PathoSEEK® Listeria Detection Assay with MaGiC Lysis for Detection in Cannabis Flower and MIP Matrices

Method Developer Validation

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Abstract

Background:

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

Objective:

To evaluate the PathoSEEK® *Listeria* Assay v2, using the MaGiC Lysis protocols for the presence/absence detection of *Listeria monocytogenes* in cannabis flower (delta 9-tetrahydrocannabinol >0.3%; 1g) and marijuana-infused products (MIP).

Results:

Inclusivity and exclusivity results showed the PathoSEEK® Listeria Detection Assay v2 method is highly specific in discriminating target organisms found in cannabis flower and infused products from non-target organisms. The MaGiC Lysis kit and PathoSEEK® Listeria Detection Assay v2 was successfully validated through an internal matrix study. The kit was further verified by running NSI Certified Reference Materials, part numbers CRM 7973 and FM-627. Results show that the MaGiC Lysis kit and PathoSEEK® Listeria Detection Assay v2 accurately detected *Listeria monocytogenes* when present.

Materials

PathoSEEK® Listeria Detection Assay v2 with MaGiC Lysis Kit - **P/N 420527**

(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	3 Vials (67 rxns/each)	RT / -20 ºC*
PathoSEEK® Listeria Assay v2	1 Tube (200 µL)	-20 ºC

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 -20 ºC for up to 3 months.

Additional Required Reagents Not in Kit:

Item P/N	Item Name	Qty Provided	Storage Conditions
420226	Listeria Enrichment Broth (via Hardy Diagnostics)*	99 mL	2-8°C
420337	Internal Cannabis Control	1 Tube (50 µL)	-20 ºC
420385	PathoSEEK® Listeria Positive Control	1 Tube (50 µL)	-20 ºC
420184	PCR Grade Water	500 mL Bottle	2-25°C

*Listeria Enrichment Broth is packaged in cases of 20 bottles and is drop shipped from the manufacturer. The minimum order is 4 cases. This item is a Made-To-Order product that requires a minimum order quantity as listed above. The lead time between date of order and ship date is approximately 3-4 weeks.

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Methodology

For inclusivity two different American Type Culture Collection (ATCC) *Listeria monocytogenes* species were evaluated. Strains were grown for 24 hours at 37 ± 1°C in *Listeria* enrichment broth. The cultures were then diluted to 100 x the Limit of Detection (LOD) of the method. For the exclusivity evaluation, a total of 45 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.

Inclusivity and Exclusivity Evaluation

Detailed results for the inclusivity and exclusivity evaluations are presented in Tables 1 and 2. For the inclusivity evaluation, all strains of *Listeria monocytogenes* were detected by the assay using the PathoSEEK *Listeria* Detection Assay v2 with MaGiC lysis on the CFX-96, AriaMx, and the BMS Mic qPCR instruments. Table 1 shows an inclusivity study performed using an *in silico* analysis of the PathoSEEK *Listeria* Detection Assay primers against NCBI genomes. The table also includes results from testing *Listeria* species that were available from ATCC. For the exclusivity evaluation, all 45 of the organisms were correctly excluded.

Table 1: PathoSEEK® *Listeria* Inclusion

Organism	Source	NCBI taxid	Max Alignment Score Percent	qPCR
Listeria monocytogenes	ATCC # 35152	N/A	N/A	Positive
Listeria monocytogenes	ATCC # 7647	N/A	N/A	Positive
Listeria monocytogenes FSL R2-561	N/A	393126	100	N/A
Listeria monocytogenes serotype 1/2a str. 11-4254	N/A	1386005	100	N/A
Listeria monocytogenes serotype 1/2a str. 88-0478	N/A	1196176	100	N/A
Listeria monocytogenes serotype 1/2c str. 10-5026	N/A	1196194	100	N/A
Listeria monocytogenes serotype 1/2a str. 10-5024	N/A	1196192	100	N/A
Listeria monocytogenes SLCC2479	N/A	882020	100	N/A

