PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 with MaGiC Lysis Kit

Method Developer Validation

Real Time PCR (qPCR) assay for the detection of *Pseudomonas aeruginosa and Staphylococcus aureus* 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 *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 with MaGiC Lysis Kit will detect these species on two separate optical channels using a qPCR (Quantitative Polymerase Chain Reaction) assay allowing for speciation of the two in cannabis flower, cannabis concentrates, infused edibles and infused non-edibles. An Optional Grim Reefer (free DNA removal) step may be employed to reduce positive results caused by dead target organisms.

Objective:

To evaluate the PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2, using the MaGiC Lysis protocols with optional Grim Reefer for the presence/absence detection of *Pseudomonas aeruginosa and Staphylococcus aureus* species in cannabis flower (delta 9-tetrahydrocannabinol >0.3%; 1g) and marijuana-infused products (MIP).

Results:

Inclusivity and exclusivity results showed the PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 with MaGiC Lysis 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 FM 628, 629, 632, and 636. Results show that the MaGiC Lysis kit and PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay accurately detected *Pseudomonas aeruginosa and Staphylococcus aureus* when present.

Materials

PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 with MaGiC Lysis Kit

P/N 420526 (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® Staph and Pseudo Multiplex Detection Assay v2	1 Tube (200 µL)	-20 ºC

Additional Required Reagents Not in Kit:

Item P/N	Item Name	Qty Provided	Storage Conditions
420337	Internal Cannabis Control	1 Tube (50 µL)	-20 ºC
420338	PathoSEEK® Staph and Pseudo Positive Control	1 Tube (50 µL)	-20 ºC
420205	Tryptic Soy Broth	CS/10 x 500mL bottles	2-25°C
420184	PCR Grade Water	500 mL Bottle	2-25°C

Additional **Optional** Reagents Not in Kit:

Item P/N	Item Name	Qty Provided	Storage Conditions
420145	Grim Reefer Free DNA Removal Enzyme and Buffer	100 Reactions	-20 ºC
	Grim Reefer Enzyme	1 Bottle (2.5 mL)	-20 ºC
	Grim Reefer Buffer	1 Bottle (12.75 mL)	-20 ºC

420150	Grim Reefer Deactivation Buffer	1 Bottle (12.75 mL)	-20 ºC

Method Developer Validation

Wet Laboratory Methodology

For the inclusivity evaluation, ATCC strains of *P. aeruginosa* and *S. aureus* were evaluated. Target strains were cultured in Tryptic Soy Broth for 16 hours at 37º C followed by MaGiC Lysis and qPCR with the PathoSEEK P. aeruginosa and S. aureus Multiplex Detection Assay v2.

For exclusivity, 37 organisms were evaluated. Target strains were cultured under optimal conditions for growth of the organism followed by MaGiC DNA lysis and qPCR with the PathoSEEK *P. aeruginosa* and *S. aureus* Multiplex Detection Assay v2.

Results

Table 1: *Staphylococcus aureus* Inclusivity Results

Organism	ATCC Number	Origin	Result
Staphylococcus aureus	12600	*Pleural fluid*	*x*

Table 2: *Pseudomonas aeruginosa* Inclusivity Results

Organism	ATCC Number	Origin	Result
Pseudomonas aeruginosa	15442	*Water bottle in animal room*	*x*

Table 3: Exclusivity Results

Organism	ATCC Number	Origin	Results	
			Staphylococcus	Pseudomonas
Rhizopus oryzae	52748	N/A	ND	ND
Pantoea agglomerans	33243	N/A	ND	ND
Penicillium chrysogenum	10106	Cheese	ND	ND
Aeromonas hydrophila	7966	Tin of milk with fishy odor	ND	ND
Proteus mirabilis	43071	Rectum	ND	ND
Citrobacter koseri	25408	Throat	ND	ND
Ralstonia insidiosa	49129	Clinical isolate	ND	ND
Salmonella enterica	13311	Feces; food poisoning	ND	ND
Pseudomonas fluorescens	13525	Pre-filter tanks	ND	ND
Purpureocillium lilacinum	10114	Soil	ND	ND
Bacillus subtilis	23857	N/A	ND	ND
Escherichia coli	25922	Clinical isolate	ND	ND
Aureobasidium pullulans	62921	N/A	ND	ND
Pseudomonas putida	47054	N/A	ND	ND
Escherichia hermannii	700368	N/A	ND	ND
Morganella morganii	25829	Stool from Providence City Hospital	ND	ND
Penicillium citrinum	9849	N/A	ND	ND
Klebsiella pneumoniae	BAA-2146	Urine	ND	ND
Trichosporon mucoides	204094	N/A	ND	ND
Burkholderia multivorans	17616	Soil enriched with anthranilate at 41°C	ND	ND
Scopulariopsis acremonium	58636	Chicken house soil	ND	ND
Erwinia rhapontici	23376	Rheum rhaponticum	ND	ND
Candida albicans	10231	N/A	ND	ND
Rahnella aquatilis	33991	Man with bronchomycosis	ND	ND
Meyerozyma guilliermondii	6260	Sputum from patient with bronchomycosis	ND	ND

Edwardsiella tarda	23672	N/A	ND	ND
Botrytis cinerea	11542	Azalea flowers	ND	ND
Citrobacter braakii	BAA-3037	Urine	ND	ND
Cryptococcus neoformans	208821	Patient with Hodgkin's disease	ND	ND
Hafnia alvei	51873	Feces	ND	ND
Papiliotrema laurentii	18803	Palm wine, malaffou	ND	ND
Klebsiella oxytoca	51983	Blood	ND	ND
Rhizopus stolonifer	14037	N/A	ND	ND
Aspergillus tamarii	12669	Industrial pipe insulation	ND	ND
Acinetobacter baumannii	19606	Urine	ND	ND
Aspergillus clavatus	1007	N/A	ND	ND
Aspergillus ustus	1041	Culture contaminant	ND	ND

