DNA Microarray for 1 CFU/g Detection of *Aspergillus, Salmonella, STEC (stx1 and stx2)* in Dried Cannabis & Hemp Flower

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Abstract: Several harmful pathogens have been identified in cannabis flower that pose a risk to consumers such as Aspergillus, Salmonella, and STEC+ Escherichia coli. The majority of states with approved medical or recreational cannabis require 1 CFU/g detection of these pathogens. The objective was to conduct an AOAC PTM matrix study of PathogenDx **Detect**[X] Combined method in dried cannabis flower (delta 9- tetrahydrocannabinol >0.3%; 10 g sample size) and hemp flower. The **Detect**[X] Combined assay was tested following AOAC Official Methods of Analysis Appendix J validation guidelines and the AOAC Cannabis Aspergillus, Salmonella and STEC protocol for 1 CFU/g detection in cannabis and hemp flower. Inclusivity and exclusivity, product consistency and assay robustness were also evaluated.

INTRODUCTION:

As the cannabis industry expands and becomes more heavily regulated, the need for tools to detect microbial contamination increases. The majority of states with approved medical or recreational cannabis require 1 CFU/g detection of those pathogens. The purpose of this study was to conduct an AOAC PTM matrix study of PathogenDx **Detect**[X] Combined method in dried cannabis flower and in hemp flower

METHODS:

Detect[X]-Combined is a microarray-based end point PCR assay, was used to simultaneously identify both bacterial and fungal organisms in hemp and cannabis flower. To compare the effectiveness of the **Detect**[X]-Combined test method, each sample was assessed by traditional microbiological plating, qPCR, serologic methods, biochemical methods and by the PathogenDx **Detect**[X]-Combined microarray.

The detailed IFU for the **Detect**[X]-Combined assay are available via 2D barcode scan, based on the recently approved AOAC PTM (Right, Lower). Briefly, 1gr of dried Cannabis flower was hydrated by addition of 9ml of PBS. (1)ml of that re-hydrate was recovered, centrifuged to generate a pellet which is then heat-lysed, neutralized and treated with ProK. 2uL of the resulting Pro K treated lysate was then subjected to a multiplexed, asymmetric PCR reaction. The resulting Cy-3 labelled (single stranded) multiplex PCR product was then mixed with Hybridization Buffer and applied directly to (1) well of the **Detect**[X]-Combined microarray. Hybridization proceeded at RT for 30 minutes, followed by 3x wash steps and imaging of the entire 96-well plate on a Sensospot Imager. Central Panel, Upper.

TEST CONTENT:.

The **Detect**[X]-Combined array contains probes for (7) Bacteria and (4) Fungi. Hybridization probes for each are printed in triplicate as a 12x12 microarray, one microarray each on the bottom of (1) well of a standard (8x12) 96-well plate. The fraction of the test content presently validated by AOAC is shown in Bold.

Bacterial Organisms	Fungal Organisms
Salmonella spp	***Aspergillus flavus***
***Escherichia coli* specific (Stx1/2)**	***Aspergillus fumigatus***
Escherichia coli/ Shigella spp	***Aspergillus terreus***
Staphylococcus aureus	***Aspergillus niger***
Pseudomonas aeruginosa	
Listeria spp	
Clostridium botulinum	

Bold=Organisms in **Detect**[X] **Combined** Certified here by AOAC (PTM 012201)
Other organisms are included in the **Detect**[X] **Combined** assay, to be validated later.

SCAN ME

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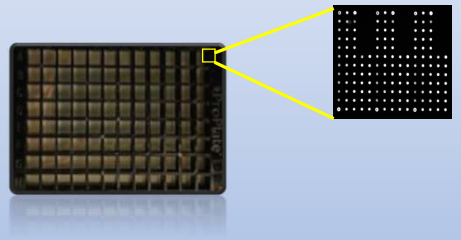

PathogenDx Process Workflow: The PathogenDx Detect[X] Combined **assay is AOAC validated for Cannabis and Hemp without the need for sample enrichment.** The DNA microarray technology, by PathogenDx, combines multiplexed PCR with specific single stranded DNA probes fixed to a glass microscope slide to detect and quantify a variety of bacterial and fungal pathogens in under 6 hours without the need for sample enrichment.
The 12 x12 layout of the **Detect**[X] Combined microarray spots are detected by imaging of Cy-5 added to each microarray probe, and is used for QC and automated image analysis on dedicated Augury software. Each spot comprises a single unique synthetic oligonucleotide, each printed as a 100um spot, 250um on center.

