404	Blueberry	Positive
5	*Aspergillus caesiellus*	ATCC 42693	Dried Chilies	Positive
6	*Aspergillus flavus*	ATCC 6943	Shoe Sole	Positive
7	*Aspergillus fumigatus*	QL[b] 021116.3	Flour Tortilla	Positive
8	*Aspergillus niger*	ATCC 6275	Not Available	Positive
9	*Aspergillus oryzae*	ATCC 10124	Not Available	Positive
10	*Aspergillus terreus*	ATCC 1012	Soil	Positive
11	*Aureobasidium* species (pullulans)	ATCC 15233	Painted wood	Positive
12	*Botrytis cinerea*	ATCC 11542	Azalea Flowers	Positive
13	*Byssochlamys fulva*	ATCC 24474	Canned Grape Juice	Positive
14	*Candida albicans*	ATCC 10231	Man with Bronchymycosis	Positive
15	*Candida lusitaniae*	QL 15166-2	Tea	Positive
16	*Candida tropicalis*	ATCC 13803	Tea	Positive
17	*Chaetomium globosum*	ATCC 6205	Stored Cotton	Positive
18	*Cladosporium halotolerans*	ATCC 58927	Air Sample	Positive
19	*Cladosporium* species (herbarum)	ATCC 58927	Air Sample	Positive
20	*Cryptococcus laurentii*	ATCC 18803	Palm Wine	Positive
21	*Cryptococcus neoformans*	ATCC 14116	Pigeon Nest	Positive
22	*Curvularia lunata*	ATCC 12017	N/A	Positive
23	*Debaryomyces hansenii*	ATCC 60978	Cheese and Milk	Positive
24	*Dekkera bruxellensis*	ATCC 200341	Kombucha	Positive

25	*Fusarium oxysporum*	QL 0567126A	Environmental Isolate	Positive
26	*Fusarium proliferatum*	QL 0567112.1C	Environmental Isolate	Positive
27	*Fusarium solani*	QL 345317.4B	Environmental Isoalte	Positive
28	*Geotrichum candidum*	ATCC 34614	Clotted Carrot	Positive
29	*Geotrichum silvicola*	QL 14282-1A	Milk	Positive
30	*Kloeckera species*	QL 15079-1A	Tea	Positive
31	*Kluyveromyces lactis*	ATCC 8563	Creamery	Positive
32	*Mucor circinelloides*	ATCC 24905	Rice Fermentations	Positive
33	*Mucor hiemalis*	ATCC 34334	Cow Dung	Positive
34	*Paecilomyces* species (marquandii)	ATCC 10525	Soil	Positive
35	*Paecilomyces variotii*	ATCC 1114	Leather	Positive
36	*Penicillium chrysogenum*	ATCC 10106	Cheese	Positive
37	*Penicillium rubens*	QL 14280-2A	Guar Gum	Positive
38	*Penicillium venetum*	ATCC 16025	*Hyacinthus* sp. Bulb	Positive
39	*Phytophthora infestans*	ATCC MYA 1113	Potato Tuber	Positive
40	*Purpureocillium* species (lilacinum)	ATCC 10114	Soil	Positive
41	*Rhizopus oryzae*	ATCC 9363	Soy Sauce	Positive
42	*Rhizopus stolonifera*	QL 14181-2A	Not Available	Positive
43	*Rhodotorula mucilaginosa*	ATCC 9449	N/A	Positive
44	*Saccharomyces kudriavzevii*	ATCC 2601	N/A	Positive
45	*Scopulariopsis acremonium*	ATCC 58636	Chicken House Soil	Positive
46	*Talaromyces flavus*	ATCC MYA 288	N/A	Positive
47	*Talaromyces pinophilus (Penicillium pinophilum)*	NRRL[c] 11797	Corn	Positive
48	*Wickerhamomyces anomala*	ATCC 2349	N/A	Positive
49	*Yarrowia lipolytica*	ATCC 9773	Not Available	Positive
50	*Zygosaccharomyces bailii*	ATCC 36947	Salad Dressing	Positive

[a]Method developers must test 50 total species to meet inclusivity requirements.

[b]ATCC = American Type Culture Collection, Manassas, VA.

[c]QL = Q Laboratories, Inc., Cincinnati, OH.

[d]NRRL = Agricultural Research Service Culture Collection, Peoria, IL.

