

# **PathoSEEK<sup>®</sup> *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**

### **Table of Contents**

|                                                                |   |
|----------------------------------------------------------------|---|
| Abstract                                                       | 3 |
| Materials                                                      | 4 |
| Inclusivity and Exclusivity                                    | 5 |
| Wet Laboratory Methodology                                     | 5 |
| Results                                                        | 5 |
| Table 1: Staphylococcus aureus Inclusivity Results             | 5 |
| Table 2: Pseudomonas aeruginosa Inclusivity Results            | 5 |
| Table 3: Exclusivity Results                                   | 5 |
| Limit of Detection                                             | 7 |
| Table 4: Limit of Detection and Plate Count Comparison Results | 7 |
| Proficiency Testing / Certified Reference Material             | 8 |
| Table 5: NSI CRM Data                                          | 8 |
| Grim Reefer Free DNA Removal Validation                        | 8 |
| Table 6: Grim Reefer Comparison Data                           | 9 |
| Conclusions                                                    | 9 |

## **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 non-viable 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 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. The kit was further verified by running NSI Certified Reference Materials, part numbers FM 628, 629, 632, and 635. Results show that the MaGiC Lysis kit and PathoSEEK® *Pseudomonas aeruginosa* and *Staphylococcus aureus* Multiplex Detection Assay v2 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 °C*       |
| PathoSEEK® Staph and Pseudo Multiplex Detection Assay v2 | 1 Tube (200 µL)        | -20 °C             |

Additional Required Reagents Not in Kit:

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

## Inclusivity and Exclusivity

### 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 37° 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

Table 1: *Staphylococcus aureus* Inclusivity Results

| Organism                     | ATCC Number | Origin        | Result |
|------------------------------|-------------|---------------|--------|
| <i>Staphylococcus aureus</i> | 12600       | Pleural fluid | x      |

Table 2: *Pseudomonas aeruginosa* Inclusivity Results

| Organism                      | ATCC Number | Origin                      | Result |
|-------------------------------|-------------|-----------------------------|--------|
| <i>Pseudomonas aeruginosa</i> | 15442       | Water bottle in animal room | x      |

Table 3: Exclusivity Results

| Organism                       | ATCC Number | Origin                      | Results        |             |
|--------------------------------|-------------|-----------------------------|----------------|-------------|
|                                |             |                             | Staphylococcus | Pseudomonas |
| <i>Rhizopus oryzae</i>         | 52748       | N/A                         | ND             | ND          |
| <i>Pantoea agglomerans</i>     | 33243       | N/A                         | ND             | ND          |
| <i>Penicillium chrysogenum</i> | 10106       | Cheese                      | ND             | ND          |
| <i>Aeromonas hydrophila</i>    | 7966        | Tin of milk with fishy odor | ND             | ND          |
| <i>Proteus mirabilis</i>       | 43071       | Rectum                      | ND             | ND          |

