PathoSEEK Total Yeast & Mold Screening Assay with MaGiC Lysis Kit

User Guide

Real Time PCR (qPCR) assay for the qualitative detection of Yeast and Mold in food, cannabis, and nutraceutical products.

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Introduction

Total yeast and mold (TYM) testing is commonly used as an indicator of microbiological quality in food, cannabis, and nutraceutical products. The PathoSEEK® Total Yeast and Mold (TYM) Screening Assay with MaGiC™ Lysis uses quantitative polymerase chain reaction (qPCR) technology to detect yeast and mold DNA across a variety of matrices, including cannabis-derived products, food ingredients, finished food products, and nutraceutical materials.

The assay provides rapid and reliable screening for total yeast and mold contamination and is designed to support routine quality control and product release testing.

Process Overview

The PathoSEEK® Total Yeast and Mold (TYM) Screening Assay with MaGiC™ Lysis is a multiplex qPCR assay that detects yeast and mold using the FAM channel and includes an Internal Control (IC) detected in the HEX channel. The IC is added during lysis and carried through the workflow to monitor sample processing and qPCR performance, helping identify potential inhibition or processing failures that could lead to false-negative results.

The method includes an enrichment step to improve detection sensitivity. Samples with a presence result are reflexed to a culture-based enumeration method to confirm viability and determine colony counts.

The assay is compatible with qPCR instruments that support FAM and HEX detection and has been validated on the BioMolecular Systems Mic, Agilent AriaMx, and Bio-Rad CFX96 platforms. A simplified overview of the MaGiC™ Lysis workflow is shown in **Figure 1**, followed by the qPCR detection workflow in **Figure 2**.



Figure 1: Overview of MaGiC Lysis

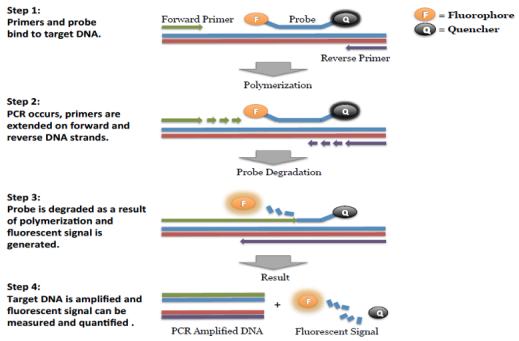


Figure 2: Overview of qPCR

Kit Components

PathoSEEK Total Yeast & Mold Screening Assay with MaGiC Lysis Kit, **P/N 420552**

(contains sufficient reagents for 200 reactions)

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)
PathoSEEK Amplification Mix *Includes 2 tubes nuclease-free water for resuspension*	4 Vials (50 rxns/each)	RT (20–28 °C) / -15 to -20 °C*
PathoSEEK Total Yeast and Mold Screening Assay	1 Tube (200 µL)	-15 to -20 °C

Note: Some actual fill volumes include overage. Some components are stored at different temperatures.

*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 °C for up to 3 months.

Additional **Required** Reagents Not Included 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 °C
420303	PathoSEEK Total Yeast and Mold Positive Control	1 Tube (50 µL)	-15 to -20 °C
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

Equipment:

- Bio-Molecular Systems Myra Liquid Handling System with Mic 4-Channel Real-Time PCR Instrument - **Medicinal Genomics P/N 420241**
    - Mic 4-Channel Real-Time PCR Instrument - **Medicinal Genomics P/N 420241**
    - Myra Liquid Handling System - **Medicinal Genomics P/N 420252**
    - Latest Version of BMS Workbench Software - Free Download from BMS
    - Lab supplied Windows PC (See vendor's requirements)
    - Myra 50 µL Robot Tips - **Medicinal Genomics P/N 420254**
    - Mic Tubes and Racked Caps - **Medicinal Genomics P/N 420244**
    - Mic Tubes and Caps (Bulk) - **Medicinal Genomics P/N 420243**
    - Eppendorf Mastercycler X40 or similar if using Mic Thermal Cycler - **Medicinal Genomics P/N 420035**
- Agilent AriaMx Real-Time PCR System G8830A—Containing the following Optical Channels: FAM and HEX
    - Software version 3.1.2306.0602
    - Agilent HP Notebook PC option 650 or lab supplied Windows PC
    - Optical Strip Caps.—Agilent #401425.

      Note: If using adhesive seals use Applied Biosystems MicroAmp Optical Film Compression Pad, Fisher Scientific, #43-126-39 to prevent evaporation and cross contamination between wells.
- Bio-Rad CFX96 Touch™ Real-Time System.
    - Software version 10.0.26100
    - Bio-Rad supplied or lab supplied Windows PC
- Bio-Rad CFX-Opus
    - Software version 10.0.26100
    - Bio-Rad supplied or ab supplied Windows PC (See vendor's requirements)
- Adjustable, variable volume pipettes (single or multichannel).—P10, P20, P200, and P1000

- Adjustable, variable volume filter pipettes tips.—For P10, P20, P200, and P1000
- Freezer—Capable of maintaining -20 ± 2 °C
- Plate centrifuge - USA Sci. Part # 4062-0572 - Eppendorf 5430 centrifuge w/ rotary knobs, non-refrigerated, with Rotor A-2-MTP swing bucket rotor
- Tabletop Mini Centrifuge—VWR #10067-588 or equivalent
- Vortex-Genie Pulse—Scientific Industries, SKU: SI-0236 or equivalent
- 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
- Scientific Scale—Capable of measuring to milligrams
- Refrigerator—Capable of maintaining 2–8 °C

Supplies:

- 96-well Optical qPCR plate — **Medicinal Genomics P/N 100164**
    a. Optically clear qPCR tubes may be substituted
- Adhesive optical seals for qPCR plates — **Medicinal Genomics P/N 100177**
- Filter Bags—Whirl-Pak bags or equivalent enrichment containers of suitable dimensions. It is strongly advised to utilize enrichment bags featuring integrated filter technology. **Medicinal Genomics P/N 100008** (Whirl-Pak #B01385WA)
- Crushed ice
- 1.5 mL Eppendorf Tubes
- 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

Safety Precautions and Recommendations for Best Results

Environment

The quality of results depends on the strict compliance with Good Laboratory Practices (for example, the EN ISO 7218 standard), especially concerning PCR:

- Never circulate lab equipment from one workstation to another.

- Always use a positive and negative control for each series of amplification reactions.
- Periodically verify the accuracy and precision of pipettes, as well as correct functioning of the instruments.
- Change gloves often, especially if you suspect contamination.
- 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.

Safety Precautions

- Assay users should observe standard microbiological practices and safety precautions when performing this assay. Wear protective gloves, lab coats, eye/face protection as indicated by your quality system.
- It is the responsibility of each laboratory to handle waste and effluents processed according to their nature and degree of hazardousness and to treat and dispose of them in accordance with applicable local, state, and federal regulations.

Intended User

The PathoSEEK Total Yeast and Mold Screening Assay with MaGiC Lysis Kit and all components required to perform the method are intended for use by trained personnel familiar with laboratory techniques associated with pathogenic organism detection.

Sample Preparation

1. Prepare the workspace. Wipe down the benchtop and all equipment with a 10% bleach solution before beginning.
2. Aliquot Potato Dextrose Broth (PDB). If not already at room temperature, remove PDB from the 2-8 °C refrigerator (it should come to room temperature, 20–28 °C, before use).
   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, so as not to contaminate the stock bottle.
3. Thoroughly homogenize the sample before weighing the test portion to ensure representative sampling.
4. Prepare samples according to the Table below.

Table 1: Matrices and Sample Prep Conditions

Matrix	Sample Preparation	Enrichment*	Dilution before qPCR (use nuclease free water)
MCT Oil and Gummy (*n* grams)	Homogenize *n* g of sample into 9 x *n* mL of PDB	Incubate for **24 - 48 h** at 37 ± 2 °C	1:10 dilution required
Chocolate (*n* grams)	Homogenize *n* g of sample into 9 x *n* mL of PDB	Incubate for **24 - 48 h** at 37 ± 2 °C	1:100 dilution required

***Enrichment Time and Matrix Considerations:** Recommended enrichment times are based on the matrices and action limits evaluated during Medicinal Genomics (MGC) validation studies. **Refer to the applicable MGC Validation Document for the specific matrices, action limits, and enrichment times evaluated and demonstrated during validation.** Laboratories should verify that the recommended enrichment conditions provide acceptable detection at the applicable action limit under their own laboratory conditions. This is particularly important when testing alternative matrices not included in the MGC validation, as matrix composition may affect microbial recovery, enrichment, and assay performance. Laboratories should evaluate and document appropriate enrichment conditions for these matrices as part of their internal method validation or verification.*

5. *Test Portion, **n** grams* —Weigh test portion into a 15 mL conical tube, 50 mL conical tube, or Whirl-Pak bag.
6. 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.
7. Homogenize the sample with PDB then place it into the incubator.

Sample Lysis

Preparation of the Internal Control (IC) Dilution

Dilute Internal Control (IC) to 1:50,000 for all matrices **except chocolate**

Note: Before starting, ensure the stock IC is fully thawed, vortexed, and pulse-spun in a mini-centrifuge.

1. Step 1 — Make a 1:100 dilution Add 2 µL of stock IC to 198 µL of nuclease-free water. Vortex thoroughly and pulse spin.
2. Step 2 — Make a 1:5,000 dilution: Add 2 µL of the 1:100 dilution to 98 µL of nuclease-free water. Vortex thoroughly and pulse spin. **This is the working IC at 1:5,000 for chocolate matrices only.**

3. Step 3 — Make the final 1:50,000 dilution Add 10 µL of the 1:5,000 dilution to 90 µL of nuclease-free water. Vortex thoroughly and pulse spin. **This is the working IC at 1:50,000 for all other matrices.**

MaGiC Lysis

1. Remove enriched samples from the incubator (contained in the Whirl-Pak Bag, 15 or 50 mL conical tube).
2. Homogenize the samples.
    a. **Infused Products/ Food Matrices:** 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.
    b. **Concentrates:** Vortex conical tube thoroughly for 30 seconds.

    Note: *Mold* 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. Transfer 50 µL of enriched test portion into a well of a 96-well PCR plate.
4. Add 100 µL of MaGiC Lysis Reagent to all samples being tested, using a fresh pipette tip for each transfer. Pipette mix 15 times to ensure the sample is mixed with the lysis reagent.
5. Add 5 µL of 1:50,000 diluted IC control to each sample. If testing chocolate, add 5 µL of 1:5,000 diluted IC control.
6. Repeat for all sample wells, using a fresh tip for each transfer.
7. Seal the top of the plate and briefly spin down in a centrifuge.
8. 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.
9. 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.
10. Using a new pipette tip for each sample, pipette mix the lysed sample 5 times, then transfer **50 µL** to the corresponding well of a new plate

11. Add **100 µL** of MaGiC Stabilization Buffer to each well containing the transferred sample. Use a new pipette tip for each sample and pipette mix 5 times to mix the sample and stabilization buffer together.
12. Briefly centrifuge the plate to collect the contents at the bottom of each well. The samples are now ready for qPCR set up, see Real - Time Quantitative PCR (qPCR) Setup Protocol.
13. 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 °C for 1 month.
    b. Thaw frozen samples at room temperature. Sample lysates which have been frozen must be pipette mixed 15 times prior to setting up qPCR or performing dilutions.

Preparation of Matrix Appropriate qPCR Dilutions

14. Refer to Table 1 for matrix-specific dilution requirements prior to qPCR setup.
    a. For MCT Oil and Gummy - Dilute the stabilized sample 1:10 in nuclease-free water.
        i. Load 5 µL of the undiluted sample per reaction
        ii. Load 5 µL of the diluted sample per reaction.
    b. For Chocolate - dilute the stabilized sample 1:100 in nuclease-free water.
        i. Load 5 µL of the diluted sample per reaction.

