Please refer to http://www.medicinalgenomics.com/product-literature/ for updated protocols and Material Safety Data Sheets (MSDS). Consult MSDS before using any new product.

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PathoSEEK™ Analysis Quick Reference Tables:

FLOWER

Table 1: Flower Samples ONLY – No Decontamination Step

PathoSEEK™ Assay	Cq Value (High CFU count)	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
E. coli (with 4+ hour enrichment)	≤ 40	FAM	> 40	100
E. coli (no enrichment)	≤ 33.1	FAM	> 40	100
Salmonella	≤ 40	ROX	> 40	Presence/Absence
Total Aerobic Count	≤ 20.7	FAM	> 30	100,000 (10^5)
Total Coliform	≤ 30.5	FAM	> 40	1,000 (10^3)
Total Enterobacteriaceae	≤ 28.1	ROX	> 40	1,000 (10^3)
Total Yeast and Mold	≤ 24.2	FAM	> 40	10,000 (10^4)
Internal Control*	≤35	HEX	*Internal control verifies the presence or absence of plant DNA	
Assay Positive Controls	≤35	FAM/ROX		

NON – FLOWER Matrices

Table 2: All Concentrates, MIP Samples – No Decontamination Step
(Except gummy, see table 3)

PathoSEEK™ Assay	Cq Value (High CFU count)	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
E. coli (with 4+ hour enrichment)	≤ 40	FAM	> 40	100
E. coli (no enrichment)	≤ 37.6	FAM	> 40	100
Salmonella	≤ 40	ROX	> 40	Presence/Absence
Total Aerobic Count	≤ 22.0	FAM	> 35	100,000 (10^5)
Total Coliform	≤ 31.1	FAM	> 40	1000 (10^3)
Total Enterobacteriaceae	≤ 28.5	ROX	> 40	1000 (10^3)
Total Yeast and Mold	≤ 31.6	FAM	> 40	10,000 (10^4)
Internal Control*	≤40	HEX	*Internal control verifies the presence or absence of spiked plant positive control (SCCG)	
Assay Positive Controls	≤35	FAM/ROX		

GUMMY

Table 3: Gummy – No Decontamination Step

PathoSEEK™ Assay	Cq Value (High CFU count)	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
E. coli (with 4+ hour enrichment)	≤ 40	FAM	> 40	100
Salmonella	≤ 40	ROX	> 40	Presence/Absence
Total Aerobic Count	≤ 25.5	FAM	> 35	100,000 (10^5)
Total Coliform	≤ 35.0	FAM	>40	1000 (10^3)
Total Enterobacteriaceae	≤ 29.9	ROX	> 40	1000 (10^3)
Total Yeast and Mold	≤ 33.1	FAM	> 40	10,000 (10^4)
Internal Control*	≤40	HEX	*Internal control verifies the presence or absence of spiked plant positive control (SCCG)	
Assay Positive Controls	≤35	FAM/ROX		

Detailed Assay Data Analysis

1. **Presence / Absence Multiplex Assay: *E.coli* (with enrichment) & Salmonella**

NOTE: If running Salmonella as a singleplex assay, Salmonella will be detected on the FAM channel

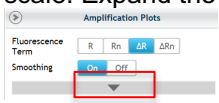
1.1. Open the Data Analysis window when the run is complete.

