PathoSEEK® Total Coliform and Enterobacteriaceae Detection Assay v2 with SenSATIVAx® Extraction Protocol for Detection in Cannabis Flower and MIP Matrices

Method Developer Validation

Table of Contents

Abstract

Background

Coliforms and Enterobacteriaceae can cause deterioration and decomposition of cannabis, and certain species of bacteria, such as Shiga Toxin-producing E. coli, can cause infections in humans. Coliforms and Enterobacteriaceae are good indicator organisms for the assessment of the overall quality of a finished product. The PathoSEEK® Total Coliform and Enterobacteriaceae v2 Detection assay is a qPCR detection assay for the rapid detection and/or enumeration of these bacteria in cannabis matrices.

Objective

To evaluate the PathoSEEK® Total Coliform and Enterobacteriaceae Detection Assay, using the SenSATIVAx® flower extraction protocols, for the enumeration of total Coliform and Enterobacteriaceae in cannabis flower (delta 9-tetrahydrocannabinol >0.3%; 1g). To evaluate the PathoSEEK® Total Coliform and Enterobacteriaceae Detection Assay as a screen for the presence or absence in infused products.

Results

Inclusivity and exclusivity results showed that the PathoSEEK® Coliform and Enterobacteriaceae method is highly specific in discriminating target organisms found in cannabis flower and infused products from non-target organisms.

The SenSATIVAx® flower extraction kit and PathoSEEK® Total Coliform and Enterobacteriaceae Detection Assay v2 were validated through the development of an enumeration curve using ten distinct bacterial species, with subsequent plating on 3M™ Petrifilm™ Enterobacteriaceae (EB) Count Plates. Following curve establishment, a certified reference material (CRM) for Quantitative EB in Hemp was procured from NSI and analyzed employing the SenSATIVAx flower extraction kit and PathoSEEK Coliform and Enterobacteriaceae qPCR detection Assay v2. The resultant qPCR Cq values were converted to colony-forming units per gram (CFU/g) utilizing a conversion equation and compared with data obtained via 3M™ Petrifilm™ Coliform Count Plates, as well as the NSI value provided on the CRM Certificate of Analysis. The results demonstrated comparability with those from 3M and alignment with the specifications detailed by NSI.

Materials

SenSATIVAx® Flower & Leaf DNA Purification Kit Components - **P/N 420001**

Component Name	Qty Provided	Storage Conditions
MGC Lysis Buffer	1 Bottle (12 mL)	RT (20–28°C)
MGC Binding Buffer	1 Bottle (48 mL)	Refrigerate (2-4 ºC)
MGC Elution Buffer	1 Bottle (12 mL)	RT (20–28°C)

SenSATIVAx® Infused Product DNA Purification Kit Components - **P/N 420004**

Component Name	Qty Provided	Storage Conditions
SenSATIVAx® Solution A	1 Bottle (350 mL)	RT (20–28°C)
SenSATIVAx® Solution B	1 Bottle (25 mL)	RT (20–28°C)
MGC Binding Buffer	1 Bottle (48 mL)	Refrigerate (2-4 ºC)
MGC Elution Buffer	1 Bottle (12 mL)	RT (20–28°C)

PathoSEEK® Total Coliform and Enterobacteriaceae Detection Assay v2 Kit - **P/N 420538**

Component Name	Qty Provided	Storage Conditions
PathoSEEK Amplification Mix *Includes 2 tubes nuclease free water for resuspension*	4 Vials (50 rxns/each)	RT (20-28 ºC)/ -15 to -20 ºC*
PathoSEEK® Total Coliform & Entero Detection Assay v2	1 Tube (200 µL)	-15 to -20 ºC

Optional: Grim Reef Free DNA Removal Kit - **P/N 420145**

Component Name	Qty Provided	Storage Conditions
GR Enzyme	1 Bottle (2.5 mL)	-15 to -20 ºC
GR Buffer	1 Bottle (12.5 mL)	-15 to -20 ºC

Inclusivity and Exclusivity Testing

Wet Laboratory Methodology

For the inclusivity evaluation, 33 strains of bacteria were evaluated. Target strains were either cultured in Tryptic Soy Broth for 24 hours at 37º C followed by extraction of DNA or purified DNA from ATCC was used. For the exclusivity, 14 organisms were evaluated. Target strains were either cultured under optimal conditions for growth of the organism, followed by extraction of DNA, or purified DNA from ATCC was used. Inclusivity and exclusivity cultures were randomized, blind coded, and analyzed by the PathoSEEK® Total Coliform and Enterobacteriaceae method.

