



One Health Diagnostics™

Nisha Corrigan¹, Alexandra Tudor², Leo Horine², and Casey Simmons¹
Hygiena®, 2 Boulden Circle, New Castle, DE 19720
1. 2. TEQ Analytical Laboratories, Inc., Aurora, CO 80045

INTRODUCTION:

The introduction of the hemp pilot program in the 2014 Farm Bill led to the authorization of hemp production through the 2018 Farm Bill. This in turn increased state-level legalization of cannabis for medicinal and recreational uses.

Although the majority of US states (n=37)¹, allow for at least medical marijuana use, there are no federal regulations around pathogen testing of these product types. This leaves pathogen testing guidelines up to each state to manage. Due to this varied oversight from state to state, regulations have ranged from rigid to lax (e.g., Florida requires <1 CFU for *Aspergillus*, STEC, and *Salmonella*, while Delaware requires none of these tested for medical marijuana).²

With the rising legalization of recreational and medical marijuana, the chance of possible outbreaks also increases. A 7-year survey (2012-2019) conducted across state-regulated dispensaries in Northern California, showed an over 14% fail rate (100,000 CFU/g microbial load). Further testing revealed a 3% detection rate of *E. coli* and a 10.6% detection rate of coliforms in cannabis flower samples.³

Due to the ever-increasing popularity to inhale, ingest and topically apply these cannabis and hemp products, pathogen detection has become a vital need to ensure the health and safety of all, especially immunocompromised users.

PURPOSE:

The independent lab study, performed by TEQ Analytical Laboratory (Aurora, CO), assessed the performance of real-time PCR assays to detect *Salmonella* and STEC (including *E. coli* O157:H7) in dried cannabis [> 0.3% delta 9-tetrahydrocannabinol (THC)] and hemp flowers (≤ 0.3% THC). Since there are no federal reference methods for pathogen testing in these matrices, the newly published guidance for confirmation in AOAC SMPR 2020.002 and 2020.012 was used.

REGISTERED TRADEMARKS:

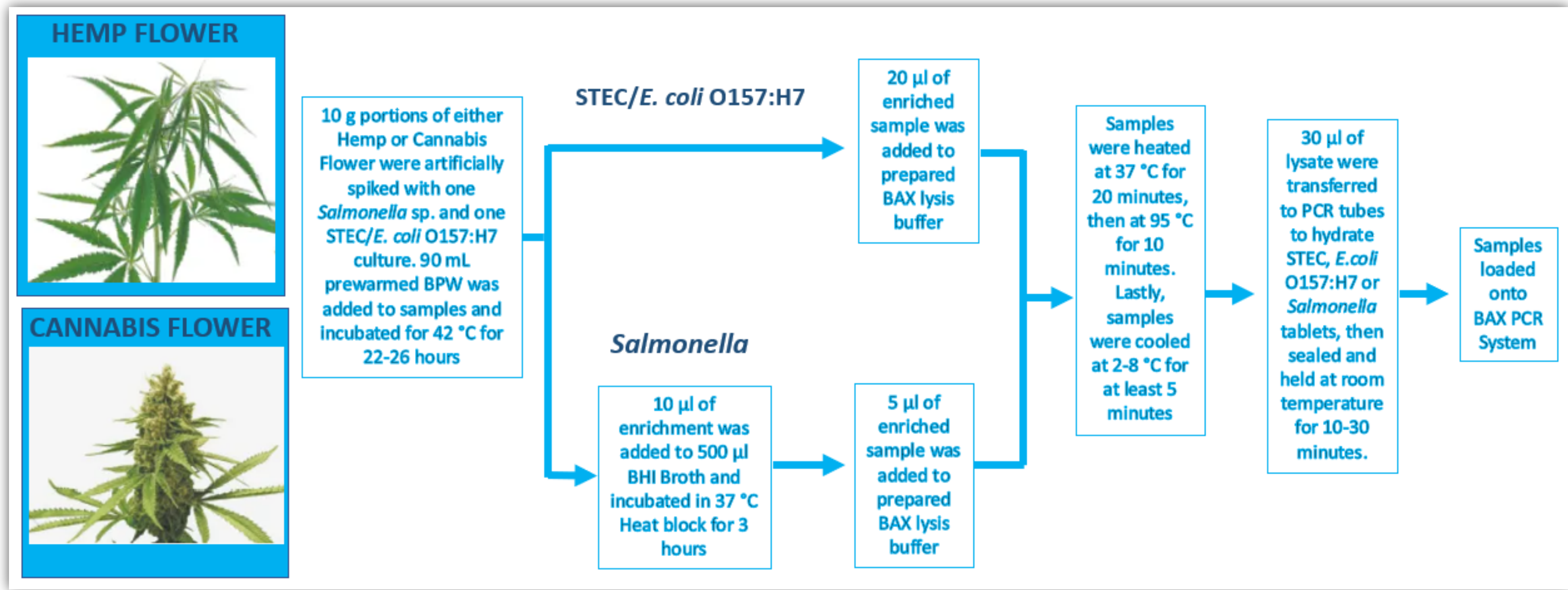
BAX® is a registered trademark of Hygiena for its line of equipment, reagents and software used to analyze samples for microbial contamination.

