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

Method Developer Validation

Real-Time PCR (qPCR) Assay for the qualitative detection and speciation of *P. aeruginosa* and *S. aureus* in nutraceutical, dietary supplement, and cannabis matrices.

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Abstract

Background:

Regulations in certain jurisdictions require cannabis flower and non-inhaled cannabis products (among other food products, dietary supplements, and raw ingredients) 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 pathogens in a single test. An optional Grim Reefer (free DNA removal) step may be employed to reduce positive results caused by non-viable target organisms.

Objective:

To evaluate the PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 using the MaGiC Lysis Kit for the qualitative presence/absence detection of Pseudomonas aeruginosa and Staphylococcus aureus in cannabis flower (delta-9-tetrahydrocannabinol >0.3%; 1g) and marijuana-infused products (MIPs), including oil and chocolate. Validation parameters include inclusivity and exclusivity panel testing, limit of detection with plate count comparison, proficiency testing against NSI Certified Reference Materials, and optional Grim Reefer free DNA removal step verification across flower, oil, and chocolate matrices. In addition, a separate independent USP equivalency study has been completed for this method against a variety of dietary supplement matrices and is available on our website.

Summary:

The PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 with MaGiC Lysis was evaluated across a comprehensive set of validation parameters, demonstrating strong analytical performance for the qualitative presence/absence detection of both target organisms in cannabis flower and cannabis-infused product matrices.

Materials

PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 with MaGiC Lysis Kit - **P/N 420526**

Inclusivity and Exclusivity

Methodology

For the inclusion 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 Lysis and qPCR with the PathoSEEK P. aeruginosa and S. aureus Multiplex Detection Assay v2.

Results

Inclusivity and exclusivity results show that 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. Results are presented in Tables 1 and 2.

Table 1: *Staphylococcus aureus* Inclusivity Results

Organism	ATCC Number	Origin	Result
Staphylococcus aureus	12600	*Pleural fluid*	*x*
Staphylococcus aureus	43300	*Clinical isolate*	*x*
Staphylococcus aureus	25923	*Clinical isolate*	*x*
Staphylococcus aureus	29213	*Wound*	*x*
Staphylococcus aureus	12598	*Septic arthritis*	*x*
Staphylococcus aureus	10832	*N/A*	*x*
Staphylococcus aureus	700698	*Sputum from lung cancer patient with MRSA pneumonia*	*x*
Staphylococcus aureus	6538 P	*N/A*	*x*
Staphylococcus aureus	49230	*Patient with chronic osteomyelitis*	*x*

Table 2: *Pseudomonas aeruginosa* Inclusivity Results

Organism	Source	Origin	Result
Pseudomonas aeruginosa	15442	*Water bottle in animal room*	x
Pseudomonas aeruginosa	10145	*N/A*	x
Pseudomonas aeruginosa	27853	*Blood culture*	x
Pseudomonas aeruginosa	BAA-1744	*Clinical isolate*	x
Pseudomonas aeruginosa	47085	*N/A*	x
Pseudomonas aeruginosa	BAA-3248	*Abscess*	x
Pseudomonas aeruginosa	BAA-3279	*Abscess*	x
Pseudomonas aeruginosa	BAA-3280	*Respiratory; sputum*	x
Pseudomonas aeruginosa	9027	*Ear infection*	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

Organism	ATCC Number	Origin	Results	
			Staphylococcus	Pseudomonas
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

Note: Sanger sequencing of the assay amplicon confirmed cross-reactivity with *Pseudomonas rhizosphaerae*, an off-target species. This organism was not included in the original primer exclusion criteria, as it is not represented in common microbial reference collections such as ATCC. Because *P. rhizosphaerae* is not commercially available for purchase or propagation, it cannot be incorporated into formal exclusivity testing. To date, no clinical evidence has linked *P. rhizosphaerae* to human disease, although its presence in inhaled agricultural products warrants continued evaluation. Because *P. rhizosphaerae* is not commercially available from ATCC, its colony-forming characteristics cannot be

readily assessed. As a result, the degree to which this species may contribute to inflated CFU/g estimates in the P. aeruginosa assay remains undetermined.

