

PathoSEEK® STEC E. Coli Detection Assay v2 with MaGiC Lysis Kit

Method Developer Validation

Real Time PCR (qPCR) assay for the detection of STEC *E. coli* in dried cannabis flower and infused products matrices

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Abstract

Background

Current regulations require cannabis flower and cannabis products to be free of species of STEC *E. Coli*. The PathoSEEK® STEC E. coli Detection Assay v2 with MaGiC Lysis Kit will detect these species using a qPCR (Quantitative Polymerase Chain Reaction) assay in cannabis flower, cannabis concentrates, infused edibles and infused non-edibles.

Objective

To evaluate the PathoSEEK® STEC E. coli Detection Assay v2 with MaGiC Lysis Kit for the presence/absence detection of STEC E.coli species in cannabis flower (delta 9-tetrahydrocannabinol >0.3%; 1g) and marijuana-infused products (MIP).

Results

Inclusivity and exclusivity results showed the PathoSEEK® STEC E. coli Detection Assay v2 with MaGiC Lysis Kit is highly specific in discriminating target organisms found in cannabis flower and infused products from non-target organisms. The kit was further verified by running NSI Certified Reference Materials, part numbers CMQC-028 and CMQC-030. Results show that the MaGiC Lysis kit and PathoSEEK® STEC E. coli Detection Assay v2 accurately detected STEC E. coli when present.

Materials

PathoSEEK® STEC E. coli Detection Assay v2 with MaGiC Lysis Kit - P/N 420534

(Kit contains sufficient reagents for 200 reactions)

Kit Components

Component Name	Qty Provided	Storage Conditions
MaGiC Lysis Reagent	1 Bottle (12 mL)	RT (20-28 °C)
MaGiC Stabilization Buffer	1 Bottle (24 mL)	RT (20-28 °C)
PathoSEEK Amplification Mix <i>Includes 2 tubes nuclease free water for resuspension</i>	4 Vials (50 rxns/each)	RT (20-28 °C)/ -15 to -20 °C*
PathoSEEK® STEC E. coli Detection Assay v2	1 Tube (200 µL)	-15 to -20 °C

Note: Actual fill volumes include overage

*The PathoSEEK Amplification Mix can be stored lyophilized at Room Temperature for up to 2 years. Once re-hydrated it must be stored at -15 to -20 °C for up to 3 months.

Method Developer Validation

Inclusivity and Exclusivity

For inclusivity evaluation, 51 strains of STEC *E. Coli* were tested. Strains were grown for 16 hours at 37 ± 1°C in TSB. For the exclusivity evaluation, a total of 46 non-targeted strains were tested. Exclusivity organisms were cultured under optimal conditions to achieve growth at the stationary phase. Inclusivity and exclusivity strains were randomized and blind coded prior to analysis.

Results

Detailed results for the inclusivity and exclusivity evaluations are presented in Tables 1 and 2. For the inclusivity evaluation, all 51 strains of STEC *E. coli* were detected by the assay using MaGiC Lysis on the CFX-96, AriaMx, and the BMS Mic qPCR instruments. For the exclusivity, 46 of the organisms were correctly excluded on the STEC *E. coli* channel.

Table 1: STEC *E. Coli* Inclusivity Results

Organism	Source	Origin	Result
<i>Escherichia coli</i> O3	TW01413c	Germany	Positive
<i>Escherichia coli</i> O3	TW01414	Germany	Positive
<i>Escherichia coli</i> O5	TW00021	Cow (MI)	Positive
<i>Escherichia coli</i> O5	TW05097	Cow (CA)	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2205	Feces	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2196	Feces	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2204	Feces	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2181	Not Available	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2188	Feces	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2193	Feces	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2189	Feces	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2185	Not Available	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2202	Feces	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2198	Feces	Positive
<i>Escherichia coli</i> O91:H21	ATCC 51434	Not Available	Positive
<i>Escherichia coli</i> O91:H21	ATCC 51435	Clinical Isolate	Positive
<i>Escherichia coli</i> O103:H11	ATCC BAA-2215	Not Available	Positive
<i>Escherichia coli</i> O103:H11	ATCC BAA-2200	Not Available	Positive
<i>Escherichia coli</i> O103:H11	NJDPH 151297-1	Not Available	Positive
<i>Escherichia coli</i> O103:H11	NJDPH 130928-3	Not Available	Positive
<i>Escherichia coli</i> O103:H11	TW08869	Human Isolate	Positive
<i>Escherichia coli</i> O103:H11	TW08872	Human Isolate	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-2201	Feces	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-2180	Feces	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-184	Feces	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-180	Feces	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-181	Feces	Positive
<i>Escherichia coli</i> O113	ATCC BAA-177	Feces	Positive
<i>Escherichia coli</i> O113	ATCC BAA-176	Feces	Positive
<i>Escherichia coli</i> O113	ATCC BAA-183	Urine	Positive
<i>Escherichia coli</i> O118	TW08644	Feces	Positive
<i>Escherichia coli</i> O118	TW08134	Cow Feces	Positive

