

Automated Cannabis DNA Purification and Microbial Safety Testing to Comply with Canadian and Individual U.S. State Requirements

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Introduction

Lack of federal cannabis testing regulation in the United States has led to a fragmented patchwork of regulations that vary across US states and Canada (Table 1). States drafting testing requirements for regulated cannabis may look to early adopters with large cannabis markets, such as California¹, or to quality control standards from non-profit educational organizations, such as the American Herbal Pharmacopoeia² for guidance. Indeed, the efficiency and experience of regulations and testing policies in these areas may eventually drive or heavily influence formalized regulations in the United States, but until that time, states may also build upon these established practices and regulations with those that reflect their unique situation and may differ by as little as a threshold value. As a result, assay protocols can vary widely. Additionally, many older methods are not specifically validated for use with cannabis samples, are not scalable, and do not include internal cannabis controls to confirm test accuracy. Labor-intensive protocol steps, such as sample preparation, limit sample throughput and risk errors due to human variability and subjective interpretation. Errors and sample mishandling may require that tests be repeated, thus consuming precious sample and incurring additional time and costly resources.

The SenSATIVax[®] Plant/Microbial DNA Purification kit^{3,4} and PathoSEEK[®] microbial detection quantitative polymerase chain reaction (qPCR) assays⁵ from Medicinal Genomics (MGC, Beverly, MA) are specifically developed for use in cannabis testing laboratories, and include the use of internal cannabis controls. Two SenSATIVax kits are available to provide rapid and simple isolation of plant and microbial DNA from fresh flower or leaf tissue and from a variety of marijuana infused product (MIP) matrices, including extracts, tinctures, or edibles. The PathoSEEK primer sets are amenable to purified DNA from either SenSATIVax extraction kit. Single or multiplex primer kits enable detection of microbial contaminants and cannabis DNA, while providing results to satisfy a broad range of regulatory requirements.

Here, we demonstrate use of the Microlab[®] STARlet automated liquid handler to automate the processing of up to 96 samples in approximately 90 minutes in the SenSATIVax workflow, and up to 192 qPCR reactions in approximately 30 minutes in the PathoSEEK workflow, compared to approximately four hours for the same using manual methods. Microlab STARlet

1. Bureau of Cannabis Control California, Required Testing Chart, 2018. State of California Department of Consumer Affairs, Bureau of Cannabis Control website. https://bcc.ca.gov/about_us/documents/17-261_required_testing_chart.pdf (accessed January 25, 2019).
2. American Herbal Pharmacopoeia. Cannabis Inflorescence, Cannabis spp. Upton R, Craker L, ElSohly M, Romm A, Russo E, Sexton M, eds. *Standards of Identity, Analysis, and Quality Control*, Scotts Valley, Calif., 2014.
3. *SenSATIVax Plant/Microbial DNA Purification Kit protocol*. Medicinal Genomics: Beverly, MA. SenSATIVax_V3_SEP2018.
4. *SenSATIVax DNA Extraction from MIP/Extracts protocol*. Medicinal Genomics: Beverly, MA. SenSATIVax_MIP_Extract_v4_OCT2018.
5. *PathSEEK Protocol for Agilent Real-Time PCR System*. Medicinal Genomics: Beverly, MA PathoSEEK_Agilent_NoDecon_MPX_SFX_Version_5

Benefits-Based Highlights

- Increase sample throughput while reducing hands-on time and complexity to help alleviate bottlenecks and lessen sample turnaround times compared to manual methods.
- Ensure consistency and quality of qPCR-based results without the risks of human error and variability that necessitate costly, time-consuming retesting.

offers flexible deck layout configurability (see Deck Layout on page 9) to suit any workflow need. The Microlab STARlet was preprogrammed with the assay methods (including the option to run the PathoSEEK setup with or without a decontamination step) to facilitate user-friendly operation and reduce operator input errors. Compressed O-Ring Expansion (CO-RE®) Technology creates an air-tight seal between the disposable tips and pipetting channel mandrels without using mechanical force to maximize sample care and integrity and also ensure accurate, reproducible liquid level dispensing. Additionally, barcode scanning provides full sample tracking and LIMS compatibility. We show that the combination of assay chemistries and automated workstation significantly increases throughput and reduces active labor time compared to manual methods without negatively impacting results.