|                                  |          |                                         |    |    |
|----------------------------------|----------|-----------------------------------------|----|----|
| <i>Citrobacter koseri</i>        | 25408    | Throat                                  | ND | ND |
| <i>Ralstonia insidiosa</i>       | 49129    | Clinical isolate                        | ND | ND |
| <i>Salmonella enterica</i>       | 13311    | Feces; food poisoning                   | ND | ND |
| <i>Pseudomonas fluorescens</i>   | 13525    | Pre-filter tanks                        | ND | ND |
| <i>Purpureocillium lilacinum</i> | 10114    | Soil                                    | ND | ND |
| <i>Bacillus subtilis</i>         | 23857    | N/A                                     | ND | ND |
| <i>Escherichia coli</i>          | 25922    | Clinical isolate                        | ND | ND |
| <i>Aureobasidium pullulans</i>   | 62921    | N/A                                     | ND | ND |
| <i>Pseudomonas putida</i>        | 47054    | N/A                                     | ND | ND |
| <i>Escherichia hermannii</i>     | 700368   | N/A                                     | ND | ND |
| <i>Morganella morganii</i>       | 25829    | Stool from Providence City Hospital     | ND | ND |
| <i>Penicillium citrinum</i>      | 9849     | N/A                                     | ND | ND |
| <i>Klebsiella pneumoniae</i>     | BAA-2146 | Urine                                   | ND | ND |
| <i>Trichosporon mucoides</i>     | 204094   | N/A                                     | ND | ND |
| <i>Burkholderia multivorans</i>  | 17616    | Soil enriched with anthranilate at 41°C | ND | ND |
| <i>Scopulariopsis acremonium</i> | 58636    | Chicken house soil                      | ND | ND |
| <i>Erwinia rhapontici</i>        | 23376    | Rheum rhaponticum                       | ND | ND |
| <i>Candida albicans</i>          | 10231    | N/A                                     | ND | ND |
| <i>Rahnella aquatilis</i>        | 33991    | Man with bronchomycosis                 | ND | ND |
| <i>Meyerozyma guilliermondii</i> | 6260     | Sputum from patient with bronchomycosis | ND | ND |
| <i>Edwardsiella tarda</i>        | 23672    | N/A                                     | ND | ND |
| <i>Botrytis cinerea</i>          | 11542    | Azalea flowers                          | ND | ND |
| <i>Citrobacter braakii</i>       | BAA-3037 | Urine                                   | ND | ND |
| <i>Cryptococcus neoformans</i>   | 208821   | Patient with Hodgkin's disease          | ND | ND |
| <i>Hafnia alvei</i>              | 51873    | Feces                                   | ND | ND |
| <i>Papiliotrema laurentii</i>    | 18803    | Palm wine, malaffou                     | ND | ND |
| <i>Klebsiella oxytoca</i>        | 51983    | Blood                                   | ND | ND |
| <i>Rhizopus stolonifer</i>       | 14037    | N/A                                     | ND | ND |
| <i>Aspergillus tamarii</i>       | 12669    | Industrial pipe insulation              | ND | ND |
| <i>Acinetobacter baumannii</i>   | 19606    | Urine                                   | ND | ND |

|                             |      |                     |    |    |
|-----------------------------|------|---------------------|----|----|
| <i>Aspergillus clavatus</i> | 1007 | N/A                 | ND | ND |
| <i>Aspergillus ustus</i>    | 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**

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    |
| <i>Pseudomonas</i> Culture | 1:10        | <i>Staphylococcus</i> | ND     | <i>Pseudomonas</i> | 22.52  | 3,220,000    |
| <i>Pseudomonas</i> Culture | 1:100       | <i>Staphylococcus</i> | ND     | <i>Pseudomonas</i> | 26.90  | 322,000      |
| <i>Pseudomonas</i> Culture | 1:1,000     | <i>Staphylococcus</i> | ND     | <i>Pseudomonas</i> | 30.08  | 32,200       |
| <i>Pseudomonas</i> Culture | 1:10,000    | <i>Staphylococcus</i> | ND     | <i>Pseudomonas</i> | 32.97  | 3,220        |
| <i>Pseudomonas</i> Culture | 1:100,000   | <i>Staphylococcus</i> | ND     | <i>Pseudomonas</i> | 36.31  | 322          |
| <i>Pseudomonas</i> Culture | 1:1,000,000 | <i>Staphylococcus</i> | ND     | <i>Pseudomonas</i> | 38.39  | 30           |

|                               |             |                       |       |                    |    |                  |
|-------------------------------|-------------|-----------------------|-------|--------------------|----|------------------|
| <i>Staphylococcus</i> Culture | 1:10        | <i>Staphylococcus</i> | 24.26 | <i>Pseudomonas</i> | ND | <i>1,460,000</i> |
| <i>Staphylococcus</i> Culture | 1:100       | <i>Staphylococcus</i> | 27.91 | <i>Pseudomonas</i> | ND | <i>146,000</i>   |
| <i>Staphylococcus</i> Culture | 1:1,000     | <i>Staphylococcus</i> | 31.26 | <i>Pseudomonas</i> | ND | <i>14,600</i>    |
| <i>Staphylococcus</i> Culture | 1:10,000    | <i>Staphylococcus</i> | 34.90 | <i>Pseudomonas</i> | ND | <i>1,460</i>     |
| <i>Staphylococcus</i> Culture | 1:100,000   | <i>Staphylococcus</i> | 38.17 | <i>Pseudomonas</i> | ND | <i>146</i>       |
| <i>Staphylococcus</i> Culture | 1:1,000,000 | <i>Staphylococcus</i> | 38.77 | <i>Pseudomonas</i> | ND | <i>16</i>        |