<i>Escherichia coli</i> O121:H19	ATCC BAA-2219	Feces	Positive
<i>Escherichia coli</i> O121:H19	ATCC BAA-2203	Feces	Positive
<i>Escherichia coli</i> O121:H19	ATCC BAA-2187	Not Available	Positive
<i>Escherichia coli</i> O121:H19	ATCC BAA-2220	Feces	Positive
<i>Escherichia coli</i> O121:H19	TW08868	Feces	Positive
<i>Escherichia coli</i> O145:NM	TW09356	Human Isolate	Positive
<i>Escherichia coli</i> O145:NM	TW07596	Feces	Positive
<i>Escherichia coli</i> O145:NM	ATCC BAA-2223	Feces	Positive
<i>Escherichia coli</i> O145:NM	ATCC BAA-2192	Feces	Positive
<i>Escherichia coli</i> O145:NM	NJDPH 17257-1	Not Available	Positive
<i>Escherichia coli</i> O145:NM	NJDPH 161296-1	Not Available	Positive
<i>Escherichia coli</i> O157:H7	ATCC 35150	Feces	Positive
<i>Escherichia coli</i> O157:H7	ATCC 43889	Feces	Positive
<i>Escherichia coli</i> O157:H7	ATCC 43894	Feces	Positive
<i>Escherichia coli</i> O157:H7	ATCC 700599	Salami	Positive
<i>Escherichia coli</i> O157:H7	TW00116	Feces	Positive
<i>Escherichia coli</i> O157:H7	TW00975	Human Isolate	Positive
<i>Escherichia coli</i> O157:H7	TW02302	Human Isolate	Positive
<i>Escherichia coli</i> O157:H7	TW04863	Feces	Positive

Table 2: Exclusivity Results, PathoSEEK® STEC *E. Coli*

Organism	Source	Origin	Results
<i>Aeromonas hydrophila</i>	7966	Tin of milk with fishy odor	Negative
<i>Aspergillus Niger</i>	66295	Jerusalem artichoke tuber	Negative
<i>Aspergillus Tubingensis</i>	1004	N/A	Negative
<i>Bacillus amyloliquefaciens</i>	23350	Soil	Negative
<i>Bacillus subtilis</i>	11774	N/A	Negative
<i>Bacillus subtilis</i>	23857	N/A	Negative
<i>Botrytis cinerea</i>	11542	Azalea flowers	Negative
<i>Burkholderia multivorans</i>	17616	Soil enriched with anthranilate at 41°C	Negative
<i>Candida albicans</i>	10321	Man with bronchomycosis	Negative
<i>Candida glabrata</i>	26512	bovine placenta	Negative
<i>Citrobacter braakii</i>	BAA-3037	Urine	Negative
<i>Citrobacter koseri</i>	25408	Throat	Negative