Materials and Methods

SenSATIVax Sample Processing — Flower Samples

Cannabis flower sample, 1 g, was weighed into a Whirl-Pak bag (P/N B01385WA, Nasco, Fort Atkinson, WI) followed by the addition of 14.2 mL of Tryptic Soy Broth (TSB, P/N 420205, Medicinal Genomics). The flower sample was then manually homogenized for one minute. Following homogenization, 6 x 285 µL aliquots of the homogenized flower/TSB mixture were transferred into six separate 1.5 mL snap cap tubes, spiked with 50,000 or 10,000 copies of *Aspergillus flavus* genomic DNA (gDNA) (P/N 16870, ATCC, Manassas, VA) and placed into the sample carriers on the Microlab STARlet deck. The SenSATIVax DNA Extraction method was selected from the Method Manager screen (Figure 1a), user information was input and sample information read from a chosen worklist. After setting up the Microlab STARlet deck with necessary reagents and materials (Figure 2a), the Microlab STARlet automatically added lysis buffer to the sample tubes. The user was prompted to remove the tubes, cap, vortex, and incubate at room temperature for two minutes followed by centrifugation to pellet cellular debris and trichomes. The tubes were then uncapped by the user and placed back in their original positions in the sample carrier(s) for further sample processing on the Microlab STARlet deck. Aliquots of the lysed samples were transferred to the extraction plate. MGC binding buffer was added to each lysed sample, mixed and incubated on deck, to allow the DNA to bind to the magnetic beads in the binding buffer. The extraction plate was then transferred to a 96-well ring magnet to allow for separation of the nucleic acid from unbound cellular components. The samples were then washed with 70% ethanol to remove any non-DNA material and eluted in aqueous buffer. The purified DNA was then transferred to a separate microplate for subsequent assay analysis.

SenSATIVax Sample Processing — Non-Flower Samples (MIP)

One g of cannabidiol (CBD) infused tincture was weighed into a 15 mL conical tube (P/N 1475-0511, USA Scientific, Ocala, FL) followed by the addition of 7 mL SenSATIVax Solution A and vigorously vortexed. Three 500 µL aliquots were then transferred into three separate 1.5 mL snap cap tubes. After aliquoting, each sample was spiked with 5 µL of a 1:5000 dilution of SCCG (single copy control gene) positive control followed by 10,000 copies of *A. flavus* gDNA and vortexed well. After the samples were spun at 14,000 xg for five minutes, aliquots were transferred to new tubes for a final chloroform addition and spin. Supernatants from each sample were transferred to an extraction plate. Solution B was automatically added to each sample followed by MGC binding buffer then mixed. The plate was automatically placed on top of a 96-well ring magnet to allow for separation on the Microlab STARlet deck. The samples were then washed with 70% ethanol to remove any non-DNA material and eluted in aqueous buffer. The purified DNA was then transferred to a separate microplate for subsequent assay analysis.

PathoSEEK qPCR Setup

The first set of qPCR reactions was run using a set of American Herbal Pharmacopoeia (AHP) recommended microbial tests. The PathoSEEK qPCR method was selected from the Microlab STARlet's Method Manager screen (Figure 1b), user information and step selection were input and sample and assay information was read from a prepared worklist. After setting up the Microlab STARlet deck with necessary prepared qPCR Assay Master Mix and materials, Microlab STARlet automatically added the purified DNA samples and controls to a microplate followed by addition of the appropriate qPCR Master Mix to each sample (Figure 2b). Samples were automatically mixed then placed in the AriaMx qPCR Detection System (Agilent, Santa Clara, CA) for qPCR analysis.

Following the same steps, a second set of qPCR reactions was run using a set of California Bureau of Cannabis Control (BCC) required microbial presence/absence tests¹, and a third set was run using three different assays on CBD infused tincture sample extracted DNA.

Results and Discussion

Detection of Potential Pathogens in Cannabis Flower — AHP Recommended Tests

Summary results from the automated PathoSEEK assays evaluated in this study are presented in Table 2. The assays were selected based on a set of AHP recommended microbial tests and were performed in triplicate on cannabis flower samples spiked with 50,000 and 10,000 genomic copies of *Aspergillus flavus* gDNA, to demonstrate primer target specificity. Using two multiplex assays, *E. coli* and *Salmonella* and Total Enterobacteriaceae and Coliform, no Cq values for any of the target bacteria were observed in the spiked cannabis flower samples, which indicated an absence of each respective bacteria in a sample well or the occurrence of non-specific primer binding of *Aspergillus flavus* gDNA. Positive controls specific to each bacteria were detected, confirming primer accuracy to target sequence. Internal cannabis DNA controls were also detected as expected in each of the flower sample assay wells, which verified a successful DNA extraction.