1.2. Highlight the wells of interest in the Analysis Criteria under Analysis, then select Graphical Display

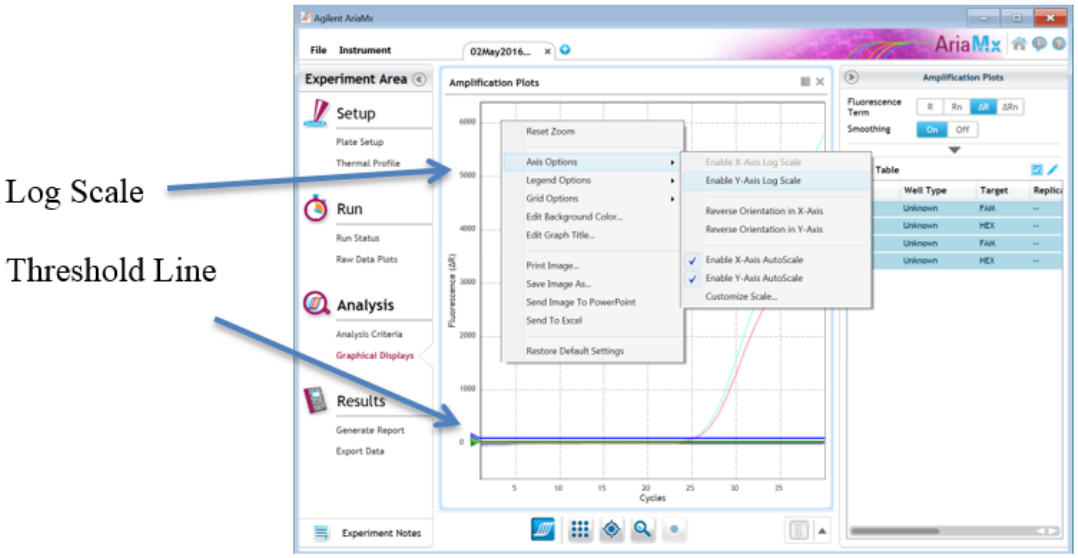
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

1.3. To analyze the results

- 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



- Manually adjust thresholds to 100 RFU for the HEX, FAM, and ROX fluorophores



- Controls
  - Assay specific Positive Control, on the FAM and ROX flourophores, should have a Cq value ≤ 35.
    - Visually confirm with the curve on the graph.
  - Assay specific Negative Controls, on the FAM and ROX flourophores, should have no Cq value.
    - Visually confirm with the curve on the graph.
- Unknown *E.coli* Target **(FAM flourophore detects *E.coli*)**
  - Internal Control, on the HEX flourophore, has a Cq value ≤ 35 for flower samples, ≤ 40 for all other matrices.
    - Visually confirm with the curve on the graph
  - A "presence" result for the unknown *E.coli* target.
    - Any Cq value for the FAM flourophore ≤ 40.
    - 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, especially when Cq reading is less than 15. (See troubleshooting guide below for more details.)
  - An "absence" result for the unknown *E.coli* target.
    - No Cq value for the FAM flourophore.
    - Visually confirm no curve on the graph.

- Unknown Salmonella Target **(ROX flourophore detects Salmonella in multiplex assay, FAM detects Salmonella in singleplex assay)**
    - Internal Control, on the HEX flourophore, has a Cq value ≤ 35 for flower samples, ≤ 40 for all other matrices.
        - Visually confirm with the curve on the graph
    - A “presence” or failing result for the unknown Salmonella target.
        - Any Cq value for the ROX flourophore ≤ 40.
        - 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, especially when Cq reading is less than 15. (See troubleshooting guide below for more details.)
    - An “absence” or passing result for the unknown Salmonella target.
        - No Cq value for the ROX flourophore.
        - Visually confirm no curve on the graph.

2. Threshold Assay: *E. coli* (NO Enrichment)

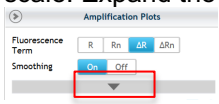
2.1. Open the Data Analysis window when the run is complete.

