A Modification of the GENE-UP® Aspergillus PRO to include a Non-Viable DNA Removal Step with IS Viability Kit: AOAC PTM 022103

Patrick Bird, John Mills, Maria McIntyre, Ron Johnson, Vikrant Dutta, BioMérieux, Inc., Hazelwood, MO.
Adam Joelsson, Ashley Brown, Invisible Sentinel, Inc., Philadelphia, PA, USA

The Emerald Conference 2023 • San Diego, CA

INTRODUCTION

Several recent surveys of cannabis products have indicated that fungal contaminants, specifically *Aspergillus,* are routinely isolated. It is estimated that between 10-20% of cannabis flower fail testing requirements, with most due to fungal contamination. This has resulted in an increase in remediation steps designed to eliminate pathogenic microorganisms, however, these steps may not eliminate the presence of their DNA in the flower. Highly sensitive methods, such as PCR, can detect this DNA from non-viable organisms.

PURPOSE

The validate the GENE-UP Aspergillus PRO method to include a protocol for removing DNA from environmental sources and non-viable cells using the IS Viability Kit. The method validation included an extension to dried hemp flower, a new supplement for the primary enrichment, the addition of PCR tubes with septum caps and a transition to the GENE-UP routine software.

METHOD

The method validation was conducted according to AOAC Appendix J validation guides for microbiology methods and AOAC SMPR 2019.001 for the detection of *Aspergillus* in cannabis.

Dried hemp flower was evaluated at two test portion sizes (10 g and 1 g). 10 g test portions were evaluated at 3 levels of contamination: control x 5 replicates, low x 20 replicates and high x 5 replicates. 1 g test portions were evaluated using naturally contaminated matrix: 2 lots x 20 g. Testing was performed with the IS Viability Kit using the current PCR tube and the new PCR tube with septum caps. All confirmation was performed according to SMPR 2019.001.

An inclusivity and exclusivity study was performed using the new enrichment, Aspergillus Enrichment Broth with supplement. 52 target strains and 30 non-target strains were evaluated.

Figure 1: GENE-UP PCR Preparation Process

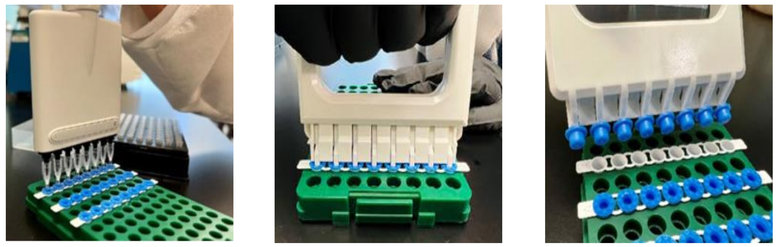


Figure 2: IS Viability DNA Removal Process

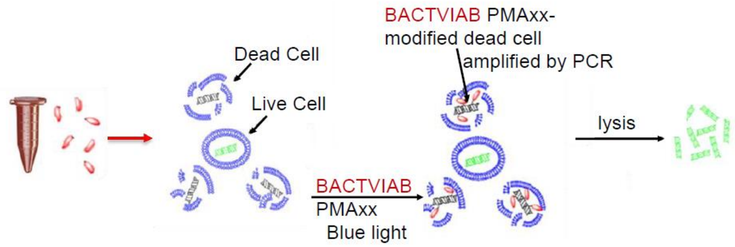
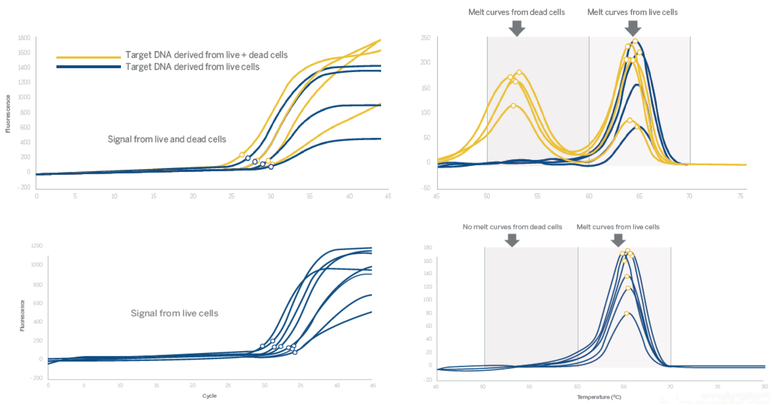


Figure 3: Impact of IS Viability DNA Removal Process on PCR and Melt Curves



INCLUSIVITY AND EXCLUSIVITY

Aspergillus Inclusivity:

1. *Aspergillus flavus* – 15 strains
2. *Aspergillus fumigatus* – 13 strains
3. *Aspergillus niger* – 13 strains
4. *Aspergillus terreus* – 12 strains

Aspergillus Exclusivity:

1. 30 total strains
2. All yeasts and molds
3. 14 non-target *Aspergillus*
4. Includes *A. tamarii* and *A. brasiliensis*

Each of the 52 target strains were correctly detected after 24 h of enrichment and each of the non-target strains was correctly excluded, including *A. tamari* and *A. brasiliensis.*

INVISIBLE SENTINEL®
A BIOMÉRIEUX COMPANY

Table 1: GENE-UP Aspergillus PRO Presumptive vs Confirmed Results (Paired) – POD Results Cannabis with IS Viability PCR Kit

Matrix and Inoculum	Time Point / Lysis	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 Hemp Flower/10g Aspergillus fumigatus ATCC[h] 1022	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.71 (0.41 - 1.19)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	(-0.13, 0.13)
		11.3 (4.13 - 18.7)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
	48 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.71 (0.41 - 1.19)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	(-0.13, 0.13)
		11.3 (4.13 - 18.7)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Dried Hemp Flower/1g Naturally Contaminated	24 h – Lot 1	2.49 (0.91, 4.90)	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0.00	(-0.13, 0.13)
	24 h – Lot 2	2.51 (0.91, 5.04)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	(-0.13, 0.13)
	48 h – Lot 1	2.49 (0.91, 4.90)	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0.00	(-0.13, 0.13)
	48 h – Lot 2	2.51 (0.91, 5.04)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	(-0.13, 0.13)

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD$_{cp}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD$_{cc}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_{cp}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]ATCC = American Type Culture Collection.

MATRIX STUDY

The GENE-UP Aspergillus PRO successfully detected *Aspergillus* species from dried hemp flower at both 24 and 48 h with both the 10 g and 1 g test portions. Test portions analyzed with the IS Viability kit reduced the presence of non-viable DNA present in the test portions as determined through culture confirmation. Results were equivalent between the current PCR tubes and the new PCR tubes with septum caps.

STUDY HIGHLIGHTS

AOAC PTM Certification for the following:

- Addition of protocol for DNA removal from non-viable cells allows for integration of remediation techniques without concern for non-confirming positives.
- Expansion of method to include dried hemp flower (10 g and 1 g).
- Integration of septum cap in PCR tube reduces the chance for cross contamination from laboratory
- Transition to routine software increases laboratory efficiency in obtaining results.