The Total Aerobic Count assay detected no aerobic bacteria in any of the cannabis flower samples or negative control well, and successfully produced a Cq value of 13.36 in the assay specific positive control. The assay also detected the internal cannabis DNA fluorophore signal, again confirming the DNA extraction was a success. Finally, evaluation made using the Total Yeast and Mold assay yielded low Cq values ranging from 19.58–19.88 in cannabis flower samples spiked with 50,000 genomic copies of *A. flavus* gDNA, and higher Cq values ranging from 23.08–23.62 in cannabis flower samples spiked with 10,000 genomic copies of the pathogenic gDNA. Lower observed Cq values for the 50,000 spike were indicative of a larger starting quantity of *Aspergillus flavus* at the start of PCR compared to the 10,000 spiked samples. At the same time, the automated assay detected cannabis DNA, with Cq values within expected ranges.

Detection of Potential Pathogens in Cannabis Flower — California BCC Required Tests

The automated PathoSEEK assays were further evaluated using six test types required by the California BCC, with results detailed in Table 3. Cannabis flower samples spiked with 50,000 and 10,000 genomic copies of *Aspergillus flavus* gDNA were again tested in triplicate. Using the multiplex assay, Shiga toxin-producing (STEC) *E. coli* and *Salmonella*, no Cq values for the target bacteria were observed in the cannabis flower samples, while the positive controls recognized levels of each respective target bacteria. Cannabis DNA was detected as expected in the assay, confirming extraction effectiveness.

The multiplex *Aspergillus* MPX assay, which detects for *A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*, yielded Cq values ranging from 18.23–18.62 in cannabis flower samples spiked with 50,000 genomic copies of *A. flavus* gDNA, and Cq values ranging from 21.04–21.75 in cannabis flower samples spiked with 10,000 genomic copies of the pathogenic gDNA. The Cq values for the pathogenic gDNA, along with the detected Cq values for cannabis DNA, were within expected ranges. Cannabis flower samples were negative for amplification in the last assay, specific to *A. niger*, thus confirming a high degree of primer specificity between *Aspergillus* strains, while cannabis DNA was also detected, confirming the assay extraction effectiveness.

Detection of Potential Pathogens in CBD Infused Tincture (MIP)

In the last evaluation, automated PathoSEEK assays were assessed using triplicate samples of non-flower CBD infused tincture (MIP) spiked with 10,000 genomic copies of gDNA from both *Salmonella* and STEC. Per Table 4, spiked target DNA was detected by each of the assays, including the multiplex assays *Salmonella* and *E. coli* and Enterobacteriaceae and Coliform, and the Bile Tolerant Gram Negative assay. Finally, the internal cannabis DNA controls were accurately detected within the CBD tincture samples.

Conclusion

The SenSATIVax Plant/Microbial DNA Purification kit and PathoSEEK qPCR-based microbial detection assays encompass cannabis-based matrices from plant tissues to MIP and are suitable for a wide range of regulatory requirements independent of locality. High-throughput, parallel sample processing and qPCR assay setup with reduced human intervention is facilitated through the Microlab STARlet. The combination of assay chemistry and automated workstation provide results that are highly reliable, accurate, and specific while reducing active labor time and the risk of variability and errors due to manual manipulations.



Figure 1a. SenSATIVax Loading Dialog

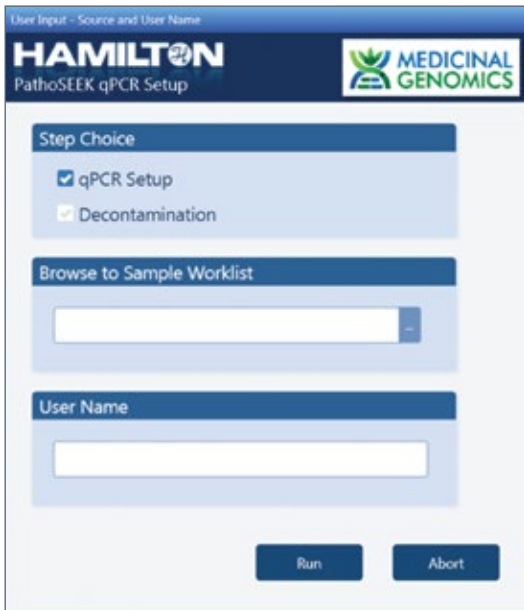


Figure 1b. PathoSEEK qPCR Dialog

MicroLab STARlet user interfaces for (a) SenSATIVax Plant/Microbial DNA purification or (b) PathoSEEK qPCR assay setup with or without decontamination step. Options selected automatically update subsequent User Dialogs.

Incorporating automated, pre-programmed methods reduces the assay set up time, and reduces or eliminates user entry errors. Additionally, the automated methods allow full sample traceability throughout sample processing.