2.2. Highlight the wells of interest in the Analysis Criteria under Analysis, then select Graphical Display

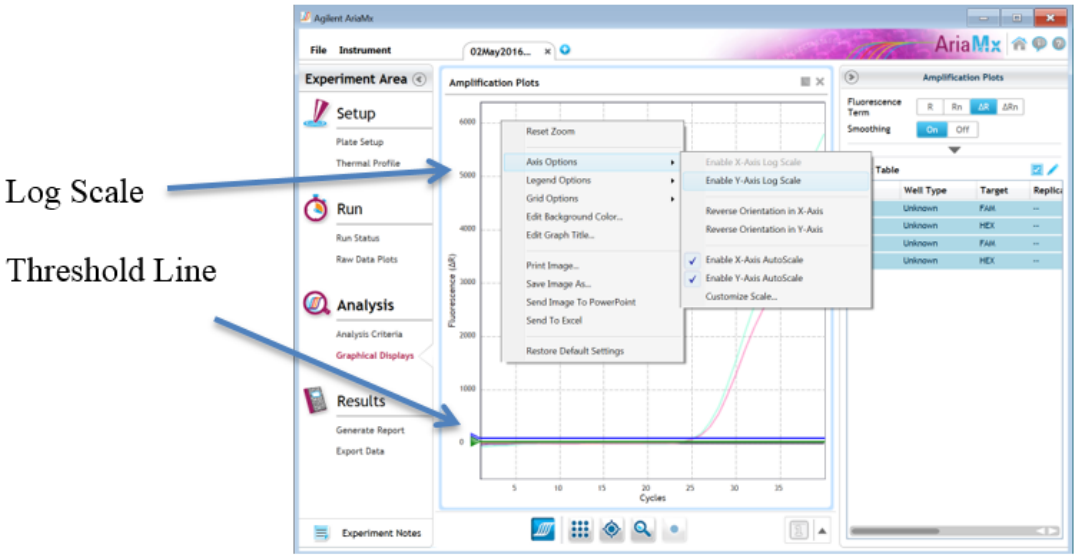
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

2.3. To analyze the results

- 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



- Manually adjust thresholds to 100 RFU for the HEX, FAM, and ROX fluorophores



- Controls
  - Assay specific Positive Control, on the FAM flourophore, has a Cq value ≤ 35
    - Visually confirm with the curve on the graph.
  - Assay specific Negative Control, on the FAM flourophore, has a Cq value of > 40 or no Cq value.
    - Visually confirm with the curve on the graph.
- Unknown Aerobic Count Target **(FAM flourophore detects *E. coli*)**
  - Internal Control, on the HEX flourophore, has a Cq value ≤ 35 for flower samples, ≤ 40 for all other matrices.
    - Visually confirm with the curve on the graph.
  - A high CFU count result for the unknown *E. coli* target.
    - **Passing Sample Result:** Check Cq Value on the FAM Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
    - Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a high CFU count occurred. Sometimes the background amplification will give a false positive reading, especially when Cq reading is less than 15. (See troubleshooting guide below for more details.)
  - A low CFU count result for the unknown *E. coli* target.
    - **Failing Sample Result:** Check Cq Value on the FAM Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
    - Visually confirm with the curve on the graph.

3. Threshold Assay: Total Aerobic Count

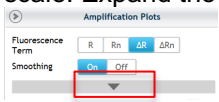
3.1. Open the Data Analysis window when the run is complete.

3.2. Highlight the wells of interest in the Analysis Criteria under Analysis, then select Graphical Display

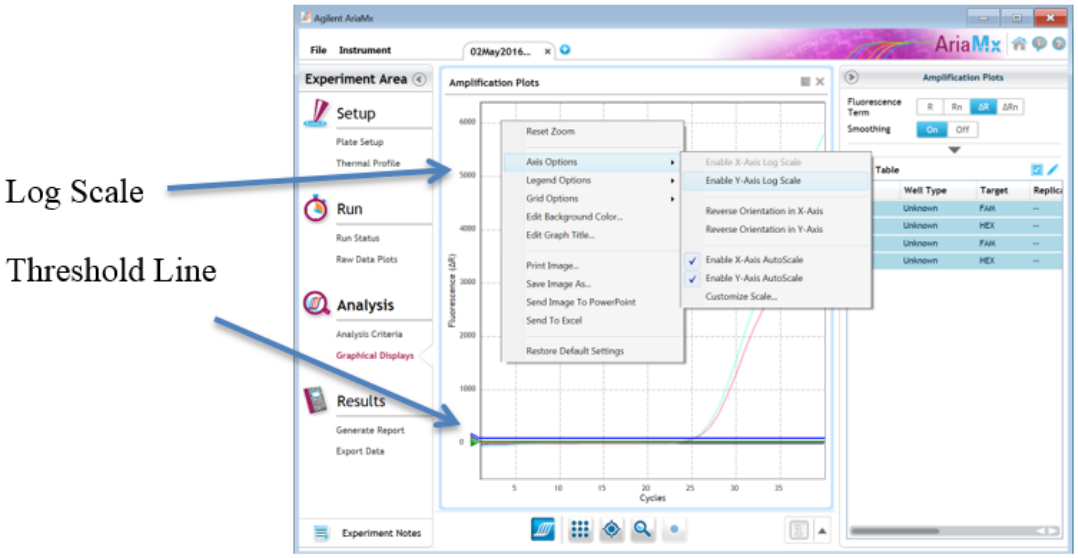
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

