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PathoSEEK® Analysis Quick Reference Tables:

FLOWER

Table 1: Flower Samples ONLY – No Decontamination Step

PathoSEEK® Assay	Cq Value (Presence or failure)	Fluor	Negative Control (Cq)	CFU threshold (CFU/g) (This may vary by location)
<i>Aspergillus</i>	≤ 40	FAM	No Cq	Presence/Absence
<i>E. coli</i>	≤ 40	FAM	No Cq	Presence/Absence
STEC <i>E. coli</i>	≤ 40	FAM	No Cq	Presence/Absence
Salmonella	≤ 40	ROX*	No Cq	Presence/Absence
<i>Pseudomonas aeruginosa</i>	≤ 40	FAM	No Cq	Presence/Absence
<i>Staphylococcus aureus</i>	≤ 40	FAM	No Cq	Presence/Absence
Total Aerobic Count	≤ 20.7	FAM	> 30	100,000 (10 ⁵)
Total Yeast and Mold Count (spin Prep, v3 MM)	≤ 24.2	FAM	No Cq	10,000 (10 ⁴)
BTGN (Singleplex)	≤ 28.9	FAM	No Cq	1,000 (10 ³)
Total Coliform (Singleplex)	≤ 30.1	FAM	No Cq	1,000 (10 ³)
Internal Control*	≤35	HEX	*Internal control verifies the presence or absence of plant DNA	
Assay Positive Control	≤35	FAM/ROX		
Grim Reefer Positive Control	≤35	Cy5		

**If running singleplex Salmonella or Entero assays, they will be detected on the FAM channel*

NON – FLOWER Matrices

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

NOTE: Grim Reefer does not work in the presence of ALL non flower matrices. Contact MGC Support for MIP SOP support@medicinalgenomics.com

PathoSEEK® Assay	Cq Value (High CFU count)	Fluor	Negative Control (Cq)	CFU threshold (CFU/g) (This may vary by location)
<i>Aspergillus</i>	≤ 40	FAM	No Cq	Presence/Absence
<i>E. coli</i>	≤ 40	FAM	No Cq	Presence/Absence
STEC <i>E. coli</i>	≤ 40	FAM	No Cq	Presence/Absence
Salmonella	≤ 40	ROX*	No Cq	Presence/Absence
<i>Pseudomonas aeruginosa</i>	≤ 40	FAM	No Cq	Presence/Absence
<i>Staphylococcus aureus</i>	≤ 40	FAM	No Cq	Presence/Absence
Total Aerobic Count	≤ 25.2	FAM	> 35	10,000 (10 ⁴)
Total Yeast and Mold Count	≤ 37.4	FAM	No Cq	1000 (10 ³)
BTGN (Singleplex)	≤ 33.9	FAM	No Cq	100 (10 ²)
Total Coliform (Singleplex)	≤ 34.7	FAM	No Cq	100 (10 ²)
Internal Control*	≤40	HEX	*Internal control verifies the presence or absence of spiked plant positive control (SCCG)	
Assay Positive Controls	≤35	FAM/ROX		
Grim Reefer Positive Control	≤35	Cy5		

* If running singleplex Salmonella or Entero assays, they will be detected on the FAM channel

GUMMY

Table 3: Gummy – No Decontamination Step

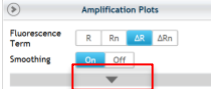
PathoSEEK® Assay	Cq Value (High CFU count)	Fluor	Negative Control (Cq)	CFU threshold (CFU/g) (This may vary by location)
<i>Aspergillus</i>	≤ 40	FAM	No Cq	Presence/Absence
<i>E. coli</i>	≤ 40	FAM	No Cq	Presence/Absence
STEC <i>E. coli</i>	≤ 40	FAM	No Cq	Presence/Absence
Salmonella	≤ 40	ROX*	No Cq	Presence/Absence
<i>Pseudomonas aeruginosa</i>	≤ 40	FAM	No Cq	Presence/Absence
<i>Staphylococcus aureus</i>	≤ 40	FAM	No Cq	Presence/Absence
Total Aerobic Count	≤ 27.8	FAM	> 35	10,000 (10 ⁴)
Total Yeast and Mold Count	≤ 38.1	FAM	No Cq	1000 (10 ³)
BTGN (Singleplex)	≤ 33.7	FAM	No Cq	100 (10 ²)
Total Coliform (Singleplex)	≤ 41.0	FAM	No Cq	100 (10 ²)
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