If using the Myra Liquid Handler and Mic thermal cycler - Proceed below.
If using the AriaMX, Bio-Rad CFX Opus 96, or CFX 96 - go to ***Real-Time Quantitative PCR (qPCR) Setup Protocol for the AriaMX, Bio-Rad CFX Opus or CFX96***

Real-Time Quantitative PCR (qPCR) Setup on the Myra Liquid Handler

1. Remove PathoSEEK Amplification Mix, Total Yeast and Mold Screening Assay, and Total Yeast and Mold positive control from the -20 ºC freezer.
    a. If lyophilized Amplification Mix has not been previously rehydrated, use the supplied Nuclease-Free Water to rehydrate with the volume specified on the side of the vial. Invert the bottle 5-10 times, vortex, or pipette mix to ensure complete resuspension.
        i. Following reconstitution, store the Amplification Mix at -15 to -20 °C when not in use. For improved access and storage stability, transfer the reconstituted

Amplification Mix to a labeled 1.5 mL or 2.0 mL microcentrifuge tube and record the rehydration date on the tube label.

b. Allow all frozen reagents to defrost at room temperature (20 to 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. Total Yeast and Mold Screening Assay tube – vortex tube quickly followed by a pulse spin down in a minicentrifuge.
    b. Total Yeast and Mold Positive Control tube – 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. Place reagents on ice.
3. For the positive control, use the Total Yeast and Mold Positive Control reagent. Dilute the positive control **1:10,000**
    a. Add 2 µL of Positive Control to 198 µL nuclease free water (found in the kit), vortex to mix and pulse spin down the tube in a mini centrifuge. This is a 1:100 positive control dilution.

    Note: It is best practice to add the largest volume reagent first.

    b. Add 2 µL of 1:100 Positive Control dilution to 198 µL nuclease free water, vortex to mix and pulse spin down the tube in a mini centrifuge. This is the 1:10,000 positive control dilution. This tube will be loaded into the instrument.
4. Dilute each sample lysate 1:10 or 1:100 with nuclease free water according to Table 1.
5. Open the Myra or Workbench software program by clicking the desktop shortcut.



Figure 3: Desktop shortcut

6. Under the “Templates” tab, double-click the appropriate run template:
    a. _MaGiC_Mic

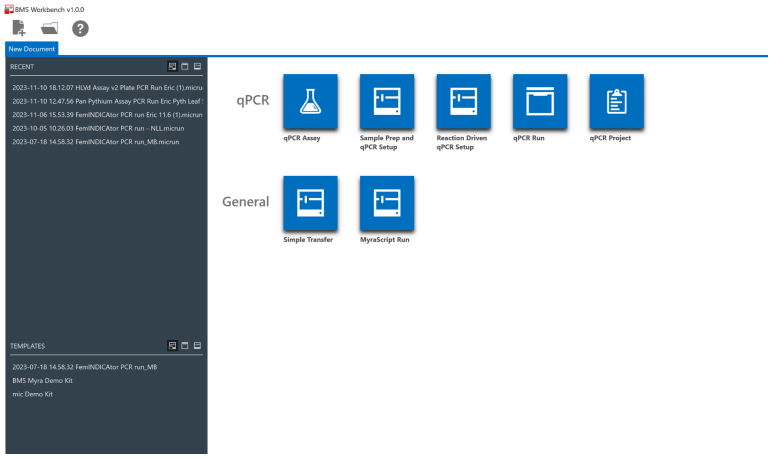


Figure 4: Template selection screen

7. Click the + Next to Assays and select the appropriate assay
    a. Total Yeast and Mold Screening Complete Assay

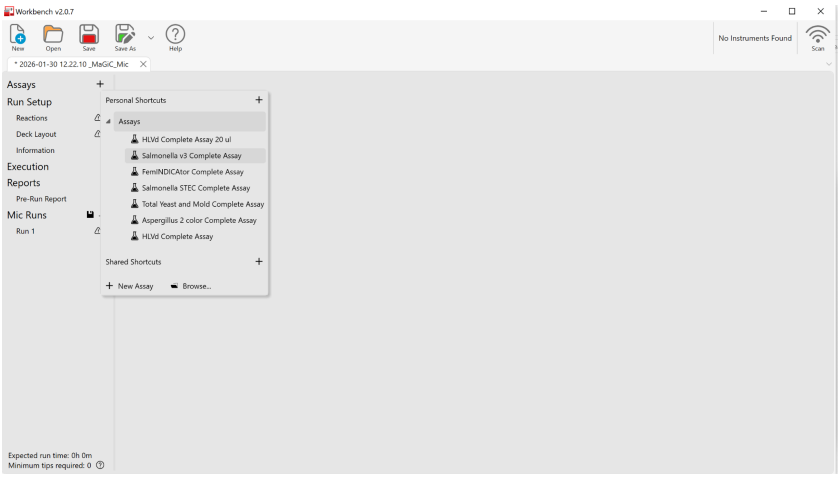


Figure 5: Example Assay selection screen

8. Connect to the Myra by using the icon in the upper right corner.



Figure 6: Connect to Myra Icon

9. Navigate to the Reactions tab and name samples, positive controls, and NTCs.
10. Use the "Type" drop down menu to identify samples as Unknown, Positive Control, or NTC.
11. For each sample, prepare **two qPCR reactions** as follows:
    a. **Reaction 1:** Undiluted sample lysate

b. **Reaction 2:** 1:10 dilution of the sample lysate in nuclease-free water

Chocolate Matrix Exception: For chocolate samples, prepare **one qPCR reaction only**, using a **1:100 dilution of the sample lysate in nuclease-free water**.

12. Use the "Assay" drop down menu to assign the Total Yeast and Mold Screening Complete Assay to all wells being tested.

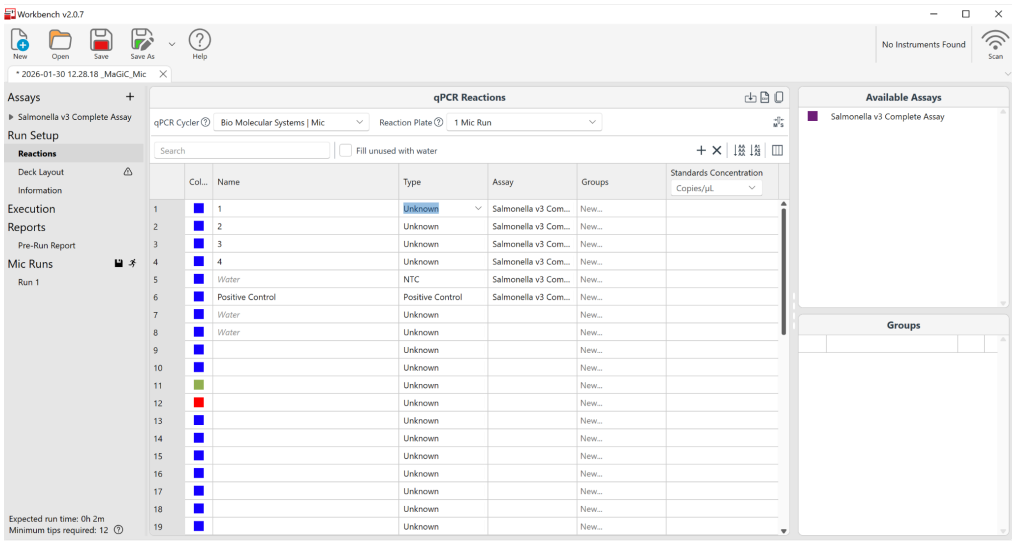


Figure 7: Example Reactions Tab

13. In the Deck Layout Screen Navigate to the Liquids Tab and allocate Samples, Reagents, Mixes, and Reactions to the appropriate deck locations:

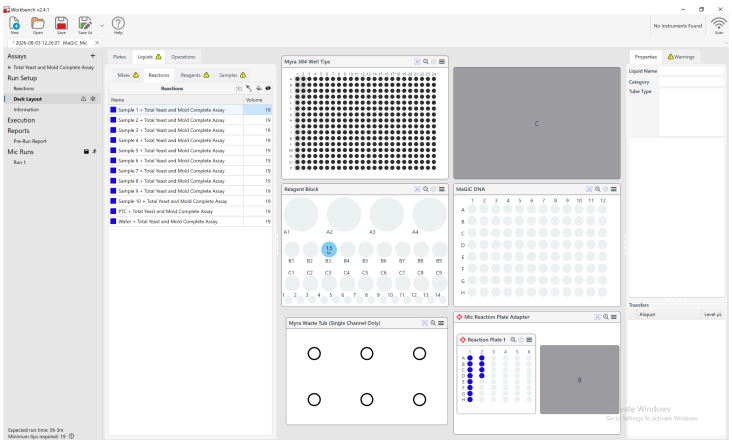


Figure 8: Example Liquids Tab

14. If stored at 4 °C, remove the Myra Multipurpose Loading Block and transfer to the correct Myra deck location according to the Deck Layout screen. If the loading block is stored at 4 °C until right before setting up a run, it will keep reagents cool while on the deck.
15. Hover over the tube positions to determine the location of each reagent tube in the multipurpose loading block. Samples will be placed just to the right of the loading block according to the deck layout. The sample lysates will be placed onto the deck in a 96 well PCR plate.
16. Hovering over the reagent tubes will also indicate volumes of each reagent needed, based on the number of samples entered into the 'Samples' tab.

    Note: Most master mixes will need additional volume over the volume indicated to account for viscosity of these reagents. It is best to load full tubes whenever you can.

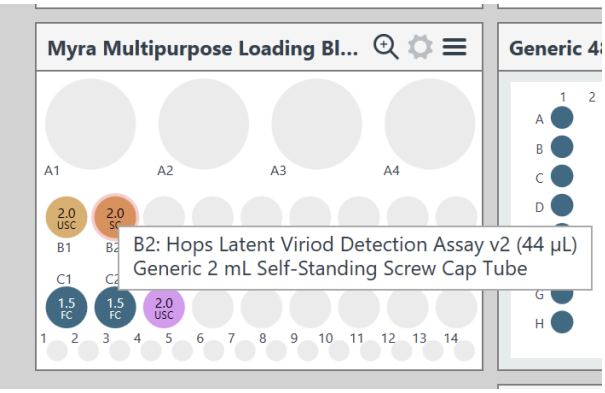


Figure 9: Example Multipurpose loading block image

17. Once the deck setup is complete, click on the Play icon to start the instrument. A "Save As" screen will allow users to save their run with a unique name.



Figure 10: Play icon

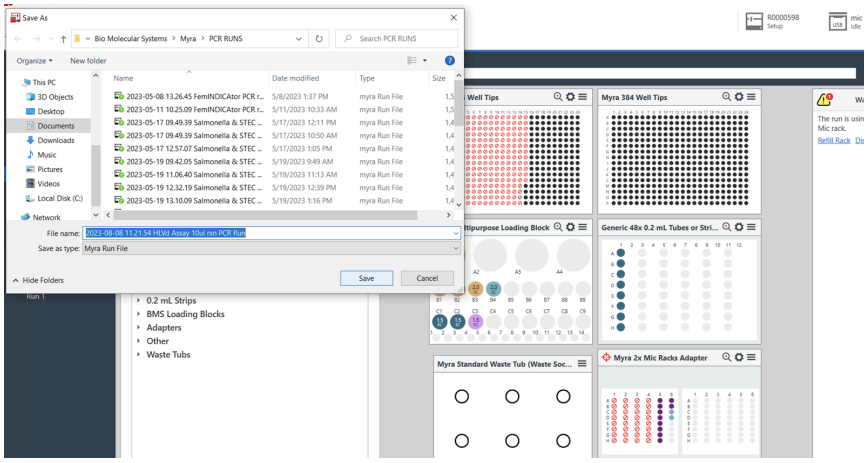


Figure 11: Example "Save As" screen

18. A pre-run checklist will appear. Check off the boxes as the steps are completed and click "Start" to initiate the run.