3.3. To analyze the results

- 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



- Manually adjust thresholds to 100 RFU for the HEX, FAM, and ROX fluorophores



- Controls
  - Assay specific Positive Control, on the FAM flourophore, has a Cq value ≤ 35
    - Visually confirm with the curve on the graph.
  - Assay specific Negative Control, on the FAM flourophore, has a Cq value of > 30 or no Cq value.
    - Visually confirm with the curve on the graph.
- Unknown Aerobic Count Target **(FAM flourophore detects Total Aerobic Count Bacteria)**
  - Internal Control, on the HEX flourophore, has a Cq value ≤ 35 for flower samples, ≤ 40 for all other matrices.
    - Visually confirm with the curve on the graph.
  - A high CFU count result for the unknown TAC target.
    - **Passing Sample Result:** Check Cq Value on the FAM Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
    - Visually confirm with the curve on the graph It is very important to confirm with the amplification curve when a high CFU count occurred. Sometimes the background amplification will give a false positive reading, especially when Cq reading is less than 15. (See troubleshooting guide below for more details.)
  - A low CFU count result for the unknown TAC target.
    - **Failing Sample Result:** Check Cq Value on the FAM Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
    - Visually confirm with the curve on the graph.

4. Threshold Assay: Yeast & Mold

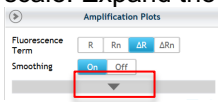
4.1. Open the Data Analysis window when the run is complete.

4.2. Highlight the wells of interest in the Analysis Criteria under Analysis, then select Graphical Display

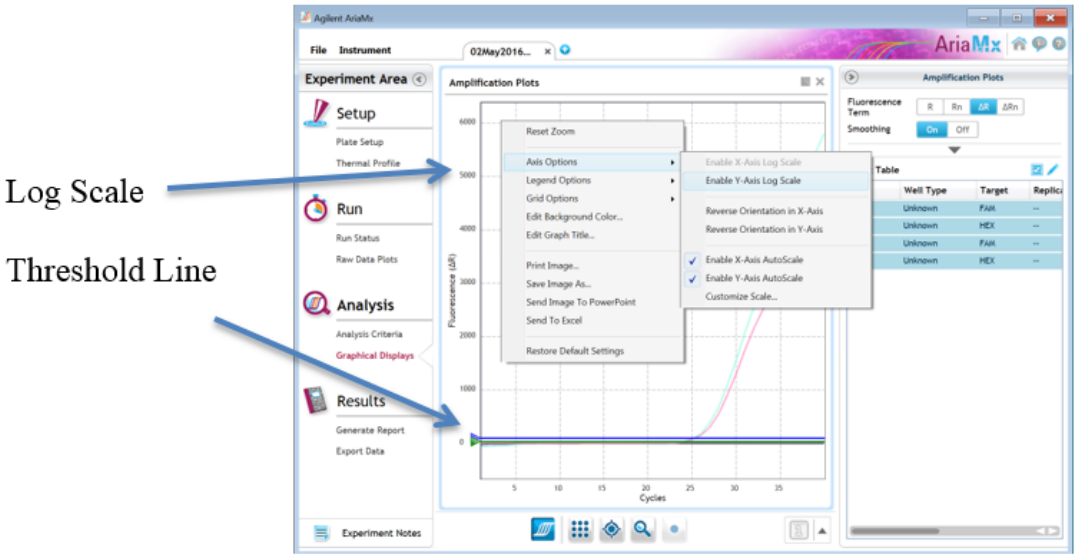
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

