PathoSEEK® Aspergillus Multiplex Detection Assays and Salmonella/STEC Assays with MaGiC Lysis Kit Single Enrichment Guide

Real Time PCR (qPCR) assay for the detection of *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. terreus, Salmonella, and Shiga Toxin producing E. coli* in dried cannabis flower and MIP matrices

Table of Contents

Please note that this alternate workflow is **not AOAC PTM-validated**. The following deviations from AOAC Performance Tested Methods 072502 and 022202 apply:

Deviations from AOAC PTM-Validated Method:

- Salmonella and STEC enrichment is performed in Potato Dextrose Broth (PDB) instead of Tryptic Soy Broth (TSB).
- Aspergillus enrichment is performed in PDB instead of PDB supplemented with chloramphenicol.
- For Salmonella-STEC testing, the sample input volume for lysis is 50 µL of enriched culture rather than 10 µL.
- For Aspergillus testing, the required enrichment time is 48–54 hours for all matrices including flower instead of 24–48 hours for flower.
- For Salmonella/STEC testing, the required enrichment time is 24-48 hours for all matrices instead of 16-24 hours.
- Free DNA Removal (Grim Reefer®) is not performed.

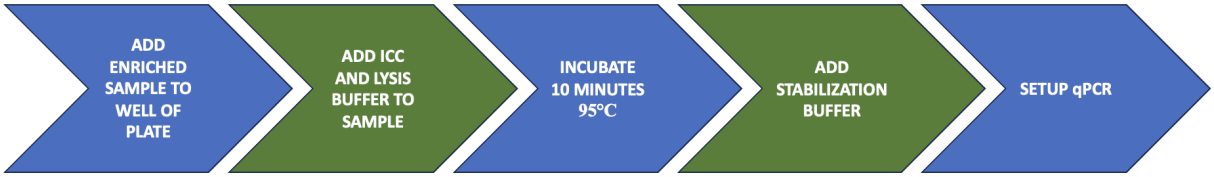
MGC recommends that each testing laboratory conduct an **internal method verification study** to evaluate the performance of this alternate workflow prior to routine implementation, as required by applicable state regulatory bodies and/or **ISO/IEC 17025:2017**.

Any deviation from a validated or standardized method constitutes a **laboratory-modified method**, and the laboratory is responsible for demonstrating that the method is **fit for its intended use**.

Process Overview

These assays use a multiplexing strategy with an internal control (IC) that is introduced at the lysis step to ensure accurate detection of four species of *Aspergillus, Salmonella and STEC* as well as the internal control DNA in every reaction. Unlike other techniques, this multiplexing strategy verifies the performance of the assay when detecting pathogens, resulting in the minimization of false negative results due to reaction setup errors or failing experimental conditions. Below is a simplified depiction of the MaGiC lysis in Figure 1 and qPCR assays in Figure 2.

Figure 1: Overview of MaGiC Lysis



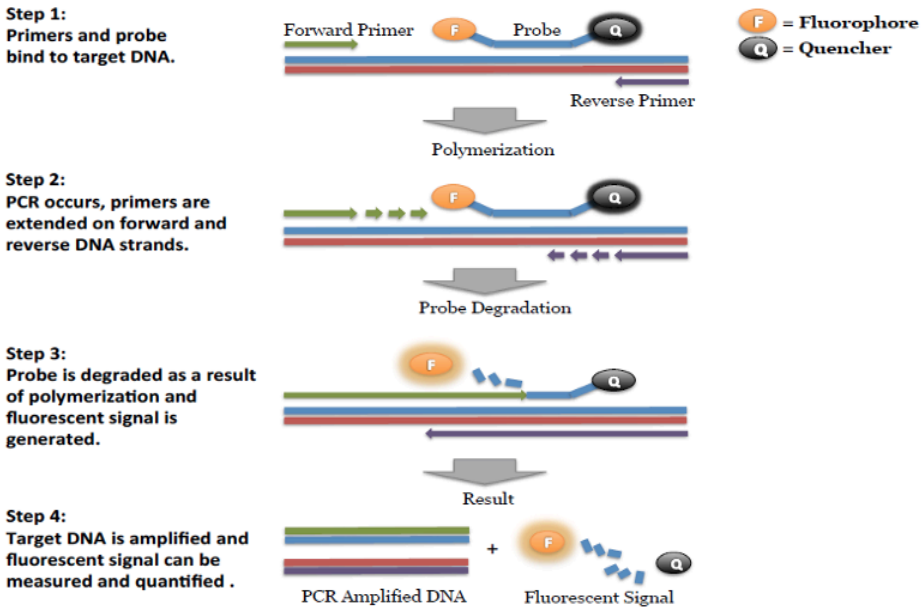


Figure 2: Overview of qPCR

Kits Referenced in this Guide

PathoSEEK 5-Color Aspergillus Multiplex - AriaMx with MaGiC Lysis Kit - **P/N 420518** (contains sufficient reagents for 200 reactions). Please note some components are stored at different temperatures.

