PathoSEEK® Aspergillus Speciation Kit User Guide

Supplemental User Guide for Aspergillus Speciation

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Introduction

Some jurisdictions which require testing for four pathogenic species of Aspergillus, *A. fumigatus*, *A. niger*, *A. flavus*, and *A. terreus*, also require the specific species of Aspergillus detected to be reported. The PathoSEEK® Aspergillus Speciation Kit allows for the individual identification of Aspergillus species, each in a separate qPCR reaction, when a positive result is obtained using the PathoSEEK® 2-Color Aspergillus Detection Assay. This supplemental guide is not included in Medicinal Genomics' Aspergillus AOAC PTM claim.

Kit Components

PathoSEEK® Aspergillus Species-Specific Assays Kit - P/N 420537

(contains sufficient reagents for 200 reactions). Please note some components are stored at different temperatures.

Component Name	Qty Provided	Storage Conditions
PathoSEEK® Amplification Mix *Includes 2 tubes nuclease free water for resuspension*	4 Vials (50 rxns each)	RT (20–28°C) / -15 to -20 ºC*
Aspergillus flavus Detection Assay v2	1 Vial (200 rxn)	-15 to -20 ºC
Aspergillus fumigatus Detection Assay v2	1 Vial (200 rxn)	-15 to -20 ºC
Aspergillus terreus Detection Assay v2	1 Vial (200 rxn)	-15 to -20 ºC
Aspergillus niger Detection Assay v2	1 Vial (200 rxn)	-15 to -20 ºC

*The PathoSEEK® Amplification Mix can be stored lyophilized at Room Temperature for up to 2 years. Once re-hydrated it must be stored at -15 to -20 ºC for up to 3 months.

Additional **Required** Reagents Not in Kit:

Item P/N	Item Name	Qty Provided	Storage Conditions
420311	PathoSEEK® Aspergillus flavus Positive control	1 Tube (50 µL)	-15 to -20 ºC
420309	PathoSEEK®Aspergillus niger Positive control	1 Tube (50 µL)	-15 to -20 ºC
420310	PathoSEEK® Aspergillus fumigatus Positive control	1 Tube (50 µL)	-15 to -20 ºC
420329	PathoSEEK® Aspergillus terreus Positive control	1 Tube (50 µL)	-15 to -20 ºC

Note: Actual fill volumes include overage

Real-Time Quantitative PCR (qPCR) Setup Protocol

1. Remove PathoSEEK® Amplification Mix, PathoSEEK® Aspergillus flavus, A. fumigatus, A. niger, and A. terreus Detection Assays v2, and A. flavus, A. fumigatus, A. niger, and A. terreus Positive Controls from the -20 ºC freezer.
    a. If lyophilized Amplification Mix has not been previously rehydrated, rehydrate with 550 µl of Nuclease Free Water. Swirl or pipette tip mix. After resuspension, store remainder Amplification Mix at -15 to -20 °C when not in use.
    b. Allow all frozen reagents to defrost at room temperature (20 - 28 °C). Once defrosted, place tubes on ice.
2. Before preparing the Master Mix, invert or vortex and pulse spin down the reagents in a mini centrifuge.
    a. Aspergillus Detection Assays – vortex tubes quickly followed by a pulse spin down in a minicentrifuge.
    b. Aspergillus Positive Controls – vortex tube quickly followed by a pulse spin down in a minicentrifuge.
    c. PathoSEEK® Amplification Mix – Invert the bottle 5-10 times to mix or briefly vortex.
    d. Return all reagents to the ice.
3. Prepare each Master Mix, one for each species, in a 1.5 mL tube (the detection assay also includes the probe for the IC). Label each tube with the appropriate Aspergillus species name. See Table 1 (PathoSEEK® Amplification Master Mix Reagent Volumes).
4. Always prepare enough Master Mix for an additional one or two reactions to account for pipetting and dead volumes. Be sure to include 2 extra reactions for the qPCR positive and negative controls. For example, if testing 10 samples, you would need to make enough Master Mix for 13 or 14 reactions, which would account for 1 or 2 excess.