4.3. To analyze the results

- 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



- Manually adjust thresholds to 100 RFU for the HEX, FAM, and ROX fluorophores



- Controls
  - Assay specific Positive Control, on the FAM flourophore, has a Cq value ≤ 35
    - Visually confirm with the curve on the graph.
  - Assay specific Negative Control, on the FAM flourophore, has a Cq value of > 35 or no Cq value.
    - Visually confirm with the curve on the graph.
- Unknown Yeast and Mold Target **(FAM flourophore detects Total Yeast & Mold)**
  - Internal Control, on the HEX flourophore, has a Cq value ≤ 35 for flower samples, ≤ 40 for all other matrices.
    - Visually confirm with the curve on the graph.
  - A high CFU count result for the unknown Y&M target.
    - **Passing Sample Result:** Check Cq Value on the FAM Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
    - Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a high CFU count occurred. Sometimes the background amplification will give a false positive reading, especially when Cq reading is less than 15. (See troubleshooting guide below for more details.)
  - A low CFU count result for the unknown Y&M target.
    - **Failing Sample Result:** Check Cq Value on the FAM Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
    - Visually confirm with the curve on the graph.

5. Threshold Multiplex Assay: Total Coliform and Entero

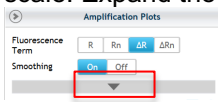
5.1. Open the Data Analysis window when the run is complete.

5.2. Highlight the wells of interest in the Analysis Criteria under Analysis, then select Graphical Display

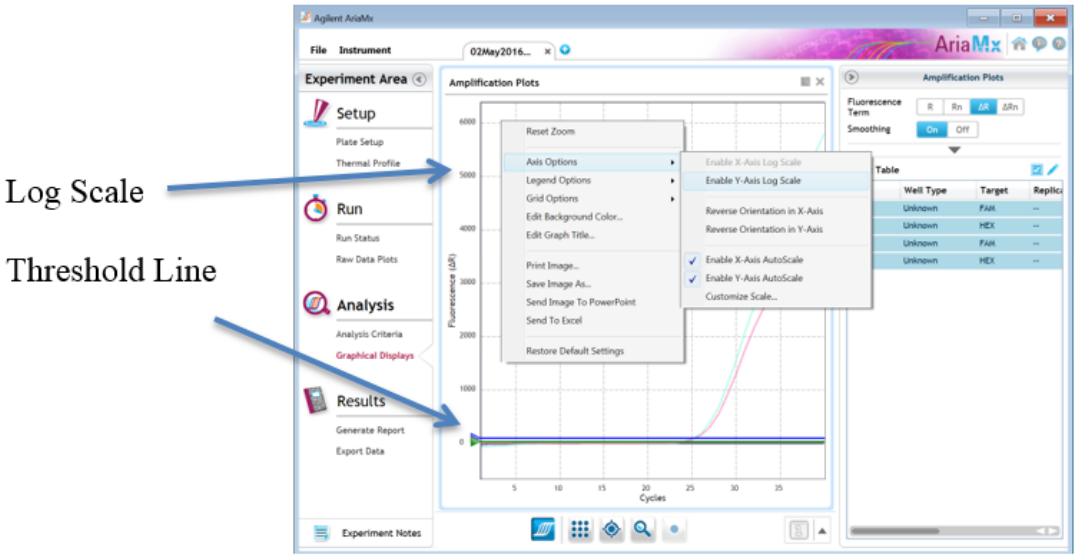
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

5.3. To analyze the results

- 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



- Manually adjust thresholds to 100 RFU for the HEX, FAM, and ROX fluorophores



- Controls
  - Assay specific Positive Control, on the FAM and ROX flourophores, have Cq values ≤ 35
    - Visually confirm with the curve on the graph.
  - Assay specific Negative Control, on the FAM and ROX flourophores, have Cq values > 35 or no Cq value.
    - Visually confirm with the curve on the graph.
- Unknown Coliform Target (**FAM flourophore detects Total Coliform)**
  - Internal Control, on the HEX flourophore, has a Cq value ≤ 35 for flower samples, ≤ 40 for all other matrices.
    - Visually confirm with the curve on the graph.
  - A high CFU count result for the unknown coliform target.
    - **Passing Sample Result:** Check Cq Value on the FAM Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
    - Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a high CFU count occurred. Sometimes the background amplification will give a false positive reading, especially when Cq reading is less than 15. (See troubleshooting guide below for more detail.)
  - A low CFU count result for the unknown coliform target.
    - **Failing Sample Result:** Check Cq Value on the FAM Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
    - Visually confirm with the curve on the graph.