Evaluation of Hygiena's BAX® System Real-Time PCR Assays for Detection of *Salmonella* spp. and STEC in Cannabis Flower and Hemp Flower for AOAC Research Institute's *Performance Tested Methods*™ Certification

BAX System Q7

METHOD:

Ten-gram portions of dried cannabis flower (n=20) and dried hemp flower (n=20) were artificially inoculated with fractional levels of one *Salmonella* spp. and one STEC/*E. coli* O157:H7 strain and held at room temperature for 2 weeks. Five uninoculated and five high-level (10X fractional) portions per matrix were also inoculated. Portions were enriched with 90 mL of prewarmed (37-42 °C) Buffered Peptone Water (BPW) and incubated at 42 °C for 22-26 hours. The BAX System lysis protocol is outlined in the flow chart below. All results were culture-confirmed according to AOAC SMPR 2020.002 and 2020.012.



RESULTS:

The real-time PCR assays successfully detected *Salmonella* and the target STEC/*E. coli* O157:H7 species in dried cannabis flower and dried hemp flower in a 10 g sample size. Difference in POD analysis for the presumptive versus confirmed positives showed no statistically significant differences, with all ranges of the 95% confidence intervals containing the zero point (See Tables 1 and 2 below).



SUMMARY:

Since the inception of the 2014 Farm Bill, the U.S. hemp and cannabis flower industries have continually expanded year after year, with 2022 set to reach \$32 billion in sales. Some industry experts project that number will hit close to \$100 billion in sales by 2030.⁴

With the rapidly booming cannabis business, coupled with a lack of U.S. federal guidelines, the need for validated methods to test these product types becomes increasingly vital.

Independent lab validation has determined the BAX System Real-Time PCR Assays (*Salmonella*, STEC Suite, *E. coli* O157 EXACT) have met the expectations as set forth in AOAC SMPR 2020.002 and 2020.012, respectively, for dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC) testing using a 10 g test portion size.

PROBABILITY OF DETECTION STATISTICAL TABLES:

Table 1. BAX System Real-Time PCR Assay for *Salmonella* presumptive vs. confirmed results in dried cannabis flower and dried hemp flower

Matrix	Inoculum	MPN ^a	Presumptive				Confirmed				
			N ^b	x ^c	POD _{cp} ^d	95% CI	x	POD _{cc} ^e	95%CI	dPOD _{cp} ^f	95% CI ^g
Dried Cannabis Flower 10 g	S. Typhimurium ATCC 14028	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.74 (0.91, 8.08)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	(-0.13, 0.13)
		4.90 (2.50, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Dried Hemp Flower 10 g	S. Enteritidis ATCC 13076	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.03 (0.46, 2.53)	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	(-0.11, 0.21)
		4.03 (2.10, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

Table 2. BAX System Real-Time PCR Assays for STEC Suite and *E. coli* EXACT presumptive vs. confirmed results in dried cannabis flower and dried hemp flower

Matrix	Inoculum	MPN ^a	Presumptive				Confirmed				
			N ^b	x ^c	POD _{cp} ^d	95% CI	x	POD _{cc} ^e	95%CI	dPOD _{cp} ^f	95% CI ^g
Dried Cannabis Flower 10 g	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		0.88 (0.40, 2.02)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
		2.96 (1.54, 9.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Dried Hemp Flower 10 g	<i>E. coli</i> O157:H7 ATCC 43890	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.48 (0.77, 3.74)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	(-0.13, 0.13)
		6.77 (3.95, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Dried Hemp Flower 10 g	<i>E. coli</i> O26 CDC 03-3014	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.15 (0.61, 2.45)	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	(-0.11, 0.21)
		2.96 (1.54, 9.78)	5	5	1.00	0.57, 1.00	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:

1. State Medical Cannabis Laws. *National Conference of State Legislatures*, (ncsl.org), (accessed 6/10/2022).
2. Cannabis Microbial Testing Regulations by State. *Medicinal Genomics*, <https://www.medicinalgenomics.com/cannabis-microbial-testing-regulations-by-state/>, (accessed June 15, 2022).
3. Robert W. Martin, Kendra Wheeler, Charisse M. Macdula, Jorge Jacobo, Emily R. Savage. *Cannabis Science and Technology*, September 2021, Volume 4, Issue 7, Pages: 16-20.
4. Cannabis Industry Statistics 2022. *Flowhub*, <https://flowhub.com/cannabis-industry-statistics>, (accessed June 20, 2022).