Inoculation level (CFU/test portion)

Matrix	Strain	MPN[a]/ Test portion	N[b]	Candidate			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Dried Hemp Flower	*Aspergillus flavus* ATCC 16883	-	5	0	0.000	0.000, 0.434	0	0.000	0.000, 0.434	0.000	-0.469, 0.469
		0.49 (0.25, 0.85)	20	17	0.850	0.639, 0.947	15	0.750	0.531, 0.888	0.100	-0.152, 0.340
		1.84 (0.91, 3.75)	5	5	1.000	0.566, 1.000	5	1.000	0.566, 1.000	0.000	-0.469, 0.469
	Salmonella Typhimurium ATCC 14028	-	5	0	0.000	0.000, 0.434	0	0.000	0.000, 0.434	0.000	-0.469, 0.469
		0.69 (0.51, 1.37)	20	7	0.350	0.181, 0.567	5	0.250	0.112, 0.469	0.100	-0.176, 0.357
		1.11 (0.60, 3.18)	5	5	1.000	0.566, 1.000	5	1.000	0.566, 1.000	0.000	-0.469, 0.469
	Escherichia coli O26:H11 BAA-2196	-	5	0	0.000	0.000, 0.434	0	0.000	0.000, 0.434	0.000	-0.469, 0.469
		0.69 (0.40, 1.14)	20	16	0.800	0.584, 0.919	15	0.750	0.531, 0.888	0.050	-0.192, 0.287
		1.84 (0.91, 3.75)	5	5	1.000	0.566, 1.000	5	1.000	0.566, 1.000	0.000	-0.469, 0.469

Table 1. PathogenDx Hemp POD table (from AOAC final report). Comparing the Candidate method Detect[X] Combined **vs the Confirmed BAM method.**

Inoculation level (CFU/test portion)

Matrix	Strain	MPN[a]/ Test portion	N[b]	Candidate			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Dried Cannabis Flower	*Aspergillus niger* ATCC 36626	-	5	0	0.000	0.000, 0.434	0	0.000	0.000, 0.434	0.000	-0.469, 0.469
		0.54 (0.29, 0.92)	20	15	0.750	0.531, 0.888	15	0.750	0.531, 0.888	0.000	-0.132, 0.132
		1.61 (0.64, 3.54)	5	5	1.000	0.566, 1.000	5	1.000	0.566, 1.000	0.000	-0.469, 0.469
	Salmonella Enteritidis ATCC 13076	-	5	0	0.000	0.000, 0.434	0	0.000	0.000, 0.434	0.000	-0.469, 0.469
		0.69 (0.51, 1.37)	20	14	0.700	0.481, 0.855	13	0.650	0.433, 0.819	0.050	-0.226, 0.317
		1.97 (0.91, 4.27)	5	5	1.000	0.566, 1.000	5	1.000	0.566, 1.000	0.000	-0.469, 0.469
	Escherichia coli O157:H7 ATCC 35150	-	5	0	0.000	0.000, 0.434	0	0.000	0.000, 0.434	0.000	-0.469, 0.469
		0.82 (0.49, 1.36)	20	14	0.650	0.481, 0.855	14	0.650	0.481, 0.855	0.000	-0.132, 0.132
		2.57 (1.15, 5.78)	5	5	1.000	0.566, 1.000	5	1.000	0.566, 1.000	0.000	-0.469, 0.469

Table 2 PathogenDx Cannabis POD table (from AOAC final report). Comparing the Candidate method Detect[X] Combined **vs the Confirmed BAM method.**

RESULTS:

A key component of the present AOAC reviewed Matrix study (Tables 1,2) demonstrates that **Detect**[X]-Combined detects fungal and bacterial organisms simultaneously with sensitivity & specificity equivalent to that obtained with analytical plate culture (Columns 6 vs 9) and that the measured Sensitivity and Specificity is unchanged when comparing dried hemp flower (Table1) vs cannabis flower (Table 2) as the matrix.

In the parallel AOAC Inclusivity study, 50 out of 50 target isolates (*Aspergillus and STEC*) were correctly detected. ***See accompanying AOAC Report.***

In the parallel AOAC Exclusivity study, 26 out of 30 *Aspergillus* and 30 out of 30 *STEC* non-target strains were correctly excluded. ***See accompanying AOAC Report.***

CONCLUSION:

In summary, these AOAC Certified data suggest that the PathogenDx **Detect**[X]-Combined assay is an effective method for simultaneous qualitative detection of *Aspergillus*, *Salmonella,* and *STEC* in a multiplex assay from dried hemp flower and cannabis flower and as a result has been awarded AOAC PTM Certification No. 012201

This study emphasizes:

- The PathogenDx **Detect**[X]-Combined microarray technology can detect bacteria and fungal spores at the limit detection previously only attainable by plate culture.
- The PathogenDx **Detect**[X]-Combined microarray technology functions without enrichment culture and with highly simplified sample preparation, thereby increasing testing throughput. i.e. raw sample to answer in less than 6 hrs on up to (7) bacterial and (4) *Aspergillus* target organisms per sample, on 96 distinct hemp or cannabis samples in parallel.


AOAC RESEARCH INSTITUTE

CERTIFICATION

AOAC® *Performance Tested*℠

Certificate No.

012201

The AOAC Research Institute hereby certifies the method known as:

PathogenDx Detect® Combined

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

This method has been evaluated in the AOAC® *Performance Tested Methods*℠ 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*℠ 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 (January 31, 2022 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

February 2, 2022
Date

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PathogenDx

Setting the standard in molecular testing