- Unknown Entero Target (**ROX flourophore detects Entero)**

- Internal Control, on the HEX flourophore, has a Cq value ≤ 35 for flower samples, ≤ 40 for non-flower matrices.
  - Visually confirm with the curve on the graph.
- A high CFU count result for the unknown Entero target.
  - **Passing Sample Result:** Check Cq Value on the ROX Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
  - Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a high CFU count occurred. Sometimes the background amplification will give a false positive reading, especially when Cq reading is less than 15. - See troubleshooting guide below for more detail.)
- A low CFU count result for the unknown Entero target.
  - **Failing Sample Result:** Check Cq Value on the ROX Flourophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
  - Visually confirm with the curve on the graph.

Table 4, Cq to CFU Conversion Equation Table

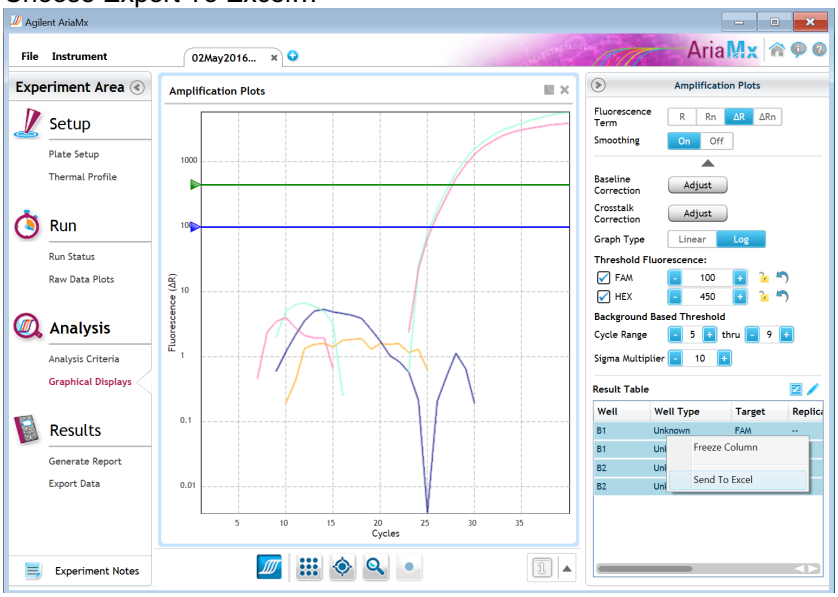
Matrix	Microbial Test	Cq to CFU/g Conversion Equation
Flower	Total Yeast and Mold	$CFU/g = 10^{[(36.671 - Cq\ Value)/3.1194]}$
Flower	Total Aerobic Count	$CFU/g = 10^{[(35.111 - Cq\ Value)/2.8883]}$
Flower	Total Coliform	$CFU/g = 10^{[(40.073 - Cq\ Value)/3.3417]}$
Flower	Total Enterobacteriaceae	$CFU/g = 10^{[(41.218 - Cq\ Value)/4.3708]}$
Flower	E. coli (no enrichment)	$CFU/g = 10^{[(40.083 - Cq\ Value)/3.4747]}$
MIP/Extract	Total Yeast and Mold	$CFU/g = 10^{[(54.972 - Cq\ Value)/5.8485]}$
MIP/Extract	Total Aerobic Count	$CFU/g = 10^{[(38.076 - Cq\ Value)/3.2249]}$
MIP/Extract	Total Coliform	$CFU/g = 10^{[(41.935 - Cq\ Value)/3.6274]}$
MIP/Extract	Total Enterobacteriaceae	$CFU/g = 10^{[(38.407 - Cq\ Value)/3.3041]}$
MIP/Extract	E. coli (no enrichment)	$CFU/g = 10^{[(43.943 - Cq\ Value)/3.1885]}$
Gummy	Total Yeast and Mold	$CFU/g = 10^{[(52.989 - Cq\ Value)/4.9718]}$
Gummy	Total Aerobic Count	$CFU/g = 10^{[(37.235 - Cq\ Value)/2.356]}$
Gummy	Total Coliform	$CFU/g = 10^{[(52.888 - Cq\ Value)/5.9643]}$
Gummy	Total Enterobacteriaceae	$CFU/g = 10^{[(44.81 - Cq\ Value)/4.9665]}$