Table 9: Results for Exclusivity of the Quant[X] Method (1)

No.	Organism	Source	Origin	Quant[X] Result
1	*Acinetobacter baumanii*	ATCC[a] 19606	Urine	Non Detected
2	*Aeromonas hydrophila*	ATCC 49140	Clinical Isolate	Non Detected
3	*Burkholderia cepacia*	ATCC 25416	Plant Derived	Non Detected
4	*Bacillus subtilis*	ATCC 6633	Not Available	Non Detected
5	*Citrobacter braakii*	ATCC 43162	Clinical Isolate	Non Detected
6	*Citrobacter farmeri*	ATCC 51633	Human Feces	Non Detected
7	*Edwardsiella tarda*	ATCC 15947	Human Feces	Non Detected
8	*Enterobacter aerogenes*	ATCC 13048	Sputum	Non Detected
9	*Enterobacter cloacae*	ATCC 13047	Spinal Fluid	Non Detected
10	*Erwinia amylovora*	ATCC 51852	Plant	Non Detected
11	*Escherichia coli*	ATCC 8739	Feces	Non Detected
12	*Escherichia coli* O157:H7	ATCC 43895	Raw Hamburger	Non Detected
13	*Escherichia hermanii*	ATCC 33650	Mouse Brain	Non Detected
14	*Escherichia vulneris*	ATCC 29943	Human Wound	Non Detected
15	*Hafnia alvei*	ATCC 51815	Milk	Non Detected
16	*Klebsiella oxytoca*	ATCC 43165	Clinical Isolate	Non Detected
17	*Klebsiella pneumonia*	ATCC 11296	Not Available	Non Detected
18	*Listeria monocytogenes*	ATCC 7644	Human Isolate	Non Detected
19	*Morganella morganii*	ATCC 25829	Human	Non Detected
20	*Pantoea agglomerans*	ATCC 19552	Sewage	Non Detected
21	*Proteus mirabilis*	ATCC 7002	Urine	Non Detected
22	*Pseudomonas aeruginosa*	ATCC 27853	Clinical Isolate	Non Detected
23	*Pseudomonas fluorescens*	QL[b] 17041.3	Raw Milk	Non Detected
24	*Pseudomonas gessardii*	QL 17041.12	Raw Milk	Non Detected
25	*Ralstonia pickettii*	ATCC 27511	Clinical Isolate	Non Detected
26	*Rahnella aquatilis*	ATCC 55046	Soil	Non Detected
27	*Salmonella* Agona	ATCC 51957	Not Available	Non Detected
28	*Stenotrophomonas maltophilia*	ATCC 13637	Patient with mouth cancer	Non Detected
29	*Staphylococcus aureus*	ATCC 6538	Human Lesion	Non Detected
30	*Serratia marcescens*	ATCC 13880	Human	Non Detected

[a]ATCC = American Type Culture Collection, Manassas, VA.
[b]QL = Q Laboratories, Inc., Cincinnati, OH.

Table 10. Quant^{X} Fungal One Step presumptive and confirmed results for testing of dried cannabis flower (1)

Matrix	Strain	Level (CFU/g)[a]	Test Threshold (CFU/g)[b]	N[c]	Quant^{X} Fungal One Step Presumptive			Quant^{X} Fungal One Step Confirmed				
					x[d]	POD_{CP}[e]	95% CI	x	POD_{CC}[f]	95% CI	$dPOD_{CP}$[g]	95% CI[h]
Dried Cannabis Flower	Naturally Contaminated	320	≥ 1,000	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			≥ 10,000	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		890	≥ 1,000[i]	20	9	0.45	0.26, 0.66	6	0.30	0.14, 0.52	0.15	-0.05, 0.35
			≥ 10,000	20	0	0.00	0.00, 0.16	0	0.00	0.00, 0.16	0.00	-0.13, 0.13
		13000	≥ 1,000[i,j]	20	18	0.90	0.70, 0.97	18	0.90	0.70, 0.97	0.00	-0.19, 0.19
			≥ 10,000	20	0	0.00	0.00, 0.16	0	0.00	0.00, 0.16	-0.05	-0.13, 0.13
		100000	≥ 1,000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
			≥ 10,000[j]	5	0	0.00	0.00, 0.43	2	0.40	0.12, 0.77	-0.40	-1.00, 0.21

[a]From aerobic viable yeast and mold plate count (DRBC).
[b]Based on dilution and volume of sample tested. A positive result indicates contamination above the test threshold level.
[c]N = Number of test portions.
[d]x = Number of positive test portions.
[e]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[f]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[g]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i] Results include presumptive positive test portions that confirmed negative
[j] Results include presumptive negative test portions that confirmed positive

DISCUSSION OF MODIFICATION APPROVED SEPTEMBER 2023 (2)

