

PathoSEEK[®] E. coli Detection Assay v2 with MaGiC Lysis Kit

Method Developer Validation

Real Time PCR (qPCR) assay for the detection of *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 *E. Coli*. The PathoSEEK® *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® *E. coli* Multiplex Detection Assay v2 with MaGiC Lysis for the presence/absence detection of *E. coli* species in cannabis flower (delta 9-tetrahydrocannabinol exceeding 0.3%; 1g) and marijuana-infused products (MIP).

Results

Inclusivity and exclusivity results showed that the PathoSEEK® *E. coli* Detection Assay v2 with MaGiC Lysis Kit is highly specific in discriminating target organisms present in cannabis flower and infused products from non-target organisms. Further validation was performed using NSI Certified Reference Materials, specifically part numbers CMQC-028 and CMQC-030. The data confirms that the MaGiC Lysis kit, in conjunction with the PathoSEEK® *E. coli* Detection Assay v2, accurately identifies *E. coli* when present.

Materials

PathoSEEK® E. coli Detection Assay v2 with MaGiC Lysis Kit - **P/N 420533**

(Kit contains sufficient reagents for 200 reactions)

Kit Components:

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

*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 *E. coli* were tested. Strains were grown for 16 hours at $37 \pm 1^\circ\text{C}$ 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, 51 strains of *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 organisms were correctly excluded on the *E. coli* channel.

Table 1: 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

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, ranging from 1:10 to 1:1,000,000, were generated from a saturated overnight culture. Ten microliters of each dilution underwent the MaGiC lysis protocol, with an equivalent volume (10 µL) subsequently plated onto 3M RAC (Rapid Aerobic Count) Petrifilm. The resultant data is presented in Table 3.

Table 3: LOD qPCR vs 3M RAC Comparison Results

Sample	Dilution	Cq Fam	Cq Hex	3M Plate RAC CFUs
<i>E. coli</i> Culture	1:10	23.17	26.49	TNTC
<i>E. coli</i> Culture	1:100	27.03	26.43	TNTC
<i>E. coli</i> Culture	1:1K	30.67	26.59	TNTC
<i>E. coli</i> Culture	1:10K	33.42	26.43	134
<i>E. coli</i> Culture	1:100K	36.93	26.46	18
<i>E. coli</i> Culture	1:1M	ND	26.39	2

Proficiency Testing/Certified Reference Material Results

Certified Reference Materials from NSI were employed to evaluate the detection of *E. coli* in various matrices. Specifically, NSI part numbers CMQC-028 and CMQC-030 were utilized. Qualitative CRMs for general *E. coli* are not available from NSI at this time and therefore STEC was used. The materials underwent resuspension in 9 mL of TSB and were subjected to enrichment for 24 hours at 37 °C.

Subsequently, each sample and its respective dilutions were analyzed using the PathoSEEK® *E. coli* Detection Assay with MaGiC Lysis. Tabulated results are presented in Table 4.

Table 4: NSI CRM Data

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

The Certified Reference Material (CRM) examined comprised five unidentified vials. Two of the five vials were confirmed positive for Shiga toxin-producing *Escherichia coli* (STEC *E. coli*). However, for part CMQC-028, *E. coli* was detected in four of the five vials. The two additional positive results are attributed to the intentional introduction of non-STEC *E. coli* into two of the five vials, an action undertaken to ensure the accurate detection of STEC *E. coli*. The objective of this validation document is the detection of *E. coli* generally, and not specifically STEC *E. coli*. Consequently, the generated results are within the expected parameters, as the *E. coli* assay encompasses the detection of both STEC and non-STEC strains of *E. coli*.

Conclusion

The PathoSEEK® *E. coli* Detection Assay v2 with MaGiC Lysis Kit, is a rapid, alternative method to traditional plating procedures for accurate detection of *E. coli* in cannabis flower and cannabis infused products.

REVISION HISTORY

Version	Date	Description
v1	April 2025	Validation of E. Coli Detection Assay v2 with MaGiC Lysis

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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