Limit of Detection

Cultures of *S. aureus* and *P. aeruginosa* were grown for 16 hours. After 16 hours a series of six 1:10 dilutions were made. Per the MaGiC lysis kit's instructions, 10 µL of each dilution in triplicate was lysed using the MaGiC lysis buffer. The dilutions were also plated on 3M Rapid Aerobic Count (RAC) plates for comparison. For plating on RAC petrifilm, 10 µl of the appropriate dilution was added to 990ul of water, keeping it consistent with the amount of enrichment going into the MaGiC Lysis, and 1 mL was added to the RAC plates and then incubated at 37°C for 24 hours. The results can be found below in Table 4.

Table 4: Limit of Detection and Plate Count Comparison Results

Sample	**Dilution**	**MaGiC Lysis**				**3M RAC Plate**
		Target Fam	**Cq Fam**	**Target Rox**	**Cq Rox**	**CFU Count**
Pseudomonas Culture	1:10	*Staphylococcus*	ND	*Pseudomonas*	22.52	*3,220,000*
Pseudomonas Culture	1:100	*Staphylococcus*	ND	*Pseudomonas*	26.90	*322,000*
Pseudomonas Culture	1:1,000	*Staphylococcus*	ND	*Pseudomonas*	30.08	*32,200*
Pseudomonas Culture	1:10,000	*Staphylococcus*	ND	*Pseudomonas*	32.97	*3,220*
Pseudomonas Culture	1:100,000	*Staphylococcus*	ND	*Pseudomonas*	36.31	322
Pseudomonas Culture	1:1,000,000	*Staphylococcus*	ND	*Pseudomonas*	38.39	30

Staphylococcus Culture	1:10	*Staphylococcus*	24.26	*Pseudomonas*	ND	*1,460,000*
Staphylococcus Culture	1:100	*Staphylococcus*	27.91	*Pseudomonas*	ND	*146,000*
Staphylococcus Culture	1:1,000	*Staphylococcus*	31.26	*Pseudomonas*	ND	*14,600*
Staphylococcus Culture	1:10,000	*Staphylococcus*	34.90	*Pseudomonas*	ND	*1,460*
Staphylococcus Culture	1:100,000	*Staphylococcus*	38.17	*Pseudomonas*	ND	146
Staphylococcus Culture	1:1,000,000	*Staphylococcus*	38.77	*Pseudomonas*	ND	16

The italicized numbers were too numerous to count and extrapolated from the higher dilutions.

Proficiency Testing/Certified Reference Material

Four different CRMs were tested:

(*S. aureus* in Hemp and Oil, and *P aeruginosa* in hemp and Edible). 9 mL of TSB was added to each pellet and incubated at 37°C for 24 hours. After enrichment, the MaGiC lysis protocol was followed and the results can be found below in Table 5.

Table 5: NSI CRM Data

CRM	Catalog #	Lot #	Dilution	Target Fam	Cq FAM	Target Hex	Cq HEX	Target Cy5	Cq Cy5
S. aureus Hemp	FM-628	240913	Straight	*S. aureus*	+	ICC	+	*P. aeruginosa*	ND
S. aureus Hemp	FM-628	240913	Straight	*S. aureus*	+	ICC	+	*P. aeruginosa*	ND
S. aureus Hemp	FM-628	240913	Straight	*S. aureus*	+	ICC	+	*P. aeruginosa*	ND
S. aureus Oil	FM-632	240307	Straight	*S. aureus*	+	ICC	+	*P. aeruginosa*	ND
S. aureus Oil	FM-632	240307	Straight	*S. aureus*	+	ICC	+	*P. aeruginosa*	ND
S. aureus Oil	FM-632	240307	Straight	*S. aureus*	+	ICC	+	*P. aeruginosa*	ND
P. aeruginosa Hemp	FM-629	230713	1:10	*S. aureus*	ND	ICC	+	*P. aeruginosa*	+
P. aeruginosa Hemp	FM-629	230713	1:10	*S. aureus*	ND	ICC	+	*P. aeruginosa*	+
P. aeruginosa Hemp	FM-629	230713	1:10	*S. aureus*	ND	ICC	+	*P. aeruginosa*	+
P. aeruginosa Edible	FM-636	230831	1:10	*S. aureus*	ND	ICC	+	*P. aeruginosa*	+
P. aeruginosa Edible	FM-636	230831	1:10	*S. aureus*	ND	ICC	+	*P. aeruginosa*	+
P. aeruginosa Edible	FM-636	230831	1:10	*S. aureus*	ND	ICC	+	*P. aeruginosa*	+

Positive Control				*S. aureus*	14.85	ICC	ND	*P. aeruginosa*	16.55
NTC				*S. aureus*	ND	ICC	ND	*P. aeruginosa*	ND

Conclusions

The MaGiC Lysis kit in conjunction with the PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 (both with and without Grim Reefer Free DNA Removal) is a rapid, alternative method to traditional plating procedures for accurate detection of *Pseudomonas Aeruginosa* and *Staphylococcus aureus* in cannabis flower and cannabis infused products.

REVISION HISTORY

Version	Date	Description
v1	December 2024	Validation for MaGiC Lysis with Pseudomonas Aeruginosa and Staphylococcus aureus Multiplex 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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