Limit of Detection

Methodology

Cultures of *S. aureus* (NSI Cat: 61-0032, Lot: 251022) and *P. aeruginosa* (NSI Cat: 10662H, Lot: 240624) were grown for 16 hours, after which six serial 1:10 dilutions were prepared from each culture. From each dilution, 10 µL was plated onto selective media — Mannitol Salt Agar for *S. aureus* and Cetrimide Agar for *P. aeruginosa* — to determine colony counts. An additional 10 µL from each dilution was inoculated into 20 mL of Buffered Peptone Water (BPW) and enriched overnight. Following enrichment, DNA extraction was performed using the MaGiC Lysis Protocol and samples were analyzed by qPCR.

Results

Results demonstrate that the PathoSEEK® Pseudomonas aeruginosa and Staphylococcus aureus Multiplex Detection Assay v2 with MaGiC Lysis reliably detected both target organisms at all inoculum levels tested. The consistency of Cq values across the dilution series is expected for a qualitative presence/absence assay and reflects the equalizing effect of overnight enrichment, which amplifies low-level inocula to a detectable and reproducible level prior to lysis and qPCR analysis. The consistent positive detection observed across replicates at the lowest dilutions therefore demonstrates robust assay performance at and near the practical limit of detection. Results are presented in Table 4.

Table 4: Limit of Detection and Plate Count Comparison Results

Sample	Dilution Enriched	MaGiC Lysis				CFU Count
		Target FAM	Cq FAM	Target Cy5	Cq Cy5	
Pseudomonas Culture	1:10	Staphylococcus	ND	Pseudomonas	20.25	*176,000*
Pseudomonas Culture	1:100	Staphylococcus	ND	Pseudomonas	20.16	*17,600*
Pseudomonas Culture	1:1K	Staphylococcus	ND	Pseudomonas	20.22	*1,760*
Pseudomonas Culture	1:10K	Staphylococcus	ND	Pseudomonas	20.09	176
Pseudomonas Culture	1:100K	Staphylococcus	ND	Pseudomonas	20.27	28

Pseudomonas Culture	1:1M	Staphylococcus	ND	Pseudomonas	21.05	3
Staphylococcus Culture	1:10	Staphylococcus	21.70	Pseudomonas	ND	*610,000*
Staphylococcus Culture	1:100	Staphylococcus	21.76	Pseudomonas	ND	*61,000*
Staphylococcus Culture	1:1K	Staphylococcus	22.05	Pseudomonas	ND	*6,100*
Staphylococcus Culture	1:10K	Staphylococcus	22.13	Pseudomonas	ND	*610*
Staphylococcus Culture	1:100K	Staphylococcus	22.48	Pseudomonas	ND	61
Staphylococcus Culture	1:1M	Staphylococcus	22.76	Pseudomonas	ND	10

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

Proficiency Testing / Certified Reference Material

Methodology

The kit was verified by evaluating NSI Certified Reference Materials representing S. aureus and P. aeruginosa in hemp, oil, and edible matrices. To establish baseline proficiency, four CRMs (FM-628, FM-629, FM-632, and FM-635) were incubated at 37 °C for 24 hours and prepared using the MaGiC Lysis protocol.

Additionally, a comparative enrichment study was conducted to evaluate the assay's performance using two different enrichment broths: Tryptic Soy Broth (TSB) and Buffered Peptone Water (BPW). For this comparison, four NSI CRMs (FM-628, CMQC-069, FM-629, and FM-635) were inoculated into 9 mL of either TSB or BPW and incubated at 37 °C for 24 hours. Following enrichment, the samples were prepared using the MaGiC Lysis protocol and evaluated across a dilution series (Straight, 1:10, and 1:100) to assess the robustness of detection across the two broths.

Results

In the baseline proficiency testing, the initial four NSI Certified Reference Materials (FM-628, FM-629, FM-632, and FM-635) were correctly detected across three replicates each. S. aureus CRMs yielded a positive FAM signal with no Cy5 signal, while P. aeruginosa CRMs yielded a positive Cy5 signal with no FAM signal. Internal controls were positive in all sample wells, and the no-template control (NTC) returned no signal on any channel. These results confirm that the assay performs as expected with commercially certified reference materials (Table 5).