Please Contact support@medicinalgenomics.com for an easy to use conversion spreadsheet

6. Export the Data

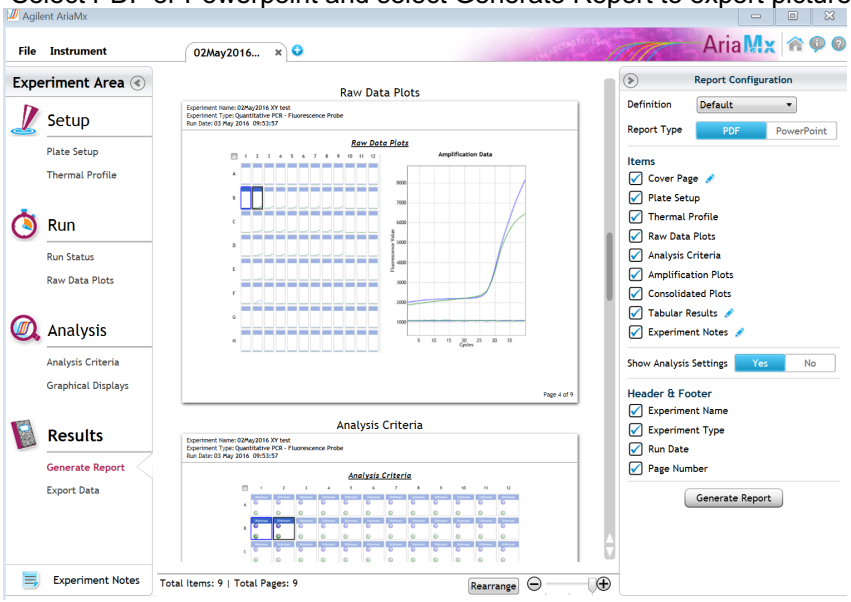
6.1. Exporting the Cq values into an Excel spreadsheet.

- To export the Cq values to an Excel spreadsheet, right- click on the chart on the bottom right of the screen.
- Choose Export To Excel…

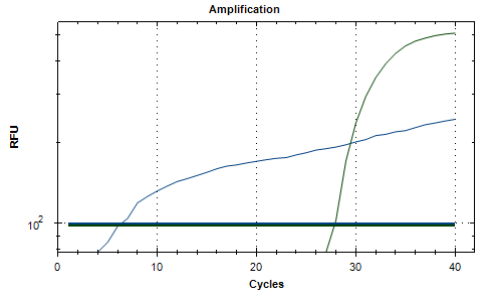


6.2. Saving a visual of the graph

- To save a picture of the graph, navigate to the Results section of the software and select Generate Report
- Select PDF or Powerpoint and select Generate Report to export pictures



Troubleshooting Guide

Symptom	Reason	Solution
Internal control (SCCG Primer) failure	Extraction Failure	Repeat SenSATIVAx™ and PathoSEEK™ by following the protocol.
	Residual ethanol in elution	Ethanol is an inhibitor to PCR. Return to the SenSATIVAx™ protocol and repeat all steps.
	Mix up in Reaction Setup	Repeat the qPCR by following the protocol.
	Missing Flourophore on plate set up	In the Data Analysis window click on View/Edit Plate Setup from the Settings drop down. All wells should have both FAM and HEX. Once completed and window is closed the analysis should automatically update.
Internal Control (SCCG) Positive result on positive or negative control samples or samples that do not contain plant DNA	Plant DNA 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 extraction area	Designate separate benches, pipettes etc. for extractions and qPCR setup
Positive Negative Control	Small Cq value <15	Visually confirm that there is an amplification curve. If not, this is low level background and is to be expected.
	Carry over	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	Repeat the qPCR by following the protocol.
Total run failure	Excessive vortex of the qPCR Master Mix	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 florescent 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 flourophore.

The **Internal Control** is added to every sample reaction where a Cq is expected. It ensures the effectiveness and efficiency of each reaction. The internal control targets plant DNA, or more specifically, a Single Copy Control Gene (SCCG), using the HEX flourophore.

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

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

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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