Table 1: PathoSEEK® Amplification Master Mix Reagent Volumes

Reagent	Volume for 1 qPCR Reaction
PathoSEEK® Amplification Mix	10 µL
Aspergillus flavus Detection Assay v2 or Aspergillus fumigatus Detection Assay v2 or Aspergillus terreus Detection Assay v2 or Aspergillus niger Detection Assay v2	1 µL
Nuclease Free Water	4 µL
Total	15 µL

5. Once the Master Mix is combined gently cap the tube and vortex to mix.
    a. Pulse spin down tube in minicentrifuge.
    b. Place the Master Mix tube on ice until used.
6. For the negative controls, use nuclease free water that was used to rehydrate your Amplification Mix.
7. For the positive controls, use the PathoSEEK® Aspergillus flavus Positive Control, PathoSEEK® Aspergillus niger Positive Control, PathoSEEK® Aspergillus fumigatus Positive Control, and PathoSEEK® Aspergillus terreus Positive Control. Dilute each positive control 1:10 in a separate tube.
    a. Add 1 µL of Positive Control to 9 µL nuclease free water (found in the kit), vortex to mix and pulse spin down the tube in mini centrifuge

    Note: It is best to add the largest volume reagent first, in this case the 9 µL water then the 1 µL of positive control, pipette mix or vortex control dilution to ensure control DNA is in solution.
8. For qPCR reactions use a 96-well optical qPCR plate or optically clear qPCR tubes.
9. Transfer source DNA into qPCR plate wells or tubes
    a. Carefully remove the seal from the source DNA plate.
    b. Transfer 5 µL of each sample into a separate tube or well, once for each species specific assay being used.

Note: If lysed samples were frozen, let the DNA thaw completely and spin the plate in centrifuge to avoid cross contamination between samples. Tip mix thawed samples wells before transferring to the qPCR plate or tubes.

10. Add 5 µL of each diluted Positive Control to the corresponding positive control well or tube for each assay being used.
11. Add 5 µL of nuclease free water to the corresponding negative control well or tube for each assay being used.

    Note: ALWAYS use a fresh tip for every liquid transfer of sample, positive, or negative control into the qPCR plate

12. Add 15 µL of Master Mix to each corresponding sample well, positive control well, and negative control well in the qPCR plate or tubes. Gently tip mix a few times after each addition of Master Mix. Be careful not to introduce bubbles during this mix. Use a fresh tip for each transfer of Mater Mix to each well.
13. Seal the plate with strip caps or an adhesive seal, or seal qPCR tubes with strip caps.
14. For the Agilent AriaMX or Bio-Rad CFX, spin down qPCR plate or tubes for at least 1 minute in plate (or tube) centrifuge to bring well contents to the bottom of wells (or tubes) and help to get rid of reaction bubbles.

    Note: Check for bubbles in the wells or tubes (minimal bubbles on the surface of the liquid is acceptable). If bubbles remain in the wells (or tubes), spin down for another minute in plate centrifuge.

15. For the Agilent Aria MX: If using an adhesive seal; place the reusable compression pad (gray side down) on the plate directly lining up the holes in the pad with the wells in the plate.
16. Place the sealed plate or tubes onto the PCR instrument.
17. Follow the software specific instructions for each qPCR platform to initiate the run with the following parameters:
    a. Hot Start at 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 15 seconds and 65 °C for 90 seconds.

Results

For detailed data analysis instructions and troubleshooting guides, refer to the appropriate 2-Color Aspergillus Detection Assay User Guide. Each Aspergillus species will be detected on FAM.

PathoSEEK® Aspergillus Detection Assay Data Analysis Quick Reference Table

PathoSEEK® Target	**Cq Value**	**Fluor**	**Negative Control (Cq)**	**CFU threshold (CFU/g)**
Aspergillus flavus	≤ 40	FAM	No Cq	Presence/Absence
Aspergillus fumigatus	≤ 40	FAM	No Cq	Presence/Absence
Aspergillus niger	≤ 40	FAM	No Cq	Presence/Absence
Aspergillus terreus	≤ 40	FAM	No Cq	Presence/Absence
Internal Control*	≤35	HEX	*Internal control verifies the presence or absence of plant DNA	
Assay Positive Control	≤35	FAM		

REVISION HISTORY

Version	Date	Description
v1	August 2025	Creation of supplemental guide for the use of singleplex Aspergillus assays to speciate results from the PathoSEEK® 2-Color Aspergillus Detection Assay.

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.

The results may vary based on laboratory conditions. Altitude and humidity are among factors known to affect the growth of bacterial and fungal species. All thresholds were determined based on the results using the BIO-RAD CFX96 Touch® Real-Time PCR Detection System. It is recommended that thresholds be calibrated for each specific laboratory setting.

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