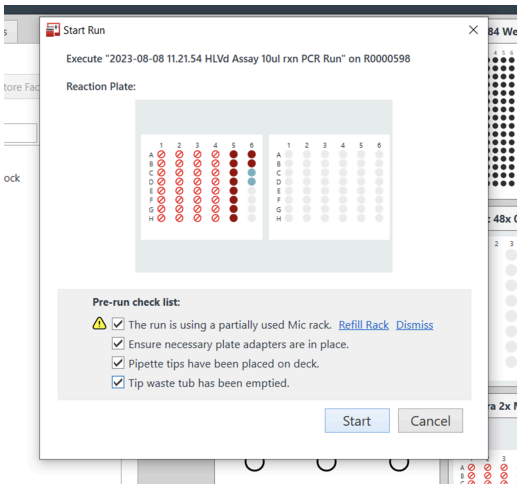


Figure 12: Example Pre-run checklist

19. Allow the instrument to run to completion.
20. Once the run has finished, the prepared Mic tubes will contain the Master mix and samples.
21. Firmly cap the tubes using the manual capper or the Rapid Cap device.

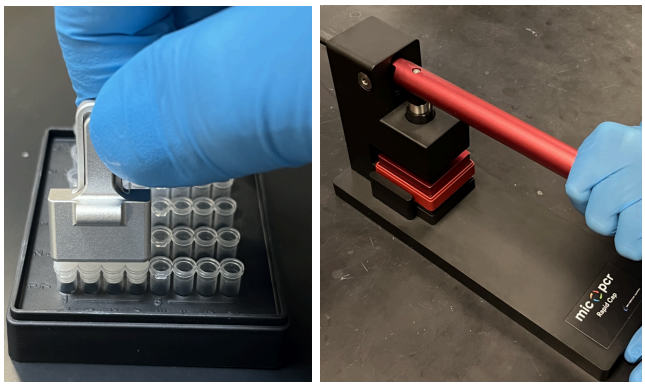


Figure 13: Capping with manual capper (left) and Rapid Cap device (right)

22. Load the Mic qPCR thermal cycler with the capped reaction tubes. Each tube is part of a strip of four, with the first tube having a small tab to ensure the strip is loaded into the instrument correctly. Start at position 1 and ensure that the small clear tab on the tubes is aiming inward towards the rotor center. If running fewer than 46 samples (48 total reactions) ensure that *water balance Mic tubes* are loaded into all unused sample locations.

    Note: Do not use old reaction tubes in the unused sample location. Not all assay reaction volumes are identical. Ensure that the proper volume of water is used in the blank tubes for each assay.

23. Replace the magnetic tube clamp and close the Mic lid when all tubes are loaded.

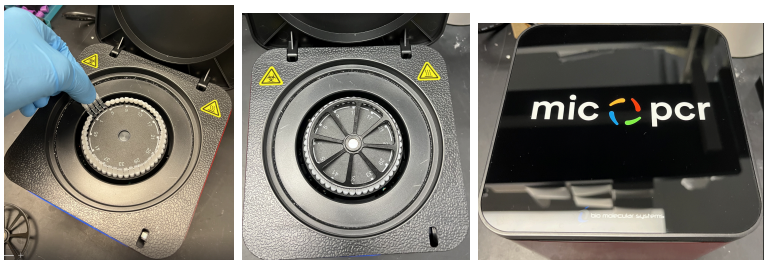


Figure 14: Loading the Mic

24. Cap the reagent tubes and sample tubes on the Myra. If another Myra run will not be performed right away, place the reagents and samples in the freezer for storage or on ice for use within the same day.
25. Click the running man figure to export the data to the Mic software.

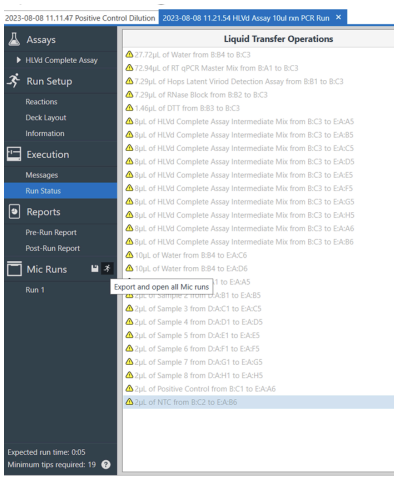


Figure 15: Example of Exporting data to the Mic

Running the BioMolecular Systems Mic

1. Open the BMS Workbench software and create a new file. If continuing from a Myra run, verify that the thermal profile matches step 3 and proceed to step 5.
2. Select qPCR Run.
3. Select the appropriate template by clicking the "+" sign next to assays or ensure that the appropriate thermal cycling conditions are entered: Hot start at 95 ºC for 5 minutes, followed by 40 cycles of 95 ºC for 15 seconds and 65 ºC for 90 seconds (data capture).

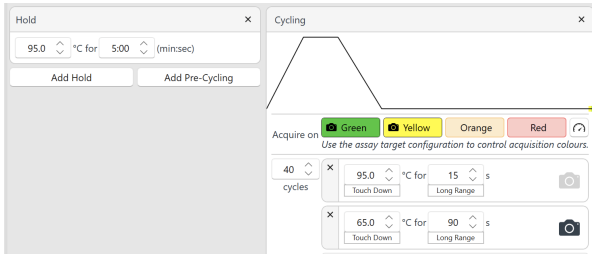


Figure 16: Thermal profile

4. Navigate to the Samples Tab and identify the samples, controls, and assay.

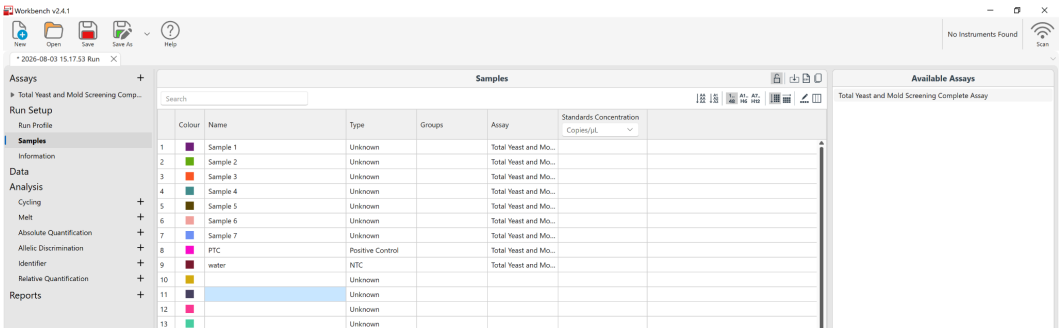


Figure 17: Example Samples Tab

5. Click on the Mic icon in the upper right-hand corner, and select “Start run” from the menu that appears.

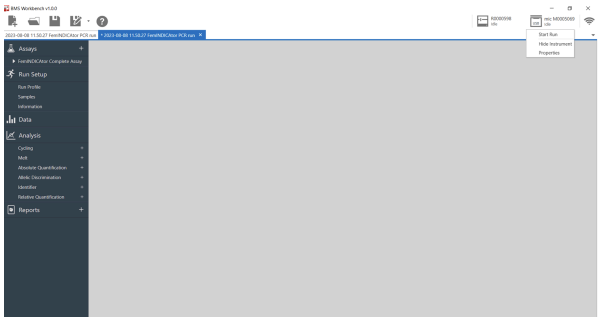


Figure 18: Example Start Run menu

6. A pop-up will appear asking for the reaction volume, which will be auto-filled with the correct volume based on the template chosen. Close the lid and the instrument will start.

   Note: If the cover isn’t closed, the program will not start.

7. Let the Mic run to completion before analyzing the data.

Data Analysis with BioMolecular System MIC

Table 2: Total Yeast and Mold Screening Assay Data Analysis Quick Reference Table - Biomolecular Systems Mic

PathoSEEK™ Assay	Cq Value	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
Total Yeast & Mold	≤ 40	FAM	No Cq	Presence/Absence
Internal Control (IC)*	≤35	HEX	*Internal cannabis control verifies the presence or absence of cannabis DNA	
Assay Positive Control	≤35	FAM		

1. Look for  next to Identifier under Analysis for the Complete Assay Analysis if it has been automatically generated. If the Complete Assay analysis is not automatically generated, click the **"+"** icon next to the **Identifier** tab and select the appropriate **Complete Assay** to display the software-generated presence/absence results.

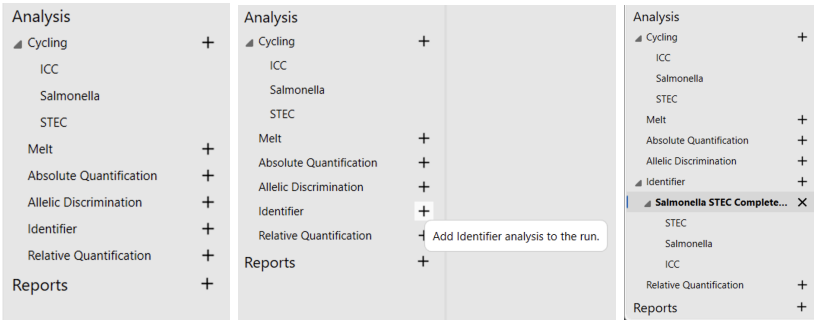


Figure 19: Add Identifier Example

2. To review a preliminary automatic report select the relevant "Complete Assay". This feature is intended to call the samples tested as detect, non-detect, or inconclusive based on the qPCR data. These results will be displayed on the right side of the screen, see screenshot below. Various factors may affect the validity of this report and further analysis is required as outlined in the following steps.

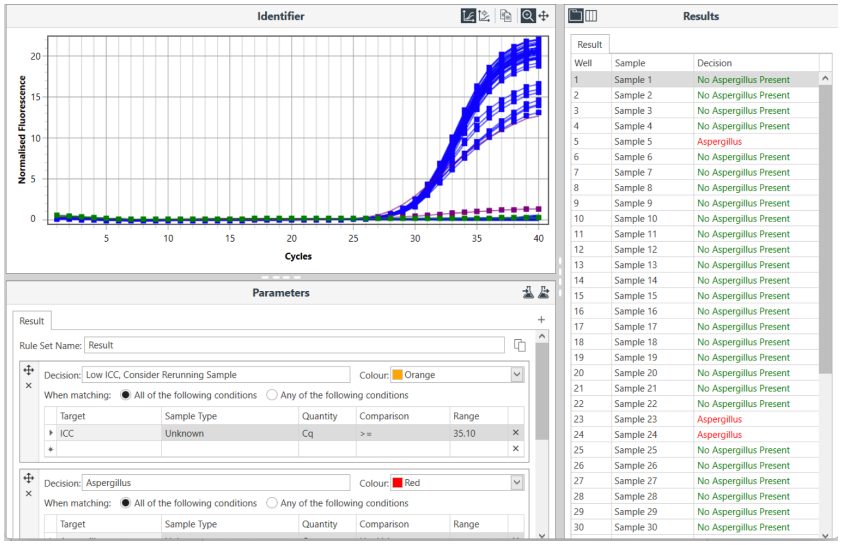


Figure 20: Example Automatic Report

3. If the automatic calls are blank, and there is amplification on all of the channels, this indicates that one of the cycles is out of threshold or the fluorescent cutoff percentage is set incorrectly. To address this, review each target under the Identifier tab.

a. In the event that the target display lacks the requisite gray horizontal threshold indicator, the user should navigate to the "Parameters" section to adjust the *Ignore Cycles Before* or *Fluorescence Cut Off* settings.

i. If the raw data shows an early rise at the beginning of the run followed by signal decay, increase or decrease the *Ignore Cycles Before* value one click at a time until a gray bar appears on the graph. End users may ignore up to 15 cycles.

ii. If a weak signal overall is observed, the *Fluorescence Cutoff* percentage should be increased to a maximum of 16% or a minimum of 0%. These adjustments may have to be made with none, one, or all of the targets.