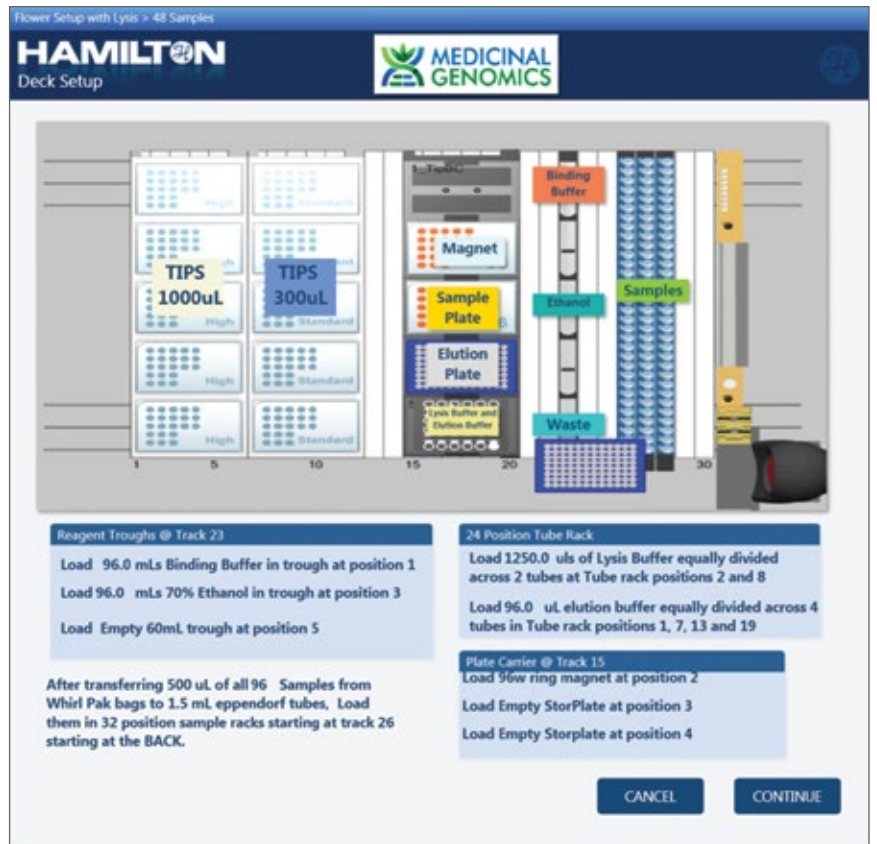


Figure 2a. SenSATIVax Setup Dialog



Figure 2b. PathoSEEK qPCR CA Asp Dialog

MicroLab STARlet user interface, indicating (a) SenSATIVax DNA extraction or (b) PathoSEEK qPCR Master Mix reagent setup volumes and instructions. The graphic user interface provides labware and reagent loading instructions. Reagent volumes are automatically scaled to account for dynamic sample throughput via worklist driven sample input.

Table 1: Microbial Testing

Microbial Target	Jurisdiction
<i>A. flavus</i>	AK, CA, HI, NM, NV, NY, VT, WV
<i>A. fumigatus</i>	AK, CA, HI, NM, NV, NY, VT, WV
<i>A. niger</i>	AK, CA, HI, NM, NV, NY, VT, WV
<i>A. terreus</i>	CA, NM, NV, NY, WV
Bile tolerant gram negative	Canada, CO, CT, IL, MA, MI, NM, NV, NY, OH, PA, WA
Coliform	Canada, AR, HI, IL, MA, MI, NV, OH, OR, RI
<i>E. coli</i>	Canada, AR, CT, FL, HI, IL, MA, MD, MI, MT, NM, NV, NY, OH, OK, OR, PA, RI, WA
<i>Pseudomonas aeruginosa</i>	NY, CT, WV
<i>Salmonella</i> spp.	AK, CA, Canada, CO, FL, HI, IL, MA, MD, MI, MT, NM, NV, NY, OH, OK, PA, RI, VT, WA
<i>Staph aureus</i>	CT, NM, WV
STEC	AK, CA, CO, VT
Total aerobic count	Canada, HI, IL, MA, MD, MI, NM, OH, OK, PA, RI, WV
Total yeast and mold	Canada, CO, FL, HI, IL, MA, MD, MI, NM, NV, OH, OK, PA, RI, WA, WV

Overlapping and various testing requirements per state or governmental market complicate a one-size-fits-all approach to microbial detection for cannabis analytical laboratories. Information compiled as of March 8, 2019.