Listeria monocytogenes serotype 1/2a str. 99-6370	N/A	1196179	100	N/A
Listeria monocytogenes SLCC2372	N/A	932920	100	N/A
Listeria monocytogenes N53-1	N/A	1234142	100	N/A
Listeria monocytogenes serotype 1/2a str. 95-0093	N/A	1196177	100	N/A
Listeria monocytogenes serotype 1/2a str. 08-7363	N/A	1386002	100	N/A
Listeria monocytogenes 08-5923	N/A	637381	100	N/A
Listeria monocytogenes serotype 1/2a str. 01-1280	N/A	1385996	100	N/A
Listeria monocytogenes serotype 1/2a str. 08-7374	N/A	1196185	100	N/A
Listeria monocytogenes serotype 1/2a str. 98-0291	N/A	1386007	100	N/A
Listeria monocytogenes serotype 1/2a str. 08-6997	N/A	1196184	100	N/A
Listeria monocytogenes serotype 1/2a str. 10-1046	N/A	1196189	100	N/A
Listeria monocytogenes serotype 1/2a str. 03-5473	N/A	1386000	100	N/A
Listeria monocytogenes 6179	N/A	1288295	100	N/A
Listeria monocytogenes R479a	N/A	1437838	100	N/A
Listeria monocytogenes LO28	N/A	393132	100	N/A
Listeria monocytogenes serotype 1/2a str. 02-5993	N/A	1196180	100	N/A
Listeria monocytogenes serotype 1/2a str. 98-2035	N/A	1196178	100	N/A
Listeria monocytogenes serotype 1/2a str. 08-7669	N/A	1196186	100	N/A
Listeria monocytogenes serotype 3c str. 10-5027	N/A	1196195	100	N/A
Listeria monocytogenes serotype 1/2a str. 10-4758	N/A	1196168	100	N/A
Listeria monocytogenes serotype 1/2a str. 01-6771	N/A	1385999	100	N/A
Listeria monocytogenes SLCC 7179	N/A	879090	100	N/A
Listeria monocytogenes Finland 1998	N/A	393127	100	N/A
Listeria monocytogenes serotype 1/2a str. 08-6056	N/A	1196182	100	N/A
Listeria monocytogenes serotype 1/2a str. 08-7362	N/A	1386001	100	N/A
Listeria monocytogenes serotype 1/2a str. 08-6569	N/A	1196183	100	N/A
Listeria monocytogenes serotype 1/2c str. 10-5025	N/A	1196193	100	N/A
Listeria monocytogenes serotype 3a str. 88-1059	N/A	1386006	100	N/A
Listeria monocytogenes J0161	N/A	393130	100	N/A
Listeria monocytogenes serotype 1/2a str. 10-1047	N/A	1196190	100	N/A
Listeria monocytogenes serotype 1/2a str. 10-0815	N/A	1196188	100	N/A

Listeria monocytogenes serotype 1/2a str. 10-4754	N/A	1196167	100	N/A
Listeria monocytogenes serotype 1/2a str. 01-5252	N/A	1385998	100	N/A
Listeria monocytogenes serotype 1/2a str. 10-0819	N/A	1386004	100	N/A
Listeria monocytogenes serotype 1/2a str. 10-0814	N/A	1196187	100	N/A
Listeria monocytogenes serotype 1/2a str. 08-7364	N/A	1386003	100	N/A
Listeria monocytogenes serotype 1/2a str. 04-5457	N/A	1196181	100	N/A
Listeria monocytogenes serotype 1/2a str. 10-1321	N/A	1196191	100	N/A
Listeria monocytogenes serotype 1/2a str. 01-1468	N/A	1385997	100	N/A
Listeria monocytogenes 08-5578	N/A	653938	100	N/A
Listeria monocytogenes serotype 1/2a str. 99-6871	N/A	1386008	100	N/A

Table 2: PathoSEEK® *Listeria* Exclusion

Organism	ATCC Source	Origin	Results
Aeromonas hydrophila	7966	Tin of milk with fishy odor	Negative
Aspergillus Niger	66295	Jerusalem artichoke tuber	Negative
Aspergillus Tubingensis	1004	N/A	Negative
Bacillus amyloliquefaciens	23350	Soil	Negative
Bacillus subtilis	11774	N/A	Negative
Bacillus subtilis	23857	N/A	Negative
Botrytis cinerea	11542	Azalea flowers	Negative
Burkholderia multivorans	17616	Soil enriched with anthranilate at 41°C	Negative
Candida albicans	10321	Man with bronchomycosis	Negative
Candida glabrata	26512	bovine placenta	Negative
Citrobacter braakii	BAA-3037	Urine	Negative
Citrobacter koseri	25408	Throat	Negative
Edwardsiella tarda	23672	N/A	Negative
Erwinia rhapontici	29290	English pink wheat grains	Negative
Escherichia hermannii	700368	N/A	Negative
Klebsiella oxytoca	51983	Blood	Negative

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

Proficiency Testing / Certified Reference Material Results

NSI Certified Reference Materials were used to compare the detection of *Listeria monocytogenes* in the presence of different matrices. NSI part numbers used were CRM 7973 (Listeria Pellet) & FM-627 (Listeria in Hemp). Both of the pellets were enriched in Listeria enrichment broth for 24 hours at 37 ℃. MaGiC extraction was performed in triplicate. The resulting extracted sample was then serially diluted by a factor of 10 out to 1:1,000 and qPCR was performed on non diluted sample, and each subsequent dilution. See Data in Table 3 below.