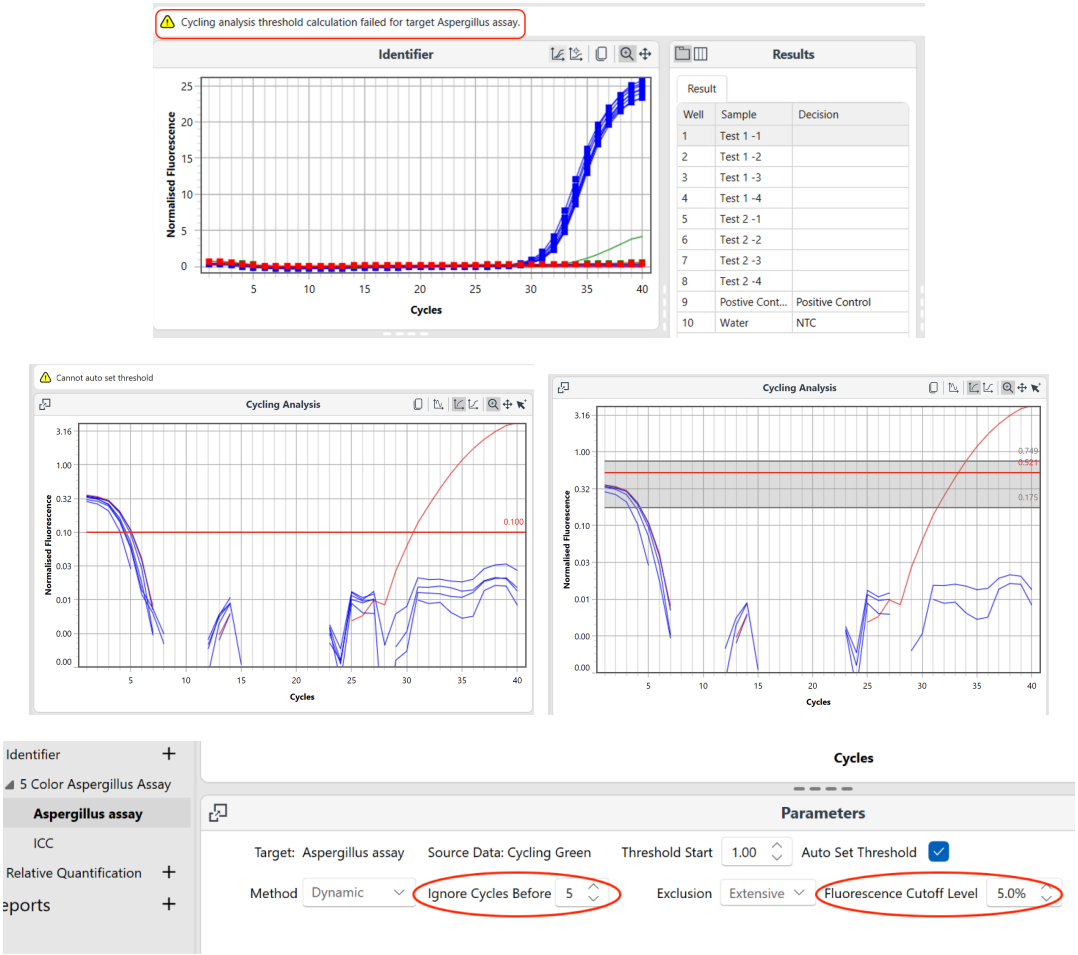


Figure 21: Example of failure to automatically set threshold

4. Conduct a manual review of the amplification curves for each sample.

a. Click the  in the top right side of the screen next to "Samples" and select Hide All.

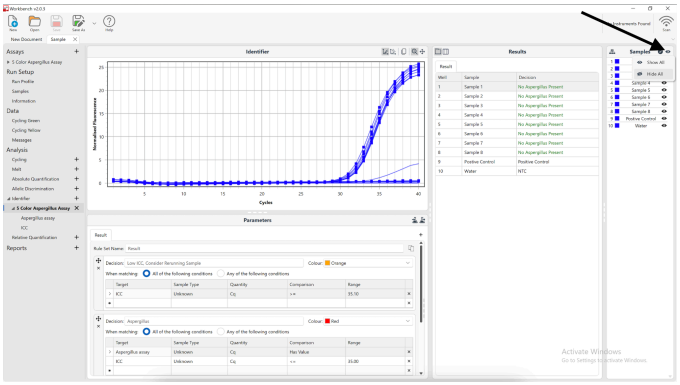


Figure 22: Example of software use

b. Next, unhide each sample, one at a time and review the curves for the Internal Control and microbial targets. Before reviewing the next sample, hide the current sample being reviewed.

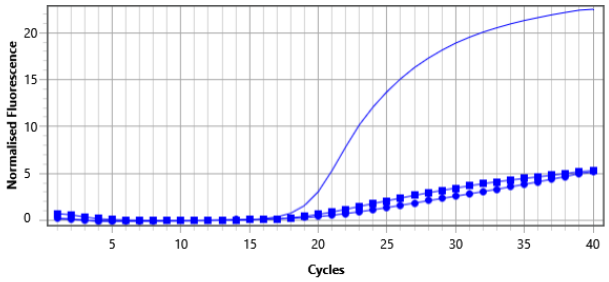


Figure 23: Example of individual sample

c. Individual targets may also be reviewed for each sample by selecting, “Display results for each target on a separate chart.”

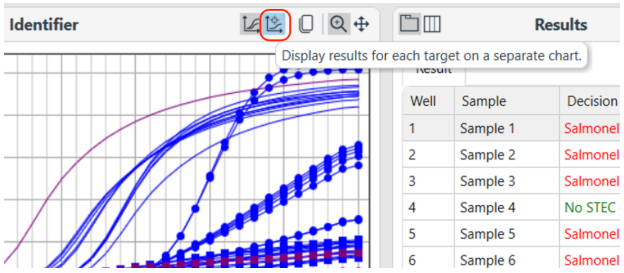


Figure 24: Example of software use

d. Confirm that the Automatic Report results and the raw amplification curves agree.

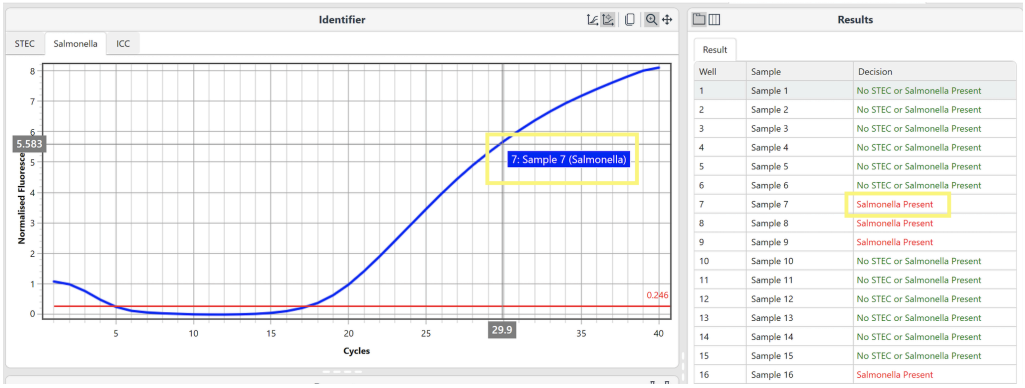


Figure 25: Example of report confirmation

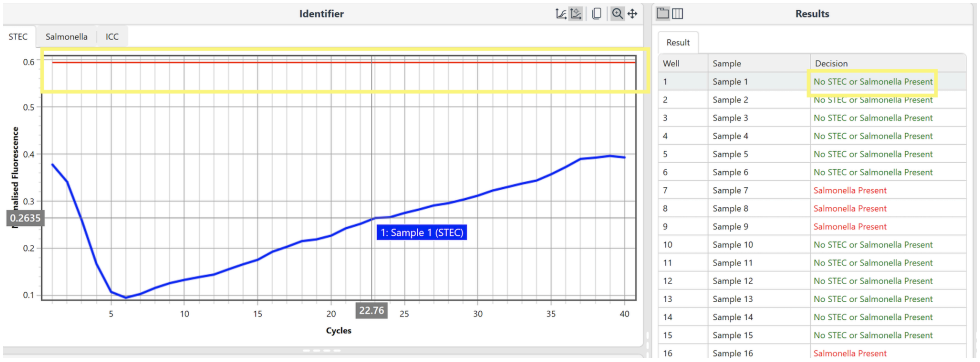


Figure 26: Example of report confirmation

e. Confirm that the Cq value generated for each target aligns with where the amplification curve crosses the threshold:

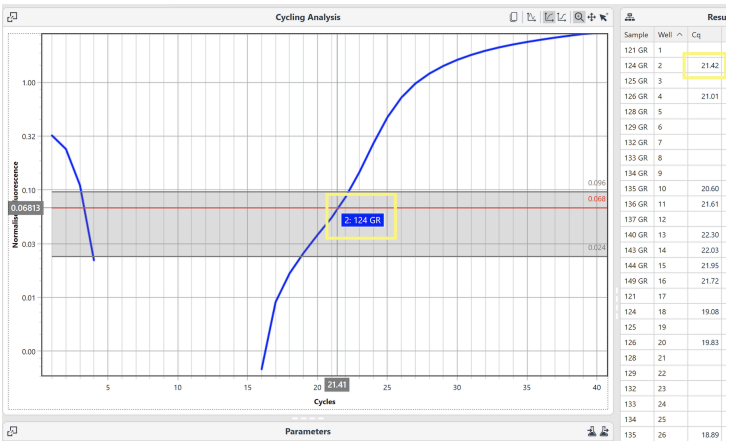


Figure 27: Example of Cq value confirmation

f. If an amplification curve is present but no Cq value is generated, the *Fluorescence Cutoff* feature or *Ignore Cycles Before* feature may require adjustment.

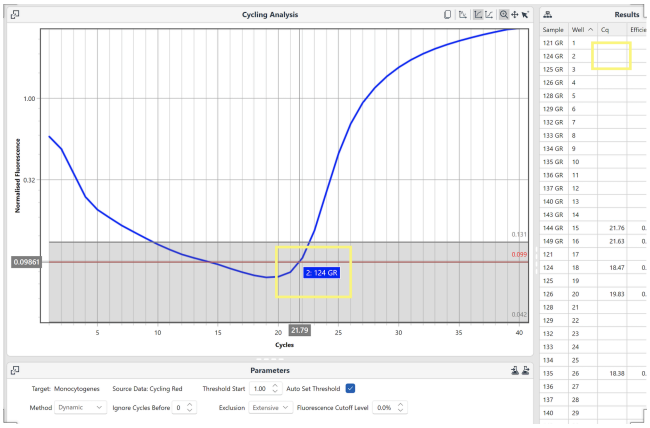


Figure 28: Example of a curve requiring adjustment of *Ignore Cycles Before* or *Fluorescence Cutoff*

g. If adjusting the *Fluorescence Cutoff* or *Ignore Cycles Before* does not allow the automatic calls to be made by the software, the already extracted DNA may be re-run on qPCR or the results may be reported manually after conducting a review of the amplification curves.