Table 2: Automated AHP Test Run

Sample Name	<i>Aspergillus flavus</i> gDNA Spike (No. Genomic Copies)	Assay	Target	Cq (ΔR)	Target	Cq (ΔR)	Target	Cq (ΔR)
Flower Sample 1	50K	<i>Salmonella/E. Coli</i>	<i>E. coli</i>	No Cq	Cannabis DNA	26.72	<i>Salmonella</i>	No Cq
Flower Sample 1	50K	<i>Salmonella/E. Coli</i>	<i>E. coli</i>	No Cq	Cannabis DNA	26.59	<i>Salmonella</i>	No Cq
Flower Sample 1	50K	<i>Salmonella/E. Coli</i>	<i>E. coli</i>	No Cq	Cannabis DNA	26.62	<i>Salmonella</i>	No Cq
Flower Sample 1	10K	<i>Salmonella/E. Coli</i>	<i>E. coli</i>	No Cq	Cannabis DNA	26.69	<i>Salmonella</i>	No Cq
Flower Sample 1	10K	<i>Salmonella/E. Coli</i>	<i>E. coli</i>	No Cq	Cannabis DNA	25.00	<i>Salmonella</i>	No Cq
Flower Sample 1	10K	<i>Salmonella/E. Coli</i>	<i>E. coli</i>	No Cq	Cannabis DNA	26.43	<i>Salmonella</i>	No Cq
Sal/ <i>E. coli</i> + C	None	<i>Salmonella/E. Coli</i>	<i>E. coli</i>	14.00	Cannabis DNA	No Cq	<i>Salmonella</i>	15.60
Sal/ <i>E. coli</i> NTC	None	<i>Salmonella/E. Coli</i>	<i>E. coli</i>	No Cq	Cannabis DNA	No Cq	<i>Salmonella</i>	No Cq
Flower Sample 1	50K	Total Aerobic Count	TAC	No Cq	Cannabis DNA	26.60		
Flower Sample 1	50K	Total Aerobic Count	TAC	No Cq	Cannabis DNA	26.73		
Flower Sample 1	50K	Total Aerobic Count	TAC	No Cq	Cannabis DNA	26.86		
Flower Sample 1	10K	Total Aerobic Count	TAC	No Cq	Cannabis DNA	26.75		
Flower Sample 1	10K	Total Aerobic Count	TAC	No Cq	Cannabis DNA	26.55		
Flower Sample 1	10K	Total Aerobic Count	TAC	No Cq	Cannabis DNA	26.49		
Total Aerobic + C	None	Total Aerobic Count	TAC	13.36	Cannabis DNA	No Cq		
Total Aerobic NTC	None	Total Aerobic Count	TAC	No Cq	Cannabis DNA	No Cq		
Flower Sample 1	50K	Total Entero and Coliform	Coliform	No Cq	Cannabis DNA	26.71	Enterobactriaceae	No Cq
Flower Sample 1	50K	Total Entero and Coliform	Coliform	No Cq	Cannabis DNA	26.78	Enterobactriaceae	No Cq
Flower Sample 1	50K	Total Entero and Coliform	Coliform	No Cq	Cannabis DNA	26.88	Enterobactriaceae	No Cq
Flower Sample 1	10K	Total Entero and Coliform	Coliform	No Cq	Cannabis DNA	26.80	Enterobactriaceae	No Cq
Flower Sample 1	10K	Total Entero and Coliform	Coliform	No Cq	Cannabis DNA	26.42	Enterobactriaceae	No Cq
Flower Sample 1	10K	Total Entero and Coliform	Coliform	No Cq	Cannabis DNA	26.40	Enterobactriaceae	No Cq
Total Entero and Coliform + C	None	Total Entero and Coliform	Coliform	12.29	Cannabis DNA	No Cq	Enterobactriaceae	14.50
Total Entero and Coliform NTC	None	Total Entero and Coliform	Coliform	No Cq	Cannabis DNA	No Cq	Enterobactriaceae	No Cq
Flower Sample 1	50K	Total Yeast and Mold	TYM	19.59	Cannabis DNA	26.86		
Flower Sample 1	50K	Total Yeast and Mold	TYM	19.58	Cannabis DNA	26.89		
Flower Sample 1	50K	Total Yeast and Mold	TYM	19.88	Cannabis DNA	26.94		
Flower Sample 1	10K	Total Yeast and Mold	TYM	23.54	Cannabis DNA	26.76		
Flower Sample 1	10K	Total Yeast and Mold	TYM	23.62	Cannabis DNA	26.73		
Flower Sample 1	10K	Total Yeast and Mold	TYM	23.08	Cannabis DNA	26.46		
Total Yeast and Mold + C	None	Total Yeast and Mold	TYM	11.67	Cannabis DNA	No Cq		
Total Yeast and Mold NTC	None	Total Yeast and Mold	TYM	No Cq	Cannabis DNA	No Cq		

Detection of select microbes and fungi in cannabis flower samples spiked with two concentrations of *Aspergillus flavus* gDNA. PathoSEEK assay selection based on testing recommendations from the American Herbal Pharmacopoeia, and adopted by several US state testing methodologies.