Results

Of the 33 inclusivity strains tested, 33 were correctly detected by the PathoSEEK® Method. Of the 14 exclusivity strains tested, all 14 were correctly excluded. Tables 1 and 2 present a summary of the results.

Table 1: Inclusivity Results

Species	ATCC#	Pathoseek Coliform/Entero Result
Aeromonas hydrophila	7965 DNA	Detected
Aeromonas hydrophila	7966	Detected
Citrobacter braakii	3037	Detected
Citrobacter freundii	8090	Detected
Citrobacter koseri	25408	Detected
Cronobacter sakazakii	BAA-894	Detected
Enterobacter aerogenes	15038 DNA	Detected
Escherichia hermannii	700368	Detected
Escherichia coli Strain 2005-3287 O145	BAA-2223	Detected
Escherichia coli Strain 2000-3039 O45:H2	BAA-2193 DNA	Detected
Escherichia coli Strain 2002-3211 O121-H19	BAA-2219 DNA	Detected
Escherichia coli Strain 2003-3014 O26:H11	BAA 2196 DNA	Detected

Escherichia coli Strain 2006-3008 O103:H11	BAA 2215 DNA	Detected
Escherichia coli Strain 99-3311 O145	BAA 2192 DNA	Detected
Escherichia coli Strain O111	BAA 2440 DNA	Detected
Hafnia alvei	51873	Detected
Klebsiella pneumonia	200721 DNA	Detected
Klebsiella oxytoca	51983	Detected
Morganella morganii	25829	Detected
Pantoea agglomerans	43348	Detected
Proteus mirabilis	43071	Detected
Proteus vulgaris	8427	Detected
Rahnella aquatilis	33991	Detected
Salmonella bongori	43975D-5	Detected
Escherichia hermannii	6962	Detected
Salmonella enterica subsp. arizonae	BAA-731D-5	Detected
Salmonella enterica subsp. diarizonae	BAA-1579D-5	Detected
Salmonella enterica subsp. houtene	BAA-1580D-5	Detected
Salmonella enterica subsp. indica	BAA-15780D-5	Detected
Salmonella enterica subsp. Salamae	BAA-1582D-5	Detected
Shigella flexneri	29903D-5	Detected
Vibrio cholerae	39315D-5	Detected
Yersinia enterocolitica	9610	Detected

Table 2: Exclusivity Results

Species	ATCC#	Pathoseek Coliform/Entero Result
Aspergillus flavus	9643	Not Detected
Aspergillus niger	1015	Not Detected
Aspergillus terreus	20542	Not Detected
Bacillus subtilis	11774	Not Detected
Candida albicans	10231	Not Detected
Clostridium sporogenes	11437	Not Detected

Lactobacillus acidophilus	4357	Not Detected
Listeria monocytogenes	19115D-5	Not Detected
Listeria seeligeri	35967D-5	Not Detected
Listeria wilshire	35897D-5	Not Detected
Penicillium chrysogenum	10160 DNA	Not Detected
Penicillium rubens	11709	Not Detected
Pseudomonas aeruginosa	9027	Not Detected
Staphylococcus aureus	6538	Not Detected

Generation of Cq to CFU Conversion Equation for Flower Samples

1. A linear regression model was derived from the quantitative polymerase chain reaction (qPCR) cycle quantification (Cq) values and colony-forming units per gram (CFU/g) obtained through plating on Petrifilm EB plates. Ten organisms were analyzed. Each qPCR and plating procedure was performed in triplicate, and the resulting data were averaged prior to plotting. The x-axis represented the qPCR data, and the y-axis represented the base-10 logarithm (log10) of the plating data. The line of best fit was determined, resulting in the equation: $y = -0.2531x + 11.6$, where y is the log10 CFU/g and x is the Cq value
2. Utilize the following linear equation to convert Cq (x) values to Log CFU (y): $y = -0.2531x + 11.6$
3. Perform an inverse logarithmic transformation of Y to obtain CFU/g
4. Multiply the derived CFU/g value by the sample's upfront dilution factor in TSB to determine the final CFU (x 20)

Empirical validation has confirmed that this derived equation produces comparable results across the Agilent AriaMX, BioRad CFX96, and BioMolecular Systems MIC quantitative PCR instruments.