5. Controls
    a. Assay-specific Positive Control on the FAM fluorophore has a Cq value ≤ 35.
        i. Visually confirm with the curve on the graph.
    b. Assay-specific Negative Control on the FAM fluorophore has no Cq value.
        i. Visually confirm with the curve on the graph.
6. Unknown Total Yeast and Mold Targets
    a. Total Yeast and Mold: FAM
        i. A “presence” or failing result for the unknown Total Yeast and Mold.
            1. Any Cq value for the FAM fluorophore ≤ 40.
            2. Visually confirm with the curve on the graph.
            3. To confirm organism viability and/or determine viable organism concentration, an appropriate culture-based enumeration method may be used. Dichloran Rose Bengal Chloramphenicol (DRBC) agar is recommended for the recovery and enumeration of viable yeast and mold; however, laboratories may use an alternative validated plating method, as appropriate.

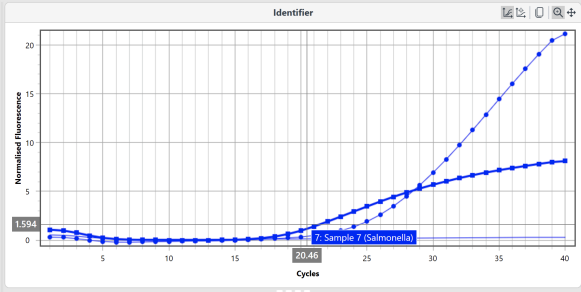


Figure 29: Example of a presence result

ii. An "absence" or passing result for the unknown targets

1. Internal Control (IC), on the HEX Fluorophore, has a Cq value ≤ 35
2. If the HEX Cq value is > 35, further dilute the lysed sample with an additional 1:2 or 1:10 dilution with nuclease-free water and rerun the qPCR.
3. No Cq value for the FAM fluorophore.
4. Visually confirm that there is no curve on the graph.

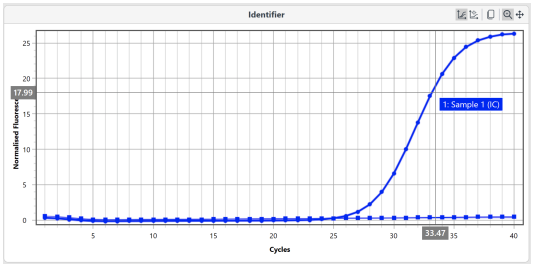


Figure 30: Example of an absence result

iii. Below is an example of acceptable amplification curves. The curves cross the threshold (horizontal red line) consistently within 40 cycles and show clear amplification consistent with typical qPCR curve shapes. The baseline remains flat until amplification occurs and a Cq is generated when the amplification curve crosses the threshold. These curves represent a positive result for their respective target.

1. Logarithmic view

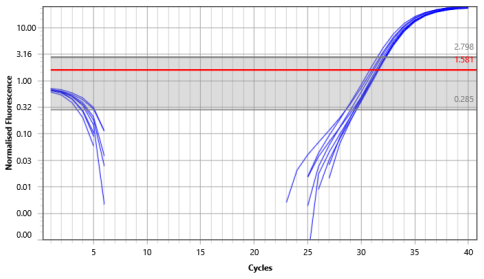


Figure 31: Example logarithmic view

2. Linear view:

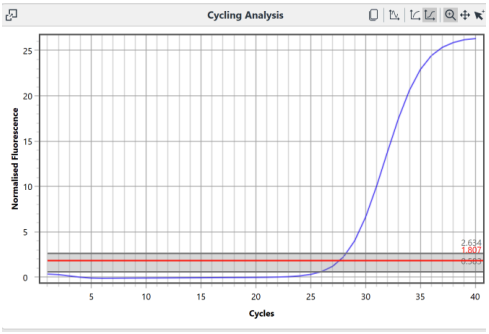


Figure 32: Example linear view

iv. Below are examples where no amplification has occurred. The curves remain generally flat and do not cross the threshold or exceed the initial background fluorescence of qPCR. No Cq value is generated. The curves below represent a non-detect result for their respective target.

1. Logarithmic view

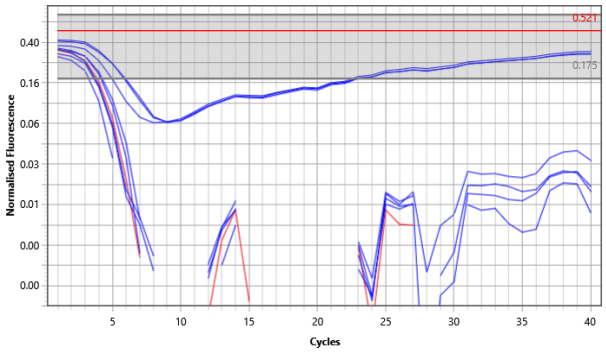


Figure 33: Example logarithmic view

2. Linear View

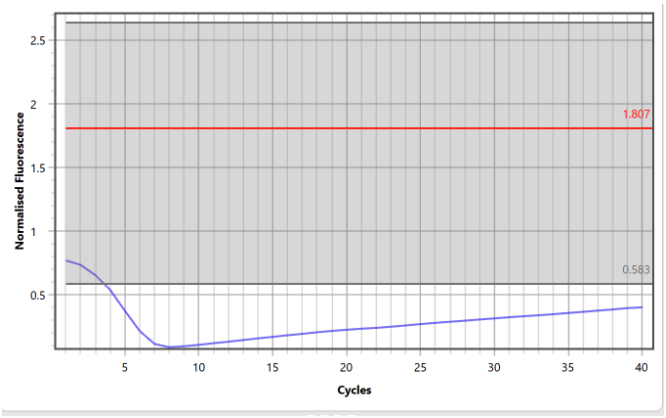


Figure 34: Example linear view

7. Raw amplification curves should be reviewed to ensure consistency with the results generated by the Identifier and that the curves are as expected. In the Data tab select Cycling Green (FAM) or Cycling Yellow (HEX) then Hide and Un-Hide samples for review. Raw data curves can be useful in determining if the "Ignore Cycles Before" or "Fluorescence Cutoff" parameters require adjusting in the "Identifier" section and whether or not amplification has occurred.

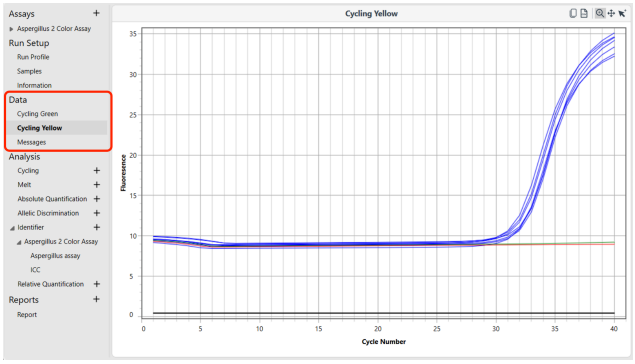


Figure 35: Example of Cycling Data

8. Data may be exported by selecting the "Report" tab, then clicking the Export icon.

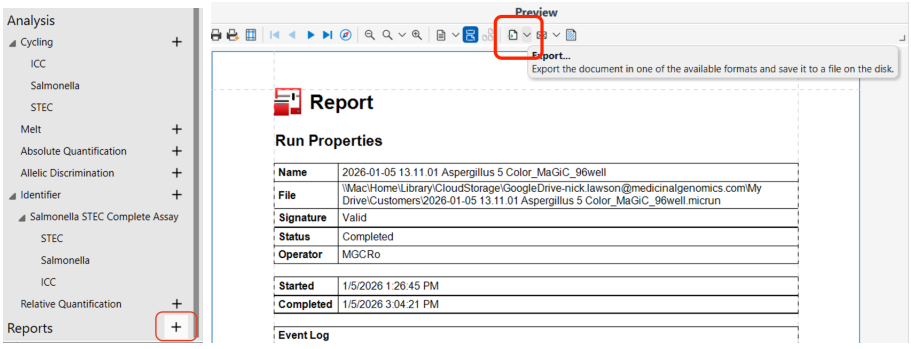


Figure 36: Example of report generation

Real-Time Quantitative PCR (qPCR) Setup Protocol for the AriaMX, Bio-Rad CFX Opus or CFX96

1. Remove PathoSEEK Total Yeast & Mold Screening Assay, PathoSEEK Amplification Mix, and Total Yeast & Mold Positive Control from the -20 ºC freezer.
    a. If lyophilized Amplification Mix has not been previously rehydrated, use the supplied Nuclease-Free Water to rehydrate with the volume specified on the side of the vial. Invert the bottle 5-10 times, vortex, or pipette mix to ensure complete resuspension.
        i. Following reconstitution, store the Amplification Mix at -15 to -20 °C when not in use. For improved access and storage stability, transfer the reconstituted Amplification Mix to a labeled 1.5 mL or 2.0 mL microcentrifuge tube and record the rehydration date on the tube label.
    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. PathoSEEK Total Yeast & Mold Screening Assay – vortex tube quickly followed by a pulse spin down in a minicentrifuge.
    b. Total Yeast and Mold Positive Control tube – 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 Master Mix in a 1.5 mL tube (the probe mix also contains the probe for the IC). Label the tube as “Master Mix”. See Table 3 (PathoSEEK Amplification Master Mix Reagent Volumes). 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, prepare enough Master Mix for 13 or 14 reactions, which would account for 1 or 2 excess.

Table 3: PathoSEEK Amplification Master Mix Reagent Volumes

Reagent	Volume for 1 qPCR Reaction
PathoSEEK Amplification Mix	10 µL
Total Yeast & Mold Screening Assay	1 µL
Nuclease-Free Water	4 µL
Total	15 µL

4. 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.
5. For the negative control, use nuclease-free water that was used to rehydrate the Amplification Mix.
6. Positive Control Preparation (1:10,000 Dilution)
    a. Ensure the Total Yeast and Mold Positive Control is completely thawed. Vortex thoroughly, then briefly centrifuge before use.
    b. Prepare a 1:100 dilution:
        i. Add 198 µL of nuclease-free water to a microcentrifuge tube.
        ii. Add 2 µL of stock Positive Control.
        iii. Vortex thoroughly, then briefly centrifuge.
    c. Prepare the final 1:10,000 dilution by making a second 1:100 dilution:
        i. Add 198 µL of nuclease-free water to a new microcentrifuge tube.
        ii. Add 2 µL of the 1:100 Positive Control prepared in Step 2.
        iii. Vortex thoroughly, then briefly centrifuge.

    The resulting solution is the **1:10,000 Positive Control** and should be used for the qPCR run.

7. For qPCR reactions use a 96-well optical qPCR plate or optically clear qPCR tubes.
8. Transfer lysed samples into qPCR plate wells or tubes.
    a. Carefully remove the seal from the Stabilized Lysis plate.

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

b. Pipette transfer 5 µL of each sample lysate into the corresponding qPCR tube or well on the qPCR plate **excluding chocolate**.
c. Dilute each sample lysate 1:10 with nuclease free water **excluding chocolate**.
d. Pipette transfer 5 µL of each 1:10 diluted sample lysate into the corresponding qPCR tube or well on the qPCR plate **excluding chocolate**.
e. Dilute each **chocolate sample lysate** 1:100 with nuclease free water.
f. Pipette transfer 5 µL of each 1:100 diluted **chocolate sample lysate** to the corresponding qPCR tube or well on the qPCR plate.
g. Add 5 µL of the diluted Positive Control to the corresponding positive control plate well or tube.
h. Add 5 µL of nuclease-free water to the corresponding negative control plate well or tube.

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

9. 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 pipette 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 Master Mix to each well.
10. Seal the plate with strip caps or an adhesive seal, or seal qPCR tubes with strip caps.
11. For the Agilent AriaMX or Bio-Rad CFX96 or Opus 96, 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.
12. For the Agilent AriaMX: 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.
13. Place the sealed plate or tubes onto the PCR instrument.
14. Follow the software specific instructions for each qPCR platform to initiate the run.