The sample preparation extension to the automated Octa™AutoPrep Station demonstrated that Quant^{X} Fungal successfully distinguished naturally contaminated cannabis at three defined action limits across five contamination levels whether the sample preparation was manual or automated. There was no statistically significant difference detected between the PathogenDx Quant^{X} Fungal One Step presumptive results and the culture confirmation, regardless of methodology. The Quant^{X} Fungal One Step assay when used in tandem with the Octa™AutoPrep System for sample processing reduced sample to answer time, plastic waste, and human error in comparison to the standard manual preparation. In a benchmarking experiment of 48 samples (data not shown) to compare automated sample preparation on the Octa AutoPrep Station against manual sample preparation, the Octa™ Tips replaced the use of spin columns, pipette tips and centrifuge tubes required for the manual procedure, resulting in less plastic consumable waste. The automated sample preparation for 48 samples was completed in 2.5 h versus 4.5 h for the manual procedure; automated preparation required under 0.5 h of operator hands-on work while the manual method required over 2.5 h of hands-on effort. This further reduction in time saves days' worth of incubation time required for the growth of fungal and yeast specimens using traditional plating methods.

Table 9. PathogenDx Quant[X] Fungal One Step Presumptive vs. Confirmed POD Results (2)

Matrix	Strain	Sample Prep Type	Level (CFU/g)[a]	Test Threshold (CFU/g)[b]	N[c]	Quant[X] Fungal Presumptive			Quant[X] Fungal Confirmed			dPOD$_{CP}$[g]	95% CI[h]
						x[d]	POD$_{CP}$[e]	95% CI	x	POD$_{CC}$[f]	95% CI		
Dried Cannabis Flower (10g)	Naturally Contaminated	Manual + ReliaPrep	323	≥1,000	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				≥10,000	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				≥100,000	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1,246	≥1,000	20	14	0.70	0.48, 0.86	13	0.65	0.43, 0.82	0.05	-0.11, 0.21
				≥10,000	20	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.13, 0.13
				≥100,000	20	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.13, 0.13
			9,715	≥1,000	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
				≥10,000	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
				≥100,000	20	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.13, 0.13
			67,750	≥1,000	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
				≥10,000	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
				≥100,000	20	4	0.20	0.08, 0.42	3	0.15	0.05, 0.36	0.05	-0.11, 0.21
			$5.04x10^5$	≥1,000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				≥10,000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				≥100,000	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.36, 0.76
		Octa™ AutoPrep	323	≥1,000	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				≥10,000	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				≥100,000	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			1,246	≥1,000	20	14	0.70	0.48, 0.86	13	0.65	0.43, 0.82	0.05	-0.11, 0.21
				≥10,000	20	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.13, 0.13
				≥100,000	20	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.13, 0.13
			9,715	≥1,000	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
				≥10,000	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
				≥100,000	20	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.13, 0.13
			67,750	≥1,000	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
				≥10,000	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.13, 0.13
				≥100,000	20	4	0.20	0.08, 0.42	3	0.15	0.05, 0.36	0.05	-0.11, 0.21
			$5.04x10^5$	≥1,000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				≥10,000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				≥100,000	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.36, 0.76

[a]From aerobic viable yeast and mold plate count (DRBC).

[b]Based on dilution and volume of sample tested. A positive result indicates contamination above the test threshold level.

[c]N = Number of test portions.

[d]x = Number of positive test portions.

[e]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.

[f]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.

[g]dPOD$_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

REFERENCES CITED

1. Katchman, B.A., Ulrich, P., Freeman, S.R., May, M., O'Brien, K., Eggers, R., Wen, F., and Hogan, M., Emergency Response Validation Study for the Pathogen Dx Quant[X] Fungal One Step Assay for the Detection of Viable Yeasts and Molds in Cannabis Flower, AOAC *Performance Tested Methods[SM]* Emergency Response Validation certification number 072105.
2. Vold, L., Rueda, A., O'Brien, K., Eggers, R., Martel, R., Lachance, E., and Hogan, M., Validation of a Sample Preparation Extension for Quant[X] Fungal One Step Assay for the Quantitative Estimation of Total Yeast and Mold in Cannabis Flower, AOAC *Performance Tested Methods[SM]* certification number 072105. Approved September 21. 2023.
3. *Standard Methods of Analysis (SMPRs®) for Viable Yeast and Mold Count enumeration in Cannabis and Cannabis Products (AOAC SMPR 2021.009)* https://www.aoac.org/wp-content/uploads/2021/06/SMPR-2021_009.pdf *(Accessed March 2023)*