C = Control

NTC = Nontemplate control

Table 3: Automated CA Test Run

Sample Name	<i>Aspergillus flavus</i> spike (No. Genomic Copies)	Assay	Target	Cq (ΔR)	Target	Cq (ΔR)	Target	Cq (ΔR)
Flower Sample 1	50K	<i>Salmonella</i> /STEC	STEC	No Cq	Cannabis DNA	25.07	<i>Salmonella</i>	No Cq
Flower Sample 1	50K	<i>Salmonella</i> /STEC	STEC	No Cq	Cannabis DNA	25.10	<i>Salmonella</i>	No Cq
Flower Sample 1	50K	<i>Salmonella</i> /STEC	STEC	No Cq	Cannabis DNA	25.46	<i>Salmonella</i>	No Cq
Flower Sample 1	10K	<i>Salmonella</i> /STEC	STEC	No Cq	Cannabis DNA	25.42	<i>Salmonella</i>	No Cq
Flower Sample 1	10K	<i>Salmonella</i> /STEC	STEC	No Cq	Cannabis DNA	25.00	<i>Salmonella</i>	No Cq
Flower Sample 1	10K	<i>Salmonella</i> /STEC	STEC	No Cq	Cannabis DNA	24.81	<i>Salmonella</i>	No Cq
Sal/STEC + C	None	<i>Salmonella</i> /STEC	STEC	10.38	Cannabis DNA	No Cq	<i>Salmonella</i>	13.77
Sal/STEC NTC	None	<i>Salmonella</i> /STEC	STEC	No Cq	Cannabis DNA	No Cq	<i>Salmonella</i>	No Cq
Flower Sample 1	50K	<i>Aspergillus</i> MPX	<i>Aspergillus flavus, fumigatus, niger, and terreus</i>	18.62	Cannabis DNA	25.56		
Flower Sample 1	50K	<i>Aspergillus</i> MPX	<i>Aspergillus flavus, fumigatus, niger, and terreus</i>	18.23	Cannabis DNA	25.47		
Flower Sample 1	50K	<i>Aspergillus</i> MPX	<i>Aspergillus flavus, fumigatus, niger, and terreus</i>	18.52	Cannabis DNA	25.42		
Flower Sample 1	10K	<i>Aspergillus</i> MPX	<i>Aspergillus flavus, fumigatus, niger, and terreus</i>	21.75	Cannabis DNA	25.48		
Flower Sample 1	10K	<i>Aspergillus</i> MPX	<i>Aspergillus flavus, fumigatus, niger, and terreus</i>	21.04	Cannabis DNA	25.09		
Flower Sample 1	10K	<i>Aspergillus</i> MPX	<i>Aspergillus flavus, fumigatus, niger, and terreus</i>	21.33	Cannabis DNA	25.10		
<i>Aspergillus</i> MPX + C	None	<i>Aspergillus</i> MPX	<i>Aspergillus flavus, fumigatus, niger, and terreus</i>	10.34	Cannabis DNA	No Cq		
<i>Aspergillus</i> MPX NTC	None	<i>Aspergillus</i> MPX	<i>Aspergillus flavus, fumigatus, niger, and terreus</i>	No Cq	Cannabis DNA	No Cq		
Flower Sample 1	50K	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	No Cq	Cannabis DNA	25.91		
Flower Sample 1	50K	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	No Cq	Cannabis DNA	25.93		
Flower Sample 1	50K	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	No Cq	Cannabis DNA	25.89		
<i>Aspergillus niger</i> + C	None	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	10.92	Cannabis DNA	No Cq		
<i>Aspergillus niger</i> NTC	None	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	No Cq	Cannabis DNA	No Cq		

Detection of select microbes and fungi in cannabis flower samples spiked with two concentrations of *Aspergillus flavus* gDNA. PathoSEEK assay selection based on testing recommendations from the California Bureau of Cannabis Control.