The comparative enrichment study (Table 6) demonstrated equivalent and robust performance between TSB and BPW. Both S. aureus and P. aeruginosa targets were reliably detected in their respective CRMs regardless of which enrichment broth was utilized. Furthermore, positive target detection was consistently achieved across all evaluated dilution levels (Straight, 1:10, and 1:100) for both broths. The internal amplification controls remained positive throughout all dilution and broth combinations, confirming that neither TSB nor BPW introduces assay inhibition and that both are highly suitable for enriching these matrices prior to qPCR analysis.

Table 5: NSI CRM Data

CRM	Catalogue #	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-635	230831	1:10	*S. aureus*	ND	ICC	+	*P. aeruginosa*	+
P. aeruginosa Edible	FM-635	230831	1:10	*S. aureus*	ND	ICC	+	*P. aeruginosa*	+
P. aeruginosa Edible	FM-635	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

Table 6: NSI CRM Data - Comparing TSB and BPW

CRM	Catalogue #	Lot #	Enrichment Broth	Dilution	Target Fam	Cq FAM	Target Hex	Cq HEX	Target Cy5	Cq Cy5
S. aureus Hemp	FM-628	240913	TSB	Straight	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	TSB	Straight	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	TSB	1:10	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	TSB	1:10	S. aureus	+	ICC	+	P. aeruginosa	ND

CRM	Catalogue #	Lot #	Enrichment Broth	Dilution	Target Fam	Cq FAM	Target Hex	Cq HEX	Target Cy5	Cq Cy5
S. aureus Hemp	FM-628	240913	TSB	1:100	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	TSB	1:100	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	BPW	Straight	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	BPW	Straight	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	BPW	1:10	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	BPW	1:10	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	BPW	1:100	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Hemp	FM-628	240913	BPW	1:100	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	TSB	Straight	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	TSB	Straight	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	TSB	1:10	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	TSB	1:10	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	TSB	1:100	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	TSB	1:100	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	BPW	Straight	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	BPW	Straight	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	BPW	1:10	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	BPW	1:10	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	BPW	1:100	S. aureus	+	ICC	+	P. aeruginosa	ND
S. aureus Oil	CMQC-069	H0326	BPW	1:100	S. aureus	+	ICC	+	P. aeruginosa	ND
P. aeruginosa Hemp	FM-629	250317	TSB	Straight	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	TSB	Straight	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	TSB	1:10	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	TSB	1:10	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	TSB	1:100	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	TSB	1:100	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	BPW	Straight	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	BPW	Straight	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	BPW	1:10	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	BPW	1:10	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	BPW	1:100	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Hemp	FM-629	250317	BPW	1:100	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	TSB	Straight	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	TSB	Straight	S. aureus	ND	ICC	+	P. aeruginosa	+

CRM	Catalogue #	Lot #	Enrichment Broth	Dilution	Target Fam	Cq FAM	Target Hex	Cq HEX	Target Cy5	Cq Cy5
P. aeruginosa Edible	FM-635	260602	TSB	1:10	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	TSB	1:10	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	TSB	1:100	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	TSB	1:100	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	BPW	Straight	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	BPW	Straight	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	BPW	1:10	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	BPW	1:10	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	BPW	1:100	S. aureus	ND	ICC	+	P. aeruginosa	+
P. aeruginosa Edible	FM-635	260602	BPW	1:100	S. aureus	ND	ICC	+	P. aeruginosa	+
Positive Control	N/A	N/A	N/A	N/A	S. aureus	23.13	ICC	ND	P. aeruginosa	24.91
NTC	N/A	N/A	N/A	N/A	S. aureus	ND	ICC	ND	P. aeruginosa	ND

Grim Reefer Free DNA Removal Validation

Methodology

To ensure that Grim Reefer free DNA removal works with PathoSEEK P. aeruginosa and S. aureus Multiplex Assay v2, live organisms were enriched in the presence of flower, oil, and chocolate for 16 hours. After 16 hours samples were tested with and without the optional Grim Reefer step according to the MaGiC Lysis protocol. The target organisms were detected in the inoculated matrices with and without Grim Reefer.