Table 3: NSI CRM Data

Sample	Dilution	Cq Fam	Cq Hex
Listeria CRM	Straight	27.59	26.68
Listeria CRM	Straight	27.59	26.84
Listeria CRM	Straight	27.86	26.72
Listeria CRM	1:10	27.82	27.02
Listeria CRM	1:10	27.45	26.87
Listeria CRM	1:10	27.88	27.04
Listeria CRM	1:100	31.61	27.03
Listeria CRM	1:100	31.58	26.91
Listeria CRM	1:100	31.32	27.01
Listeria CRM	1:1K	34.44	27.15
Listeria CRM	1:1K	34.58	27.04
Listeria CRM	1:1K	35.17	27.02
Listeria NSI in hemp	Straight	28.55	33.57
Listeria NSI in hemp	Straight	28.61	34.01
Listeria NSI in hemp	Straight	28.67	33.34
Listeria NSI in hemp	1:10	31.25	35.11
Listeria NSI in hemp	1:10	31.96	ND
Listeria NSI in hemp	1:10	31.54	35.84
Listeria NSI in hemp	1:100	34.37	ND
Listeria NSI in hemp	1:100	34.71	ND

Listeria NSI in hemp	1:100	34.28	ND
Listeria NSI in hemp	1:1K	36.18	ND
Listeria NSI in hemp	1:1K	36.75	ND
Listeria NSI in hemp	1:1K	ND	ND
Positive Control		12.42	ND
NTC		ND	ND

Limit of Detection

An overnight culture of *Listeria monocytogenes* was diluted serially 1:10, out to 1:10 million. Next, 10ul of each dilution was plated onto 3M Petrifilm Environmental *Listeria* Plates and 10 ul was processed according to the PathoSEEK *Listeria* Assay with MaGiC Lysis protocol. The plating for the dilutions 1:10 and 1:100 were too overgrown to count and the numbers were extrapolated from the 1:1K dilution. Our MaGiC protocol was able to detect down to 2 CFUs that were visible on the 3M plates.

Table 4: LOD Results

Sample	**Dilution**	**MaGiC Lysis**		**3M Environmental *Listeria* Plate**
		Target Fam	**Cq Fam**	**CFU Count**
Listeria monocytogenes	1:10	*Listeria*	22.72	~110,800
Listeria monocytogenes	1:100	*Listeria*	26.15	~11,080
Listeria monocytogenes	1:1K	*Listeria*	29.66	1108
Listeria monocytogenes	1:10K	*Listeria*	32.97	169
Listeria monocytogenes	1:100K	*Listeria*	36.81	25
Listeria monocytogenes	1:1M	*Listeria*	39.49	2
Listeria monocytogenes	1:10M	*Listeria*	ND	1

***Listeria* in Marijuana-Infused Products**

An overnight culture of *Listeria monocytogenes* was spiked into 3 different MIP matrices (gummy, oil and chocolate). After 24 hours of enrichment at 37°C each sample was tested with the PathoSEEK® Listeria Detection Assay with MaGiC Lysis. See results in Table 5 below.

Table 5: MIP Test Results

Sample	MIP	Target FAM	Cq FAM	Target HEX	Cq HEX
Listeria monocytogenes	Gummy	*Listeria*	21.61	ICC	26.59
Listeria monocytogenes	Gummy	*Listeria*	21.37	ICC	25.85
Listeria monocytogenes	Gummy	*Listeria*	21.33	ICC	26.64
Listeria monocytogenes	Oil	*Listeria*	20.21	ICC	26.58
Listeria monocytogenes	Oil	*Listeria*	20.50	ICC	25.48
Listeria monocytogenes	Oil	*Listeria*	20.20	ICC	26.22
Listeria monocytogenes	Chocolate	*Listeria*	21.36	ICC	27.06
Listeria monocytogenes	Chocolate	*Listeria*	21.34	ICC	27.16
Listeria monocytogenes	Chocolate	*Listeria*	21.35	ICC	26.72
Positive Control		*Listeria*	13.07	ICC	ND
NTC		*Listeria*	ND	ICC	ND

Listeria was detected in three infused cannabis matrices.

Conclusions

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

REVISION HISTORY

Version	Date	Description
v1	November 2024	Validation for PathoSEEK Listeria Detection Assay v2

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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