Running the Agilent AriaMX

The following species will be detected on the following Fluorophores:

Target	Optical Channel
Total Yeast & Mold	FAM
Internal Control	HEX

1. Create a New Experiment on the Agilent qPCR instrument.
    a. Select "Quantitative PCR: Fluorescence Probe" from Experiment Types.

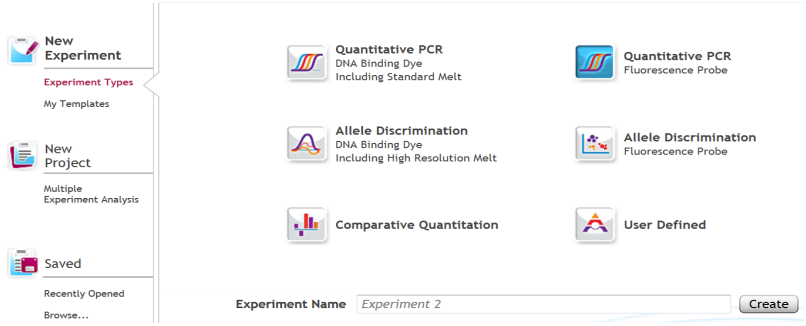


Figure 37: Example new experiment

    b. Under Setup > Plate Setup, highlight **only** wells that contain reactions and select necessary fluorophores:
        i. PathoSEEK Total Yeast & Mold Screening Assay: FAM and HEX under **Add Dyes**. Having fluorescence detection turned on in empty wells can affect the calculations which the software makes to obtain the corrected data, ΔR log.

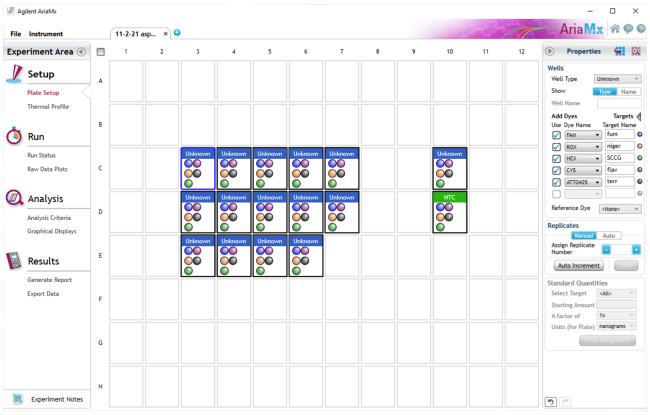


Figure 38: Example Plate Setup

2. Change the well types to reflect the plate set up. All wells should be set to Unknown except the negative control can be set to NTC well type. The positive control can be set to Unknown or Standard well type. Add Target names to the dyes under Targets:
    a. PathoSEEK Total Yeast & Mold Screening Assay
        i. FAM : Total Yeast & Mold
        ii. HEX : Internal Control
3. Under Setup > Thermal Profile, create the following PCR thermal profile
    a. Hot start at 95ºC for 5 minutes, followed by 40 cycles of 95ºC for 15 seconds and 65ºC for 90 seconds.

    Note: A compression pad should be placed on top of the plate with an adhesive seal to prevent cross contamination.

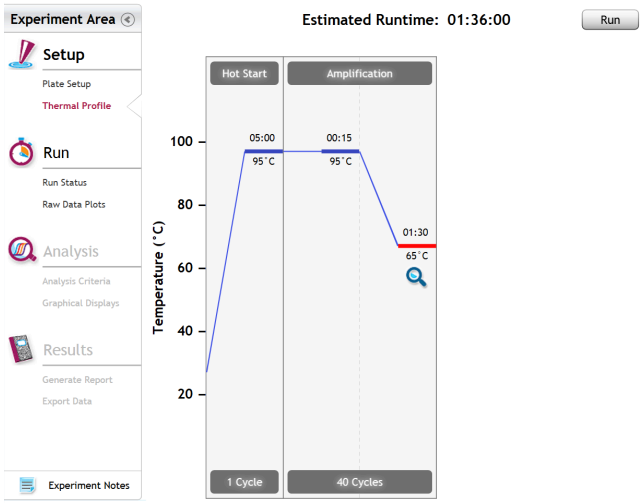


Figure 39: Thermal Profile

4. Close the lid and click “Start Run”.
5. Save the experiment with the [User] and [date].
6. When the qPCR run is complete, immediately dispose of the plate. Do not open the plate seal after the run to avoid contamination in the lab.

Data Analysis: Agilent AriaMX

Table 4: Total Yeast and Mold Screening Assay Data Analysis Quick Reference Table - AriaMX

PathoSEEK™ Assay	Cq Value	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
Total Yeast & Mold	≤ 40	FAM	No Cq	Presence/Absence
Internal Control (IC)*	≤35	HEX	*Internal cannabis control verifies the presence or absence of cannabis DNA	
Assay Positive Control	≤35	FAM		

1. Open the Data Analysis window when the run is complete.
2. Highlight the wells of interest in the Analysis Criteria under Analysis, then select Graphical Displays
    a. Amplification plots will be available for viewing
    b. The Cq values will appear to the right in the table
    c. Right click inside the graph, select Edit Legend under Legend Options

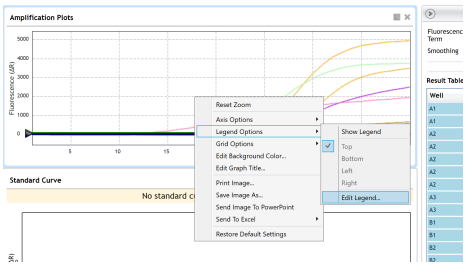


Figure 40: Example of software navigation

    d. Change “All” to “Dye”

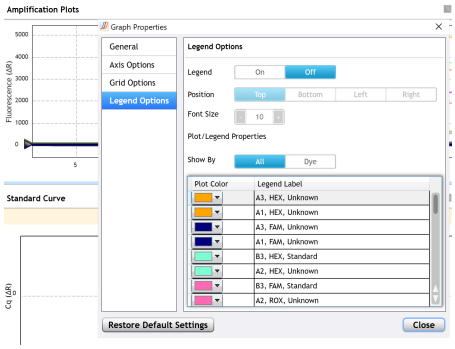


Figure 41: Example of software navigation

e. All user settings for Plot/Legend Properties will be removed. Do you want to proceed? Select “Yes”.

f. This will assign a single color to each fluorophore.

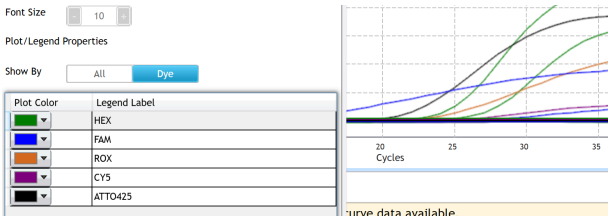


Figure 42: Example of software navigation

3. To analyze the results

   a. Start by turning the graph to Log Scale with a right click on the chart, select Axis options, enable y-axis log scale. Expand the amplification plots settings by clicking on the triangle.

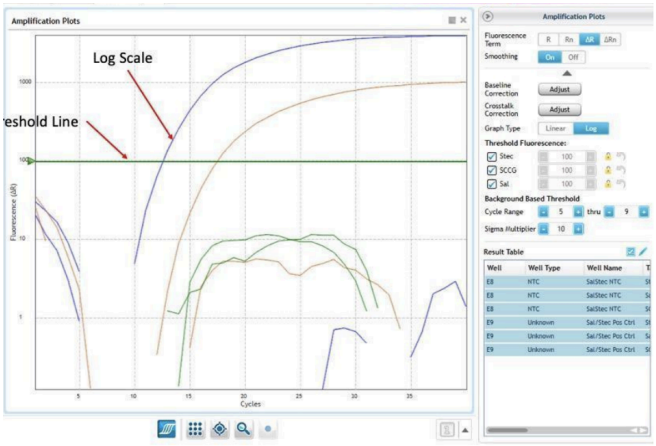


Figure 43: Example amplification curves

4. Manually adjust thresholds to 100 RFU

   a. PathoSEEK Total Yeast & Mold Screening Assay - adjust and lock thresholds to 100 RFU for the FAM and HEX fluorophores. (Target names will have previously been identified.)

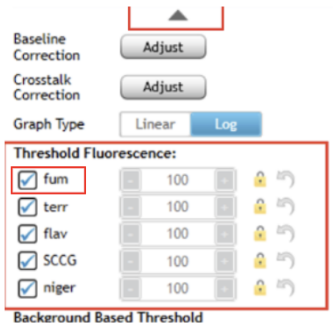


Figure 44: Example of setting the thresholds

5. Controls
    a. Assay-specific Positive Control on the FAM fluorophore has a Cq value ≤ 35.
        i. Visually confirm with the curve on the graph.
    b. Assay-specific Negative Control on the FAM fluorophore has no Cq value.
        i. Visually confirm with the curve on the graph.
6. Unknown Yeast and Mold Targets
    a. A "presence" or failing result for the unknown Total Yeast and Mold target.
        i. Any Cq value for the FAM Fluorophore ≤ 40.
        ii. To confirm organism viability and/or determine viable organism concentration, an appropriate culture-based enumeration method may be used. Dichloran Rose Bengal Chloramphenicol (DRBC) agar is recommended for the recovery and enumeration of viable yeast and mold; however, laboratories may use an alternative validated plating method, as appropriate.

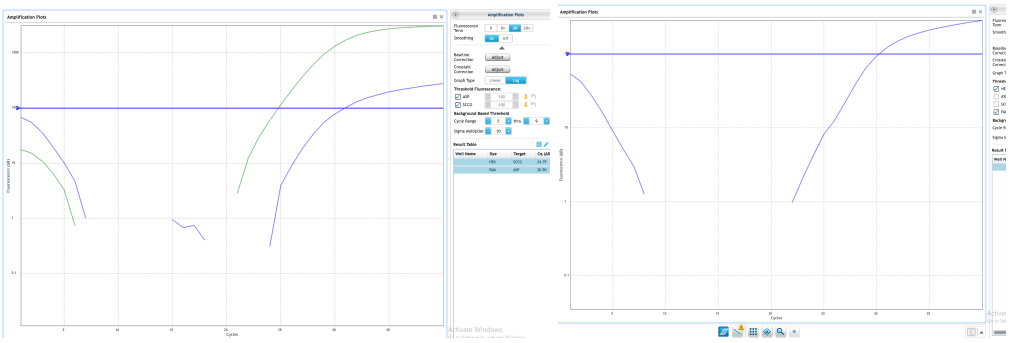


Figure 45: Examples of a presence result

1. Visually confirm with the curve on the graph. It is very important to check the raw data view (R) to confirm with the amplification curve when a presence result occurs.
    a. Review the raw data for each sample: Select the wells of interest, use the plate view of the Raw Data Plots to scroll across the plate to confirm amplification in sample wells and positive control wells. This review also ensures that thermal cycling is uniform across the plate.

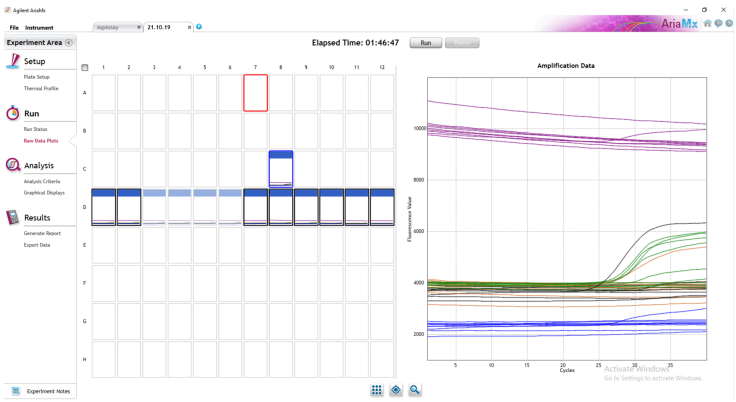


Figure 46: Raw data (R) plots for which the cycle quantification (Cq) will be generated.

    b. Look at the raw data in the "Graphical Displays" view. For every dye that gives a Cq make sure the baseline is flat and the fluorescence signal grows rapidly over 5-7 cycles as seen below. (To view the raw data, select "R" next to "Fluorescence Term" at the top right-hand side of the screen).