C = Control

NTC = Non template control

Table 4: MIP Test Run

Sample Name	<i>Salmonella</i> and STEC gDNA Spike (No. Genomic Copies)	Assay	Target	Cq (ΔR)	Target	Cq (ΔR)	Target	Cq (ΔR)
CBD Tincture	10K each	<i>Salmonella</i> / <i>E. Coli</i>	<i>E. coli</i>	29.69	Cannabis DNA	28.81	<i>Salmonella</i>	36.96
CBD Tincture	10K each	<i>Salmonella</i> / <i>E. Coli</i>	<i>E. coli</i>	28.29	Cannabis DNA	27.85	<i>Salmonella</i>	33.83
CBD Tincture	10K each	<i>Salmonella</i> / <i>E. Coli</i>	<i>E. coli</i>	28.75	Cannabis DNA	28.94	<i>Salmonella</i>	34.94
Sal/STEC + C	None	<i>Salmonella</i> / <i>E. Coli</i>	<i>E. coli</i>	11.06	Cannabis DNA	No Cq	<i>Salmonella</i>	15.87
Sal/STEC NTC	None	<i>Salmonella</i> / <i>E. Coli</i>	<i>E. coli</i>	No Cq	Cannabis DNA	No Cq	<i>Salmonella</i>	No Cq
CBD Tincture	10K each	Total Enterococci and Coliform	Coliform	30	Cannabis DNA	28.94	Enterococci	30.26
CBD Tincture	10K each	Total Enterococci and Coliform	Coliform	28.61	Cannabis DNA	28.19	Enterococci	28.75
CBD Tincture	10K each	Total Enterococci and Coliform	Coliform	29.97	Cannabis DNA	29.37	Enterococci	29.8
Total Enterococci and Coliform + C	None	Total Enterococci and Coliform	Coliform	10.88	Cannabis DNA	No Cq	Enterococci	13.77
Total Enterococci and Coliform NTC	None	Total Enterococci and Coliform	Coliform	No Cq	Cannabis DNA	No Cq	Enterococci	No Cq
CBD Tincture	10K each	Bile Tolerant Gram Negative	BTGN	27.59	Cannabis DNA	29.22		
CBD Tincture	10K each	Bile Tolerant Gram Negative	BTGN	26.38	Cannabis DNA	28.02		
CBD Tincture	10K each	Bile Tolerant Gram Negative	BTGN	27.22	Cannabis DNA	29.07		
BTGN + C	None	Bile Tolerant Gram Negative	BTGN	12.07	Cannabis DNA	No Cq		
BTGN NTC	None	Bile Tolerant Gram Negative	BTGN	No Cq	Cannabis DNA	No Cq		

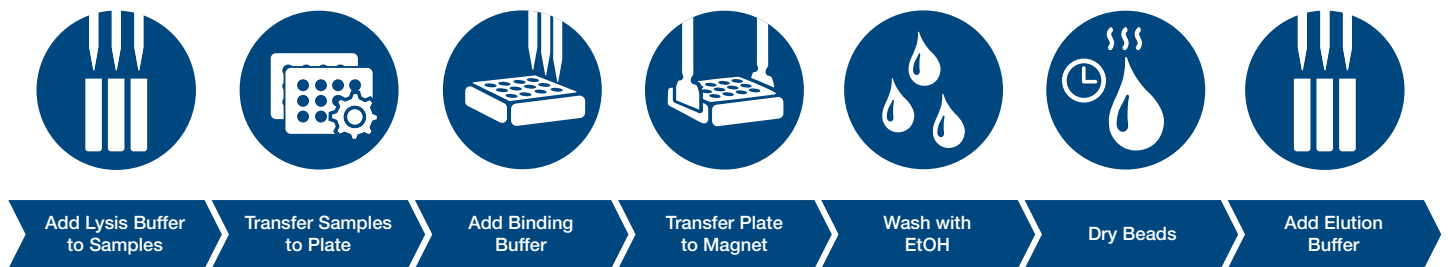
Detection of select microbes in non-flower cannabidiol infused tincture (MIP) spiked with 10K genomic copies of both *Salmonella* and STEC.