PathoSEEK 5-Color Aspergillus Multiplex - CFX with MaGiC Lysis Kit- **P/N 420519** (contains sufficient reagents for 200 reactions). Please note some components are stored at different temperatures.

PathoSEEK 2-Color Aspergillus Multiplex with MaGiC Lysis Kit, **P/N 420523** (contains sufficient reagents for 200 reactions). Please note some components are stored at different temperatures.

PathoSEEK® Salmonella & STEC E. coli Multiplex Detection Assay v3 with MaGiC Lysis Kit, **P/N 420517** (Kit contains sufficient reagents for 200 reactions). Please note some components are stored at different temperatures.

Component Name	Qty Provided	Storage Conditions
MaGiC Lysis Reagent	2 Bottles (12 mL each)	RT (20–28°C)
MaGiC Stabilization Buffer	1 Bottle (24 mL)	RT (20–28°C)

Additional **Required** Reagents Not in Kit:

Item P/N	Item Name	Qty Provided	Storage Conditions
420032	Potato Dextrose Broth (PDB)	500 mL Bottle	2-25 °C
420337	Internal Control	1 Tube (50 µL)	-15 to -20 ºC
420184	PCR Grade Water	500 mL Bottle	2-25 °C

Shelf Life and Storage

Once received, each kit component must be stored at its designated storage condition. Reagents stored properly can be used until the expiration date indicated on each component label.

Required Equipment and Supplies

- Incubator—Capable of maintaining 37 ± 2 °C, VWR #97025-630 or equivalent.
- 96-Well PCR Plate Cryogenic Rack (optional) —VWR #89004-570
- 1.5 mL Tube Benchtop Cryogenic Rack (optional if crushed ice is not an option) — VWR #89004-558 or equivalent
- Eppendorf Tube Rack

Supplies:

- 96-well Optical qPCR plate — **Medicinal Genomics P/N 100164**
- Adhesive optical seals for qPCR plates — **Medicinal Genomics P/N 100177**
- Adjustable, variable volume pipettes (single or multichannel).—P10, P20, P200, and P1000
- Adjustable, variable volume filter pipettes tips.—For P10, P20, P200, and P1000
- Filter Bags—**Medicinal Genomics P/N 100008** (Whirl-Pak #B01385WA)
- Beaker or Solo Cup. (optional)
- 15 mL or 50 mL conical tubes
- Pipette - Aid Portable Pipetting device for serological pipettes, VWR 89166-464 or equivalent
- 25 mL Serological Pipette—VWR 89130-890 or 89130-900 or equivalent
- 10% bleach
- Clean workspaces periodically with 10% bleach and other decontaminating agents.
- Use powder-free gloves and avoid fingerprints and writing on tube caps. Both can interfere with data acquisition.

Sample Preparation

1. Aliquot PDB.

   Note: PDB is a very good growth medium for microbes. Therefore, it is best to pour the approximate amount of PDB into another sterile tube or container, to not contaminate the stock bottle.
2. Wipe down the workspace with a 10% bleach solution, including the bench top and all equipment being used.
3. If not already at room temperature, remove PDB from the 2-8 °C refrigerator, the PDB should come to room temperature, 20–28 °C, before use.
4. Prepare consumables. Label all the filter bags or conical tubes with "[sample name] [date]".
5. Label lysis plate with date.
6. Before weighing out the sample(s) to be tested, make sure that the entire sample is broken up and thoroughly homogenized. A well-homogenized sample will ensure more accurate testing.
7. *Dried Cannabis flower, **n** grams* —Weigh flower sample material into one side of the mesh liner inside the Whirl-Pak bag. Add 9 x *n* mL of prepared PDB to each test portion. Close the Filter bag by folding the top over three times. Mix for 1 minute by hand.
   a. Incubate Samiples
      i. Salmonella & STEC Testing: **24 – 48** h at 37 ± 1 °C.
      ii. Aspergillus Testing: **48 - 54 h** at 37 ± 1 °C.
8. *Concentrates and MIPs, **n** grams* —Weigh concentrate or MIP into a 15 mL conical tube, 50 mL conical tube, or Whirl-Pak bag depending on the concentrate test portion size. Add 9 x *n* mL of PDB to each test portion. Homogenize the test portion until thoroughly mixed in PDB.
   a. Incubate Samples
      i. Salmonella & STEC Testing: **24 – 48** h at 37 ± 1 °C.
      ii. Aspergillus Testing: **48 - 54 h** at 37 ± 1 °C.
9. If processing multiple samples, be sure to change gloves between each sample to ensure that there is no cross-contamination between samples during the weighing process. See flower example in Figure 3.