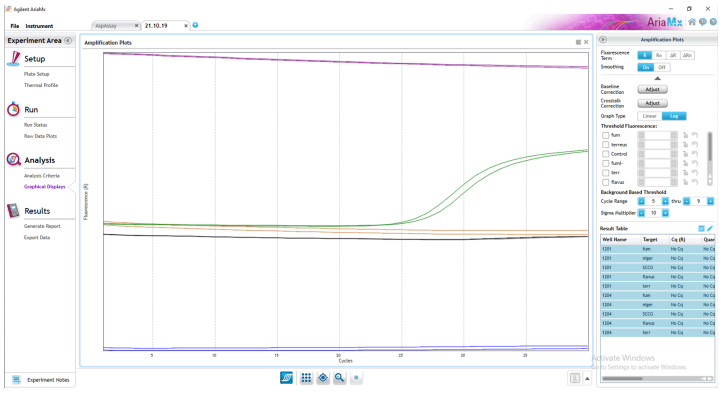


Figure 47: Example of good baseline with sigmoidal shaped curve increase in the internal control fluorescence.

c. Check the amplification plots for a semi-logarithmic curve with two distinct phases that crosses the threshold:
    i. Select the ΔR Fluorescence Term and look at the shape of the amplification plot. The graph should have the classic semi-logarithmic shape with two visually distinct phases.
d. Phase 1 shows exponential growth in the fluorescence, in a span of around 5 cycles.
e. Phase 2 is a plateau where the amplification signal growth ends but remains level.

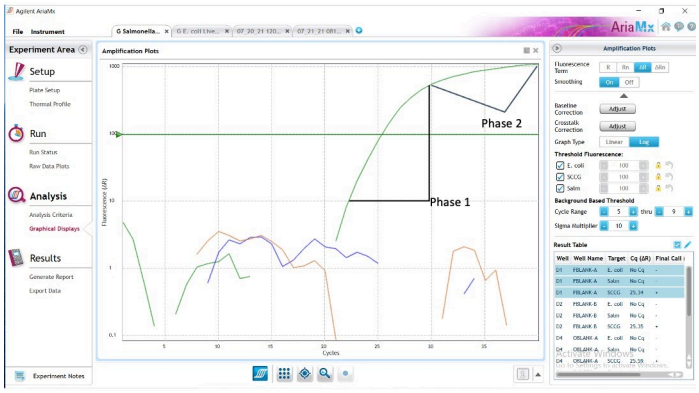


Figure 48: Example of a typical amplification curve

b. An "absence" or passing result for the samples.
    i. Internal Control (IC), on the HEX fluorophore, has a Cq value ≤ 35 for all sample types.
        1. Visually confirm with the curve on the graph.
        2. If the HEX Cq value is > 35, further dilute the lysed sample with an additional 1:2 or 1:10 dilution with nuclease-free water and rerun the qPCR.
    ii. Total Yeast and Mold (TYM) on the FAM fluorophore has No Cq value.
        1. Visually confirm that there is no curve on the graph.

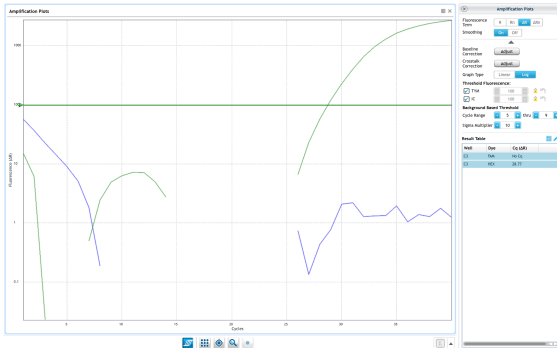


Figure 49: Example of an absence result

Running the BioRad CFX96

The following targets will be detected on the following fluorophores:

Target	Optical Channel
Total Yeast & Mold	FAM
Internal Control	HEX

1. Start the qPCR Cycling program
    a. Select User-Defined in the Startup Wizard under Run setup.

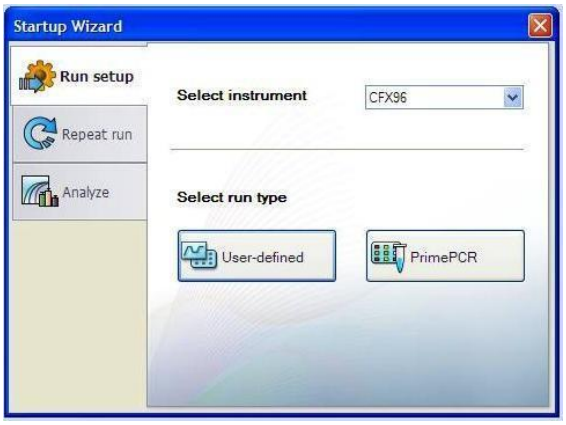


Figure 50: Startup Wizard

2. Use the Express Load dropdown menu to pick the qPCR Multiplex Detection Program and click "Next".
    a. If not already pre-programmed, create a cycling program with the following specifications and save as "qPCR Multiplex Detection".

b. Hot start at 95 ºC for 5 minutes, followed by 40 cycles of 95 ºC for 15 seconds and 65 ºC for 90 seconds.

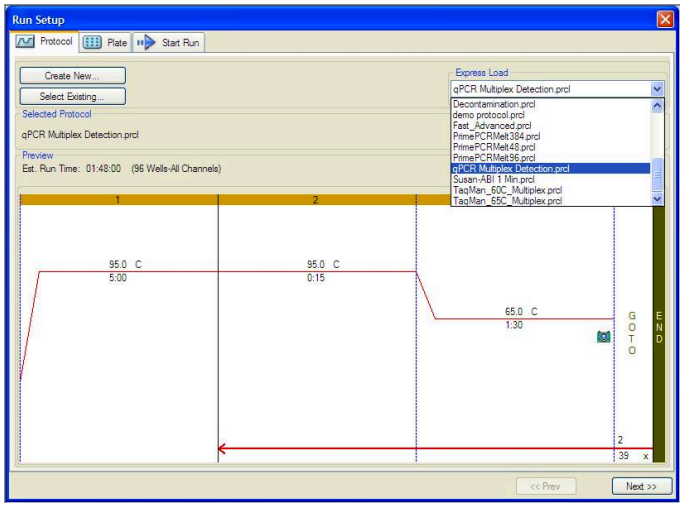


Figure 51: Thermal Profile

3. Design the plate under the plate tab in the Run Setup.
   a. Select the qPCR Multiplex Detection from the dropdown menu. If not already present, click “Create New”.

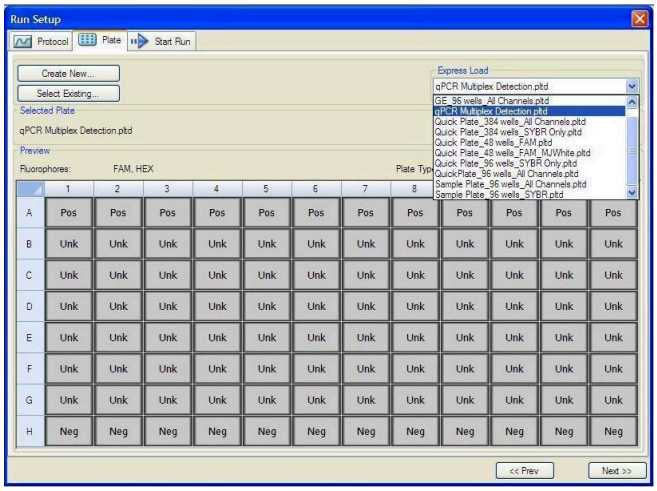


Figure 52: Example Plate Tab

   b. The plate editor window will appear.
      i. PathoSEEK Total Yeast & Mold Screening Assay - Choose FAM and HEX Fluorophores and click “OK”.

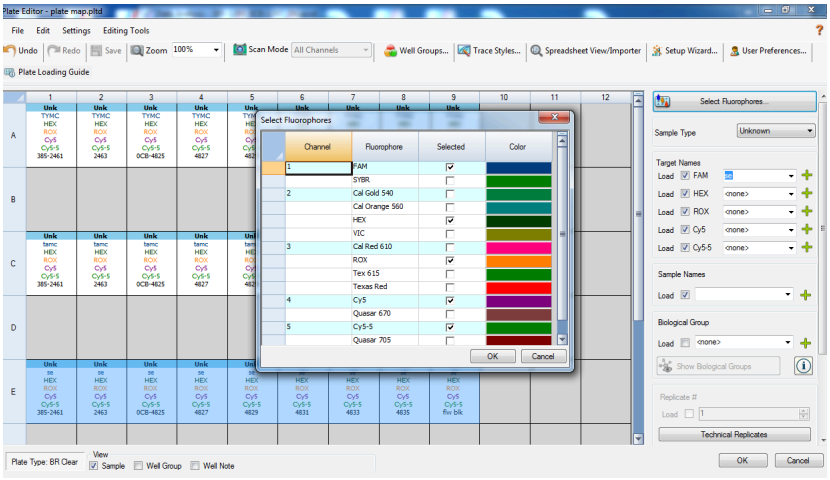


Figure 53: Example Plate Editor window

c. If the plate layout was previously saved, click "Edit Selected" to move to the Plate Editor Screen.

d. On the Plate Editor Screen, change the Sample Type to correlate with the specific plate setup.

NOTE: To select the Sample Type, highlight the wells you would like to define, then choose from the dropdown menu one of three types:

Unknown

Positive Control

Negative Control

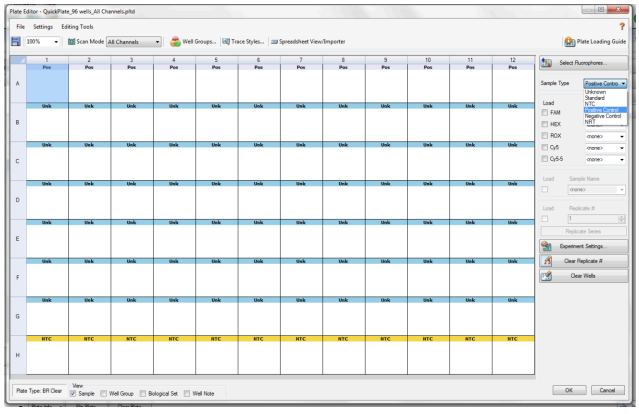


Figure 54: Sample type selection

e. Make sure All Channels is selected from the dropdown menu at the top.

f. Attach the fluorophores to the wells being used.

i. Highlight all the wells being used.

1. For PathoSEEK Total Yeast & Mold Screening Assay, Highlight the well locations and click on FAM and HEX.

g. When the plate is designed correctly, click OK.

4. Click “Yes” to save the plate. If creating plate layout for the first time, save as “qPCR Multiplex Detection”. If you do not save the plate, it will return to the default plate.