Results

The optional Grim Reefer enzymatic free DNA removal step was validated across flower, chocolate, and oil matrices inoculated with both target organisms. Target detection was consistent with and without the Grim Reefer step in all matrix and organism combinations, and internal controls remained positive throughout. These results confirm that the Grim Reefer reagents do not interfere with MaGiC Lysis efficiency or downstream qPCR performance, and that the optional step can be employed to reduce false-positive signals from non-viable organism DNA without compromising sensitivity for viable target detection. Results are presented in Table 7.

Table 7: Grim Reefer Comparison Data

Sample	Matrix	Grim Reefer	Target Fam	Cq Fam	Target Hex	Cq Hex	Target Cy5	Cq Cy5
Pseudomonas aeruginosa	Flower	No	S. aureus	ND	IC	+	P. aeruginosa	+
Pseudomonas aeruginosa	Flower	Yes	S. aureus	ND	IC	+	P. aeruginosa	+
Staphylococcus aureus	Flower	No	S. aureus	+	IC	+	P. aeruginosa	ND
Staphylococcus aureus	Flower	Yes	S. aureus	+	IC	+	P. aeruginosa	ND
Pseudomonas aeruginosa	Chocolate	No	S. aureus	ND	IC	+	P. aeruginosa	+
Pseudomonas aeruginosa	Chocolate	Yes	S. aureus	ND	IC	+	P. aeruginosa	+
Staphylococcus aureus	Chocolate	No	S. aureus	+	IC	+	P. aeruginosa	ND
Staphylococcus aureus	Chocolate	Yes	S. aureus	+	IC	+	P. aeruginosa	ND
Pseudomonas aeruginosa	Oil	No	S. aureus	ND	IC	+	P. aeruginosa	+
Pseudomonas aeruginosa	Oil	Yes	S. aureus	ND	IC	+	P. aeruginosa	+
Staphylococcus aureus	Oil	No	S. aureus	+	IC	+	P. aeruginosa	ND
Staphylococcus aureus	Oil	Yes	S. aureus	+	IC	+	P. aeruginosa	ND
Positive Control			S. aureus	14.32	IC	ND	P. aeruginosa	16.41
NTC			S. aureus	ND	IC	ND	P. aeruginosa	ND

Conclusion

This validation evaluated the PathoSEEK® P. aeruginosa and S. aureus Multiplex Detection Assay v2 with MaGiC Lysis across four parameters: inclusivity and exclusivity panel testing, limit of detection with plate count comparison, proficiency testing against NSI Certified Reference Materials, and optional Grim Reefer free DNA removal verification. All four validation parameters passed. The assay demonstrated 100% inclusivity across nine ATCC strains of each target organism and 100% exclusivity across 37 non-target organisms, with the single noted exception of P. rhizosphaerae, an organism not commercially available for formal testing. Detection was confirmed at the lowest inocula tested following overnight enrichment, all four NSI CRMs were correctly detected across three replicates each, and the optional Grim Reefer step was shown to have no adverse effect on assay sensitivity across flower, oil, and chocolate matrices. Additionally, comparative enrichment studies confirmed that both Buffered Peptone Water (BPW) and Tryptic Soy Broth (TSB) provide robust, equivalent performance for target recovery across multiple dilutions.

The PathoSEEK® P. aeruginosa and S. aureus Multiplex Detection Assay v2 with MaGiC Lysis Kit is a validated, rapid, and specific alternative to traditional culture-based methods for the qualitative detection of these two harmful pathogens, offering laboratories confident flexibility in their enrichment workflows.

REVISION HISTORY

Version	Date	Description
v1	December 2024	Validation for MaGiC Lysis with *Pseudomonas aeruginosa* and *Staphylococcus aureus* Multiplex Assay v2 Method
v2	June 2025	Addition of validation data for Grim Reefer Free DNA Removal & note about exclusivity data concerning *P. rhizosphaerae*
v3	June 2026	• Update to analysis of Pseudomonas detection Cq values • Update to Limit of Detection study • Inclusion of CRM study with BPW vs. TSB

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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