C = Control

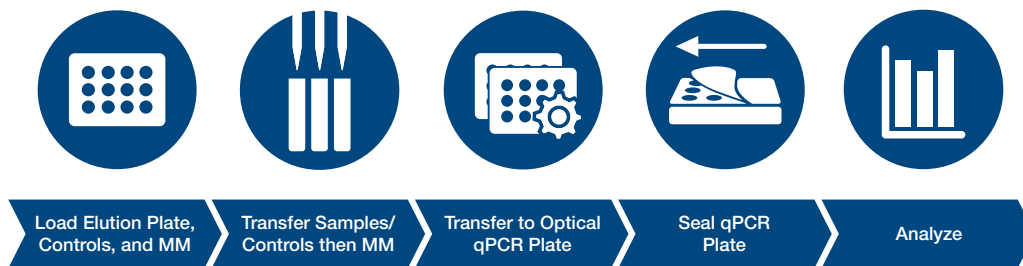
NTC = Non template control

MGC STARlet Workflow

SenSATIVax® Sample Extraction Automated Workflow



PathoSEEK® qPCR Setup Automated Workflow

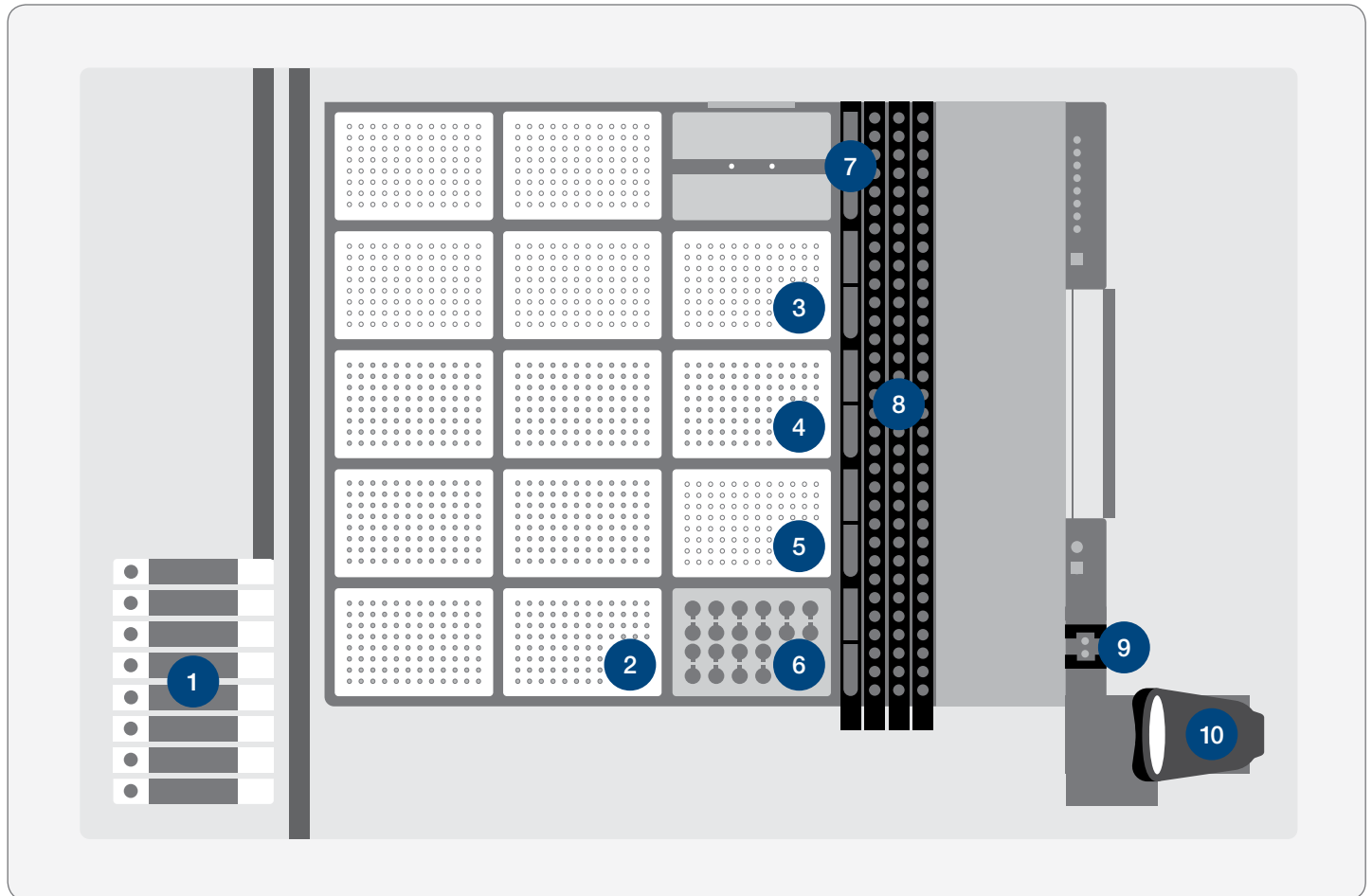


Automated workflows for the SenSATIVax sample extraction and PathoSEEK qPCR setup platforms on the Microlab STARlet.

Note: Workflows are representative of a typical extraction and qPCR method, but not all sample additions or plate movements are illustrated.

Microlab STARlet Deck Layout

Microlab STARlet deck layout for use with the SenSATIVax and PathoSEEK assays. Top-down view of the Microlab STARlet deck layout showing the various components and spatial locations available for automating the MGC SenSATIVax and PathoSEEK workflows.



- | | | | |
|---------------------------------|--------------------------------|--------------------------------------|---------------------------------|
| 1 8 Independent Channels | 4 96-Well Sample Plate | 7 Reagent Trough Carrier | 10 Orbit Barcode Scanner |
| 2 Tips | 5 96-Well Elution Plate | 8 1.5 mL Sample Tube Carriers | |
| 3 Magnet | 6 PCR Reagent Module | 9 CO-RE Gripper | |

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