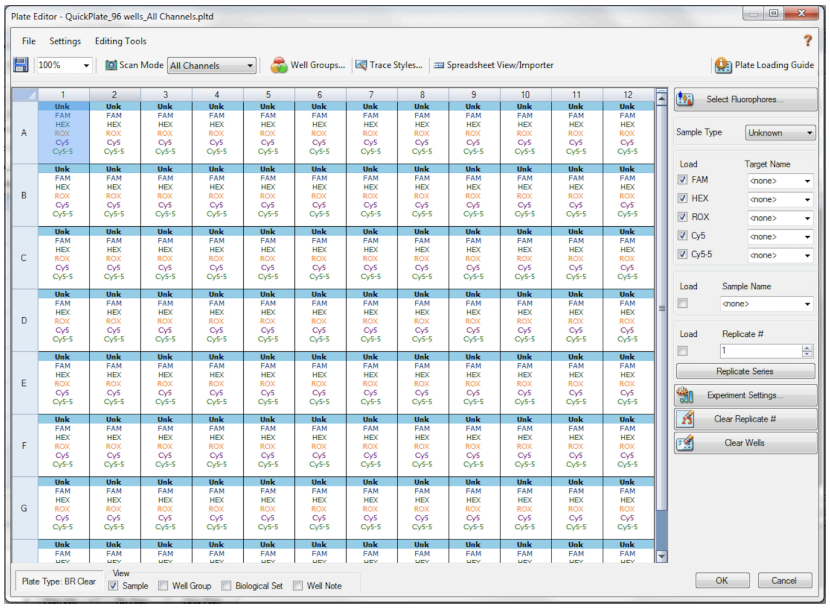


Figure 55: Plate Template

Note: Saving will override the template which is acceptable.

5. Close the lid and click Start Run.
6. Save the experiment with the [User] and [date].
7. When the run is complete, immediately dispose of the plate after qPCR. Do not remove the plate seal after the run to avoid contamination in the lab.

Data Analysis Bio-Rad CFX96 Opus or CFX96

Table 5: PathoSEEK® Total Yeast and Mold Screening Assay Data Analysis Quick Reference Table - Bio-Rad CFX96 or CFX96 Opus

PathoSEEK™ Assay	Cq Value	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
Total Yeast and Mold	≤ 40	FAM	No Cq	Presence/Absence
Internal Control (IC)*	≤35	HEX	*Internal cannabis control verifies the presence or absence of cannabis DNA	
Assay Positive Control	≤35	FAM		

1. The Data Analysis window will open automatically when the run is complete.
2. Highlight the wells of interest.
    a. The graph will appear above.
    b. The Cq values will appear to the right.
3. To analyze the results
    a. Start by turning the graph to Log Scale and manually moving the threshold to 10^2 for all fluorophores.
        i. To turn the graph to Log Scale, click on the box at the bottom right of the graph.
        ii. To adjust the threshold, click on the horizontal lines, and move them to the specified value mentioned above on the y-axis.
        iii. Alternatively, set an exact threshold value by selecting a single fluorophore at a time beneath the graph, then Settings > Baseline Threshold. In the next window select User Defined and enter 100.

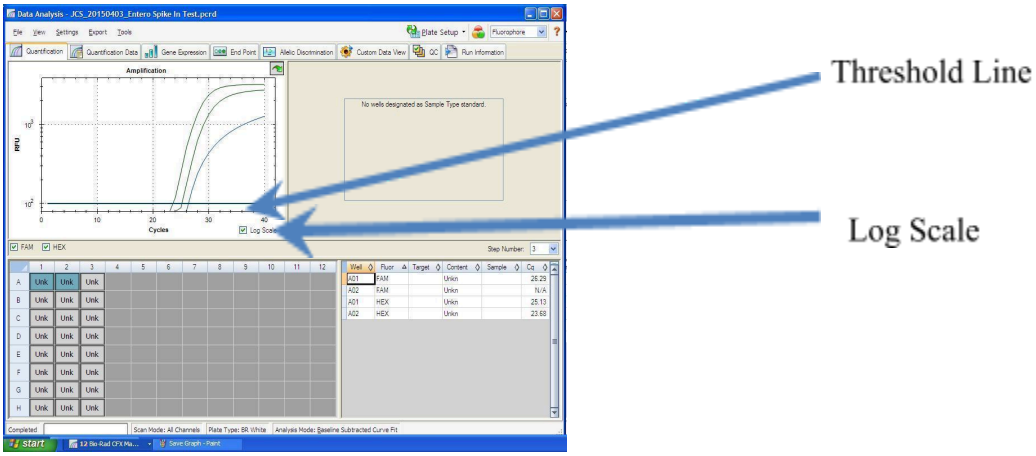


Figure 56: Example of threshold setting

4. Controls
    a. Assay-specific Positive Control on the FAM fluorophore has a Cq value ≤ 35.
        i. Visually confirm with the curve on the graph.
    b. Assay-specific Negative Control on the FAM fluorophore has no Cq value.
        i. Visually confirm with the curve on the graph.
5. Unknown Total Yeast and Mold target: The following fluorophore detects Total Yeast and Mold:
    a. Total Yeast and Mold: FAM
        i. A "presence" or failing result for the Total Yeast and Mold target.
            1. Any Cq value for the FAM fluorophore ≤ 40
            2. Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a presence result occurred. Sometimes the background amplification will give a false positive reading.
        ii. To confirm organism viability and/or determine viable organism concentration, an appropriate culture-based enumeration method may be used. Dichloran Rose Bengal Chloramphenicol (DRBC) agar is recommended for the recovery and enumeration of viable yeast and mold; however, laboratories may use an alternative validated plating method, as appropriate.

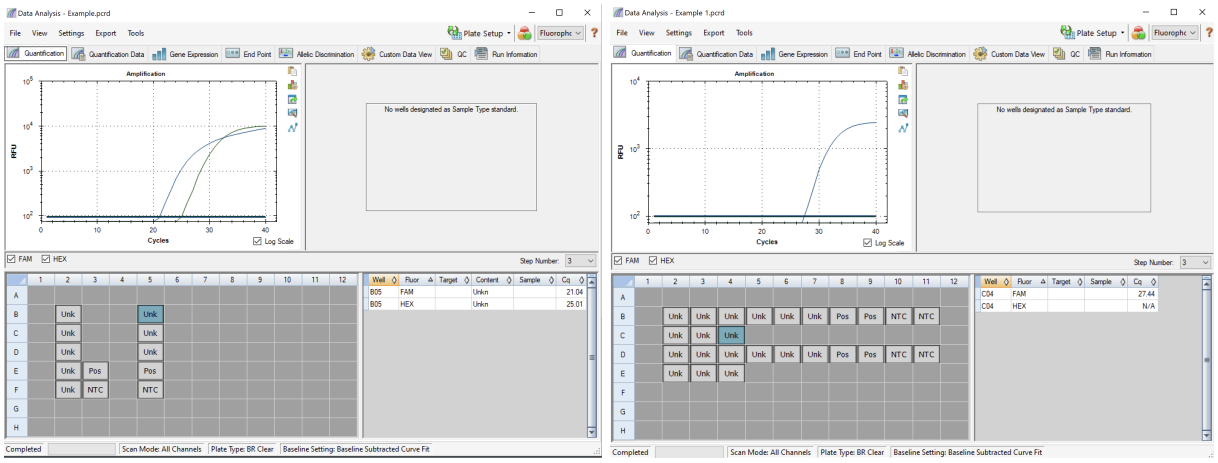


Figure 57: Examples of a presence result.

iii. An “absence” or passing result for unknown Total Yeast and Mold target.

1. Internal Control (IC), on the HEX fluorophore, has a Cq value ≤ 35 for all sample types.
2. If the HEX Cq value is > 35, further dilute the lysed sample with an additional 1:2 or 1:10 dilution with nuclease-free water and rerun the qPCR.
3. No Cq value for the FAM fluorophore.
4. Visually confirm with the curve on the graph.

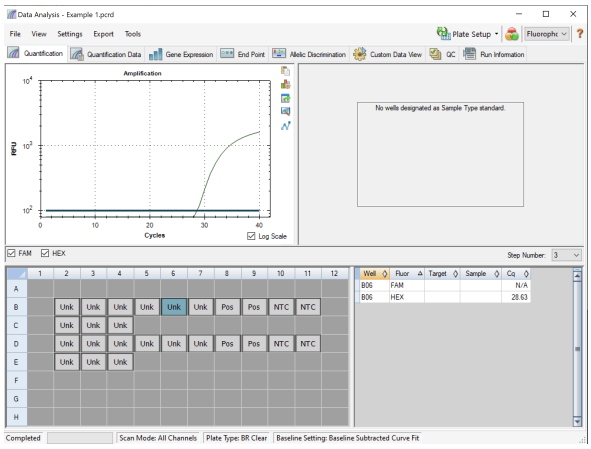


Figure 58: Example of an absence result.

Viability and Enumeration Testing

To confirm organism viability and/or determine viable organism concentration, an appropriate culture-based enumeration method may be used. Dichloran Rose Bengal Chloramphenicol (DRBC) agar is recommended for the recovery and enumeration of viable yeast and mold; however, laboratories may use an alternative validated plating method, as appropriate.

Troubleshooting Guide

Symptom	Reason	Solution
Internal control failure	IC not spiked into sample/MaGiC lysis when applicable	Repeat MaGiC Lysis and PathoSEEK by following the protocol.
	Sample not added	Repeat MaGiC Lysis and PathoSEEK by following the protocol.
	Mix up in Reaction Setup	Repeat the qPCR by following the protocol.
	Missing Fluorophore on plate set up	Check plate setup in qPCR platform data file and ensure the correct fluorophores were chosen for the assay being run.
	Optional IC spike not performed for flower samples for Aspergillus Assays	The IC spike into flower is not mandatory when running the Aspergillus MaGiC lysis process. However if samples are low quality, it may be necessary.
	qPCR inhibition	Dilute MaGiC Lysates 1:2 or 1:10 with nuclease free water and re-perform qPCR. If dilution is not successful, repeat MaGiC Lysis.
Amplification of the Internal control is not expected in the assay positive or negative control wells. No Cq or a Cq of more than 35 is acceptable. Any Cq lower than 35 constitutes a rerun	Cannabis DNA or IC contamination in a reagent	Troubleshoot which reagent was contaminated; use new reagents, thoroughly clean all pipettes and bench areas with 10% bleach solution.
	qPCR bench too close to MaGiC lysis area	Designate separate benches, pipettes etc. for lysis and qPCR setup.
Positive Negative Control	Small Cq value <15	Visually confirm that there is a true amplification curve. If not, this is a low level background and can be ignored.
	Contamination	Repeat the qPCR by following the protocol.
	Insufficient pre-setup bleaching	Wipe down the lab workspace and all equipment with 10% Bleach. Repeat qPCR.
Negative Positive Control	Mix up in Reaction Setup	Ensure correct well location was chosen for assay positive control. If yes, repeat the qPCR by following the protocol.
Background Amplification	Unclear	This is usually seen with a very low Cq reading (<15), the curve is usually missing the exponential growth phase, but rather a gradual increase of fluorescence signal. This is usually a negative result, but should be repeated.

Glossary and Definitions

Deoxyribonucleic acid (DNA) is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms.

Polymerase Chain Reaction (PCR) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

A **fluorophore** is a fluorescent chemical compound that can re-emit light upon light excitation.

The **Negative Controls** are the reactions where no Cq is expected. It helps to ensure that all assay-specific reactions are clean of contaminants.

The assay-specific **Positive Controls** are the reactions where a Cq is expected. It helps ensure that all Assay-specific reactions are working correctly. The Assay specific Positive Control is targeting the pathogen using the FAM, ROX and Cy5 Fluorophores.

Amplification of the **Internal Control** or the microbial target of interest is expected in every reaction containing DNA isolated from a cannabis sample. It ensures the DNA isolation procedure was successful or the presence of microbial contamination. The internal control targets the cannabis genome, using the HEX Fluorophore.

MIP is short for Marijuana Infused Product. A MIP is cannabis plant material or concentrate mixed into a consumable.

Revision History

Version	Date	Description
v1	September 2026	New Document - Standalone User Guide for PathoSEEK Total Yeast & Mold Screening 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.

Results may vary based on laboratory conditions. Altitude and humidity are among factors known to affect the growth of bacterial and fungal species.

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