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 vial then 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                         | Catalogue # | Lot #  | Dilution | Target Fam       | Cq FAM | Target Hex | Cq HEX | Target Cy5           | Cq Cy5 |
|-----------------------------|-------------|--------|----------|------------------|--------|------------|--------|----------------------|--------|
| <i>S. aureus</i> Hemp       | FM-628      | 240913 | Straight | <i>S. aureus</i> | +      | ICC        | +      | <i>P. aeruginosa</i> | ND     |
| <i>S. aureus</i> Hemp       | FM-628      | 240913 | Straight | <i>S. aureus</i> | +      | ICC        | +      | <i>P. aeruginosa</i> | ND     |
| <i>S. aureus</i> Hemp       | FM-628      | 240913 | Straight | <i>S. aureus</i> | +      | ICC        | +      | <i>P. aeruginosa</i> | ND     |
| <i>S. aureus</i> Oil        | FM-632      | 240307 | Straight | <i>S. aureus</i> | +      | ICC        | +      | <i>P. aeruginosa</i> | ND     |
| <i>S. aureus</i> Oil        | FM-632      | 240307 | Straight | <i>S. aureus</i> | +      | ICC        | +      | <i>P. aeruginosa</i> | ND     |
| <i>S. aureus</i> Oil        | FM-632      | 240307 | Straight | <i>S. aureus</i> | +      | ICC        | +      | <i>P. aeruginosa</i> | ND     |
| <i>P. aeruginosa</i> Hemp   | FM-629      | 230713 | 1:10     | <i>S. aureus</i> | ND     | ICC        | +      | <i>P. aeruginosa</i> | +      |
| <i>P. aeruginosa</i> Hemp   | FM-629      | 230713 | 1:10     | <i>S. aureus</i> | ND     | ICC        | +      | <i>P. aeruginosa</i> | +      |
| <i>P. aeruginosa</i> Hemp   | FM-629      | 230713 | 1:10     | <i>S. aureus</i> | ND     | ICC        | +      | <i>P. aeruginosa</i> | +      |
| <i>P. aeruginosa</i> Edible | FM-635      | 230831 | 1:10     | <i>S. aureus</i> | ND     | ICC        | +      | <i>P. aeruginosa</i> | +      |
| <i>P. aeruginosa</i> Edible | FM-635      | 230831 | 1:10     | <i>S. aureus</i> | ND     | ICC        | +      | <i>P. aeruginosa</i> | +      |
| <i>P. aeruginosa</i> Edible | FM-635      | 230831 | 1:10     | <i>S. aureus</i> | ND     | ICC        | +      | <i>P. aeruginosa</i> | +      |
| Positive Control            |             |        |          | <i>S. Aureus</i> | 14.85  | ICC        | ND     | <i>P. aeruginosa</i> | 16.55  |
| NTC                         |             |        |          | <i>S. Aureus</i> | ND     | ICC        | ND     | <i>P. aeruginosa</i> | ND     |

### **Grim Reefer Free DNA Removal Validation**

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, which can be seen in Table 6.

Table 6: Grim Reefer Comparison Data

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

### **Conclusions**

The MaGiC Lysis kit in conjunction with the PathoSEEK® *Pseudomonas aeruginosa* and *Staphylococcus aureus* Multiplex Detection Assay v2 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 <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> Multiplex Assay v2 Method      |
| v2      | June 2025     | Addition of validation data for Grim Reefer Free DNA Removal & note about exclusivity data concerning <i>P. rhizosphaerae</i> |

## 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.

## LIMITED USE LABEL LICENSE

This product is covered by at least one or more claims of US patent applications, which are exclusively licensed to Medicinal Genomics Corporation. This product is sold strictly for the use of the buyer, and the buyer is not authorized to transfer this product [or any materials made using this product] to any third party.

© 2025 Medicinal Genomics Corporation. All rights reserved.

\* All Trademarks are property of their respective owners.