Proficiency Testing / Certified Reference Material Results

To test our equation, two different CRMs supplied by NSI, Quantitative EB in hemp (Cat # FM-730), and Quantitative Coliform/E.Coli (Cat # FM-727) in hemp, were used. The reference materials were prepared

according to the user guide (with Grim Reefer) in triplicate, used the equation to convert the Cq value to CFU/g and analyzed the results. These all fell within the provided acceptable range in the CoAs.

Table 3: CFU results of the CRMs

Sample	Assay	Cq FAM	Cq to CFU/g	Within Range
Quant. Coliform/E. coli in Hemp	Total Entero v2	32.26	54,453	Yes
Quant. Coliform/E. coli in Hemp	Total Entero v2	32.61	44,406	Yes
Quant. Coliform/E. coli in Hemp	Total Entero v2	31.69	75,909	Yes
Quant. EB in Hemp	Total Entero v2	32.30	53,199	Yes
Quant. EB in Hemp	Total Entero v2	32.28	53,822	Yes
Quant. EB in Hemp	Total Entero v2	32.43	49,317	Yes

Marijuana Infused Product Testing Confirmation

To validate the functionality of the PathoSEEK Coliform and Enterobacteriaceae Detection Assay v2 with Marijuana Infused Products (MIPs), chocolate and oil samples were analyzed. When used with non-flower matrices (such as MIPs), the assay is designed for presence/absence screening. The method is not designed to report a CFU/g result in non-flower matrices.

Chocolate and oil matrices were inoculated with live Salmonella culture and processed according to the SenSATIVAx MIP protocol. Growth of the Salmonella culture was concurrently confirmed through plating on 3M RAC plates. The results may be found in Table 4.

Table 4: Results of the MIP testing

Sample	Assay	Cq FAM	Cq Hex
Salmonella in Chocolate	Total Coliform & Entero v2	(+)	(+)
Salmonella in Chocolate	Total Coliform & Entero v2	(+)	(+)
Salmonella in Chocolate	Total Coliform & Entero v2	(+)	(+)
Salmonella in Oil	Total Coliform & Entero v2	(+)	(+)
Salmonella in Oil	Total Coliform & Entero v2	(+)	(+)

Salmonella in Oil	Total Coliform & Entero v2	(+)	(+)
Positive Control	Total Coliform & Entero v2	(+)	(-)
NTC	Total Coliform & Entero v2	(-)	(-)

Conclusions

The PathoSEEK® Total Coliform and Enterobacteriaceae Detection Assay with SenSATIVAx® DNA Purification is a rapid, alternative method to traditional plating procedures for the detection of Coliform and Enterobacteriaceae on cannabis flower and infused products. The method produced comparable results to 3M Petrifilm EB and CC plates for the enumeration of Total Coliform/Enterobacteriaceae bacteria in cannabis flower.

REVISION HISTORY

Version	Date	Description
v1	June 2025	Validation date generated with the use of Amplification Mix and v2 Assay design

DISCLAIMER

This test was developed and its performance characteristics determined by Medicinal Genomics Corporation, for laboratory use. Any deviations from this protocol are not supported by MGC.

This test has not been validated on remediated (irradiated, ozone-treated, acid-treated, hydrogen peroxide-treated, etc.) samples. Samples that have undergone remediation may cause discordant results between plating methods and PathoSEEK methods. When remediated samples produce a result above the action limit on qPCR, we recommend confirming viability with an approved plating method.

Results may vary based on laboratory conditions. Altitude and humidity are factors known to affect the growth of bacterial and fungal species.

LIMITED USE LABEL LICENSE

This product is covered by at least one or more claims of US patent applications, which are exclusively licensed to Medicinal Genomics Corporation. This product is sold strictly for the use of the buyer, and the buyer is not authorized to transfer this product [or any materials made using this product] to any third party.