<i>Edwardsiella tarda</i>	23672	N/A	Negative
<i>Erwinia rhapontici</i>	29290	English pink wheat grains	Negative
<i>Escherichia hermannii</i>	700368	N/A	Negative
<i>Klebsiella oxytoca</i>	51983	Blood	Negative
<i>Klebsiella pneumoniae</i>	BAA-2146	Urine	Negative
<i>Lactobacillus leichmannii</i>	4797	N/A	Negative
<i>Listeria monocytogenes</i>	7647	Bovine	Negative
<i>Meyerozyma guilliermondii</i>	6260	Sputum from patient with bronchomycosis	Negative
<i>Morganella morganii</i>	25829	Stool from Providence City Hospital	Negative
<i>Paecilomyces variotii</i>	26820	N/A	Negative
<i>Pantoea agglomerans</i>	45340	Knee laceration	Negative
<i>Penicillium chrysogenum</i>	10106	Cheese	Negative
<i>Penicillium citrinum</i>	9849	N/A	Negative
<i>Penicillium venetum</i>	16025	Hyacinthus sp. bulb	Negative
<i>Pichia membranifaciens</i>	24249	N/A	Negative
<i>Pityrosporum ovale</i>	12078	N/A	Negative
<i>Proteus mirabilis</i>	43071	Rectum	Negative
<i>Pseudomonas aeruginosa</i>	35554	N/A	Negative
<i>Pseudomonas aeruginosa</i>	15442	Water bottle in animal room	Negative
<i>Pseudomonas fluorescens</i>	13525	Pre-filter tanks	Negative
<i>Pseudomonas putida</i>	47054	N/A	Negative
<i>Purpureocillium lilacinum</i>	10114	Soil	Negative
<i>Ralstonia insidiosa</i>	49129	Clinical isolate	Negative
<i>Rhizopus oryzae</i>	52748	N/A	Negative
<i>Rhizopus stolonifer</i>	2276	N/A	Negative
<i>Saccharomyces cerevisiae</i>	18824	Top yeast	Negative
<i>Saccharomyces kudriavzevii</i>	2601	N/A	Negative
<i>Scopulariopsis acremonium</i>	58636	Chicken house soil	Negative
<i>Sphingomonas paucimobilis</i>	29837	Hospital respirator	Negative
<i>Staphylococcus aureus</i>	12600	Pleural fluid	Negative
<i>Staphylococcus haemolyticus</i>	29970	Skin	Negative
<i>Trichosporon mucoides</i>	204094	N/A	Negative
<i>Wickerhamomyces anomalus</i>	2349	N/A	Negative
<i>Yersinia enterocolitica</i>	9610	Tissue; glanders-like infection of the face	Negative

Limit of Detection

Serial dilutions were prepared from a saturated overnight culture of STEC from 1:10 to 1:1M. 10 µL of each dilution was prepared following the MaGiC lysis protocol and tested right away while the same amount (10 µL) was plated on 3M RAC (Rapid Aerobic Count) petrifilm and grown for 24 hours at 37°C. The results are below.

Table 3: LOD qPCR vs 3M RAC Comparison Results

Sample	Dilution	Cq Fam	Cq Hex	3M Plate RAC CFU
STEC <i>E. coli</i> Culture	1:10	20.69	25.66	TNTC
STEC <i>E. coli</i> Culture	1:100	24.30	27.11	TNTC
STEC <i>E. coli</i> Culture	1:1K	27.89	27.35	TNTC
STEC <i>E. coli</i> Culture	1:10K	31.19	27.33	672
STEC <i>E. coli</i> Culture	1:100K	33.84	27.23	82
STEC <i>E. coli</i> Culture	1:1M	37.29	27.26	9

Proficiency Testing/Certified Reference Material Results

NSI Certified Reference Materials were used to compare the detection of STEC *E. coli* in the presence of different matrices. NSI part numbers CMQC 028 and CMQC-030 were used. The materials were resuspended in 9 mL of TSB and enriched for 24 hours at 37 °C. After enrichment, each sample was tested with PathoSEEK® STEC E.coli Detection Assay v2 with MaGiC Lysis Kit. Table 4 contains the resulting data.

Table 4: NSI CRM Data

Sample	Sample Number	Catalog #	Lot #	Detection
STEC in Hemp	001	CMQC-028	H0924	<i>Yes</i>
STEC in Hemp	002	CMQC-028	H0924	<i>No</i>
STEC in Hemp	003	CMQC-028	H0924	<i>No</i>
STEC in Hemp	004	CMQC-028	H0924	<i>Yes</i>
STEC in Hemp	005	CMQC-028	H0924	<i>No</i>
STEC in Oil	001	CMQC-030	H0924	<i>Yes</i>
STEC in Oil	002	CMQC-030	H0924	<i>No</i>
STEC in Oil	003	CMQC-030	H0924	<i>No</i>
STEC in Oil	004	CMQC-030	H0924	<i>No</i>
STEC in Oil	005	CMQC-030	H0924	<i>Yes</i>

Conclusion

The PathoSEEK® STEC E. coli Detection Assay with MaGiC Lysis Kit is a rapid, alternative method to traditional plating procedures for accurate detection of STEC *E.coli* in cannabis flower and cannabis infused products.

REVISION HISTORY

Version	Date	Description
v1	May 2025	Validation of MaGiC Lysis with STEC <i>E. Coli</i> Multiplex Detection Assay v2 Method

DISCLAIMER

This test was developed, and its performance characteristics determined by Medicinal Genomics Company, 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.

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