Figure 3: Homogenized cannabis flower and PDB

MaGiC Lysis

Matrices: Dried Cannabis Flower, Infused Cannabis Products, and Cannabis Concentrates

1. Dilute IC to 1:50,000
    a. Dilute stock IC to 1:100.
        i. Ensure stock IC is fully thawed and vortexed, and then pulse spin down in a mini centrifuge before use.
        Add 2 µL of stock IC to 198 µL of nuclease-free water. Vortex well and pulse spin down in a mini centrifuge.
    b. Serially dilute the 1:100 dilution of IC an additional 1:100 to make a 1:10,000 dilution.
        i. Add 2 µL of 1:100 IC to 198 µL of nuclease-free water. Vortex and pulse spin down in a mini centrifuge. This is the 1:10,000 dilution IC.
    c. Dilute the 1:10,000 IC by 1:5, which results in the final dilution of 1:50,000.
        i. Add 20 µL of the 1:10,000 IC to 80 µL of nuclease free-water. Vortex and pulse spin down in a mini centrifuge. Final dilution is 1:50,000 IC.
2. Remove samples from the incubator (contained in the Whirl Pak Bag, 15 or 50 mL conical tube).
    a. **Flower sample**: Mix thoroughly by hand manipulating and/or squeezing the contents in Whirl Pak bag for the flower enrichment for 1 minute.
    b. Infused Products: Mix thoroughly by hand manipulating and/or squeezing the contents in Whirl Pak bag for the food enrichment for 1 minute. Vortex conical tube thoroughly for 30 seconds.
    c. **Concentrates:** Vortex conical tube thoroughly for 30 seconds.

    Note: *Aspergillus* growth tends to clump together, this post enrichment homogenization and/or vortexing will aid in spreading cells throughout the media in the Whirl Pak bag or tubes.
3. Remove 50 µL of enriched test portion and transfer into the well of a 96-well PCR plate.
4. Add 100 µL of MaGiC Lysis Buffer to all wells being tested, using a fresh pipette tip for each transfer. Tip mix 15 times to ensure the sample is mixed with the lysis buffer.
5. For Infused Products and Concentrate samples, Spike IC into Samples (optional for flower samples)
    a. Add 5 µL of 1:50,000 dilution of IC to the 50 µl of each concentrate or MIP sample.
    b. OPTIONAL: Add 5 µL of the 1:50,000 dilution of IC to the 50 µL of each flower sample.
6. Seal the top of the plate and briefly spin down in a centrifuge.

7. Place plate on the thermal cycler and run MaGiC Lysis program
    a. Incubate 95 °C for 10 minutes, then 4 °C for 5 min (if 4°C is not an option, set to 25 °C for 10 min).

    Note: When using the Agilent AriaMX, a compression pad should be placed over the adhesive seal to prevent evaporation from the plate and seal.
8. Remove plate with lysed samples from the thermal cycler and briefly spin the plate down in centrifuge to remove evaporation from the plate seal. Carefully remove the plate seal.
9. Tip mix 5 times and then transfer 50 µL of lysed sample to a new plate.
10. Add 100 µL of MaGiC Stabilization buffer to each sample well using a new pipette tip for each sample. Pipette mix 5 times and briefly spin the plate down in plate centrifuge. The samples are now ready for qPCR set up, see Real - Time Quantitative PCR (qPCR) Setup Protocol with PathoSEEK Amplification Mix.
11. Seal the plate with sample lysates with a plate seal and store in -20 °C freezer if not proceeding directly to qPCR setup.
    a. Sample lysates with stabilization buffer added are stable at -20 ºC for 1 month.
    b. Thaw frozen samples at room temperature. Sample lysates which have been frozen must be tip mixed 15 times prior to setting up qPCR or performing dilutions.
12. For qPCR setup and data analysis instructions proceed to the appropriate published User Guide
    a. PathoSEEK® Salmonella & STEC E. Coli Multiplex Detection Assay With MaGiC Lysis Kit
    b. PathoSEEK® Aspergillus Multiplex Detection Assays with MaGiC Lysis Kit

Revision History

Version	Date	Description
v1	January 2026	Creation of Single Enrichment Supplemental Guide

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 among factors known to affect the growth of bacterial and fungal species.