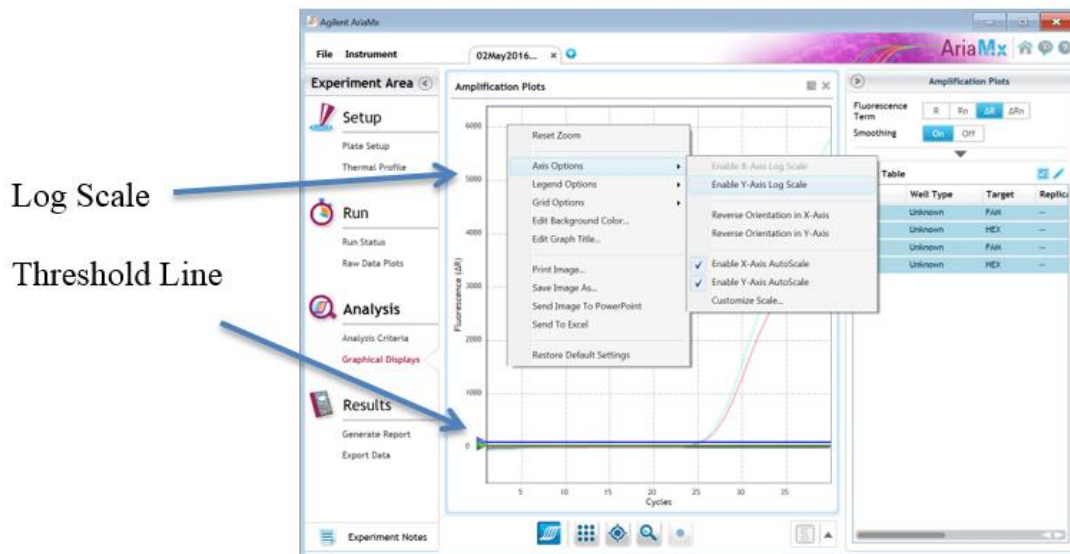
1. Presence / Absence Multiplex Assay: *E. coli* & Salmonella

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, ROX and Cy5 fluorophores.



- Controls
 - Assay specific Positive Control, on the FAM and ROX Fluorophores, should have a Cq value ≤ 35.
 - Visually confirm with the curve on the graph.
 - Assay specific Negative Controls, on the FAM and ROX Fluorophores, should have no Cq value.
 - Visually confirm with the curve on the graph.
- Unknown *E. coli* Target (**FAM Fluorophore detects *E. coli***)
 - Internal Control, on the HEX Fluorophore, 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 Fluorophore ≤ 40.
 - Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a presence result occurs. 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 Fluorophore.
 - Visually confirm no curve on the graph.

- **Unknown Salmonella Target (ROX Fluorophore detects Salmonella in multiplex assay, FAM detects Salmonella in singleplex assay)**
 - Internal Control, on the HEX Fluorophore, 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 Fluorophore ≤ 40 .
 - Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a presence result occurs. 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 Fluorophore.
 - Visually confirm no curve on the graph.

2. Presence / Absence Singleplex and Multiplex Assay: Aspergillus (includes species specific and multiplex assays)

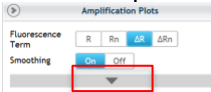
2.1. Open the Data Analysis window when the run is complete.

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

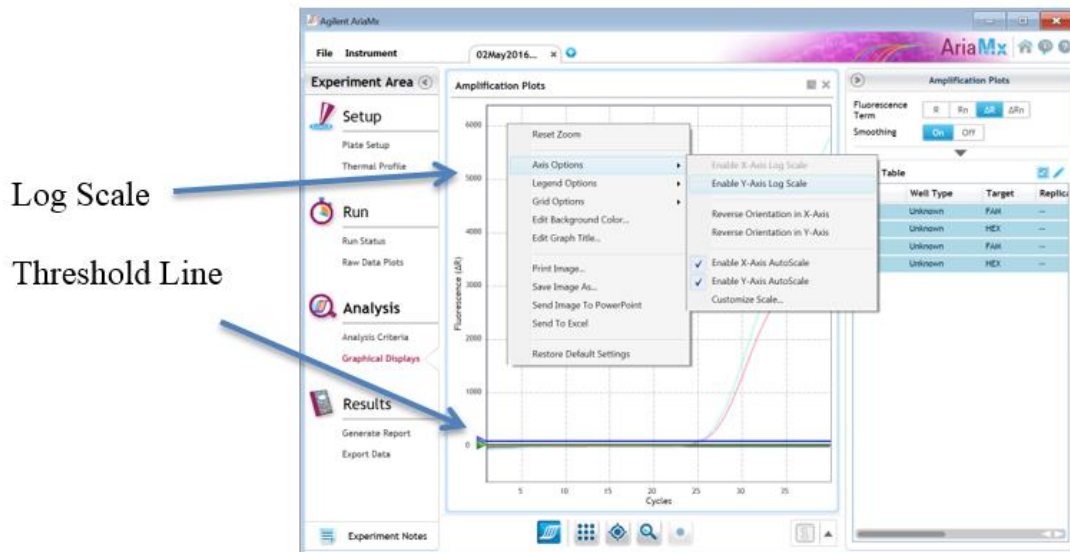
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

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, ROX and Cy5 fluorophores.



Controls

- Assay specific Positive Control, on the FAM Fluorophore, has a Cq value ≤ 35.
 - Visually confirm with the curve on the graph.
- Assay specific Negative Control, on the FAM Fluorophore, has no Cq value.
 - Visually confirm with the curve on the graph.
- Grim Reefer Positive Control on the Cy5 Channel, has a Cq value ≤ 35.
- Unknown Aspergillus Target (**FAM Fluorophore detects all 4 Aspergillus species in multiplex assay**)
 - Internal Control, on the HEX Fluorophore, 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 Aspergillus target(s).
 - Any Cq value for the FAM Fluorophore ≤ 40.
 - Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a presence result occurs. 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 Aspergillus target.
 - No Cq value for the FAM Fluorophore.
 - Visually confirm no curve on the graph.

3. Presence / Absence Multiplex Assay: *Pseudomonas aeruginosa*/Staphylococcus aureus

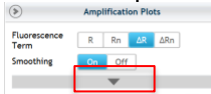
Open the Data Analysis window when the run is complete.

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

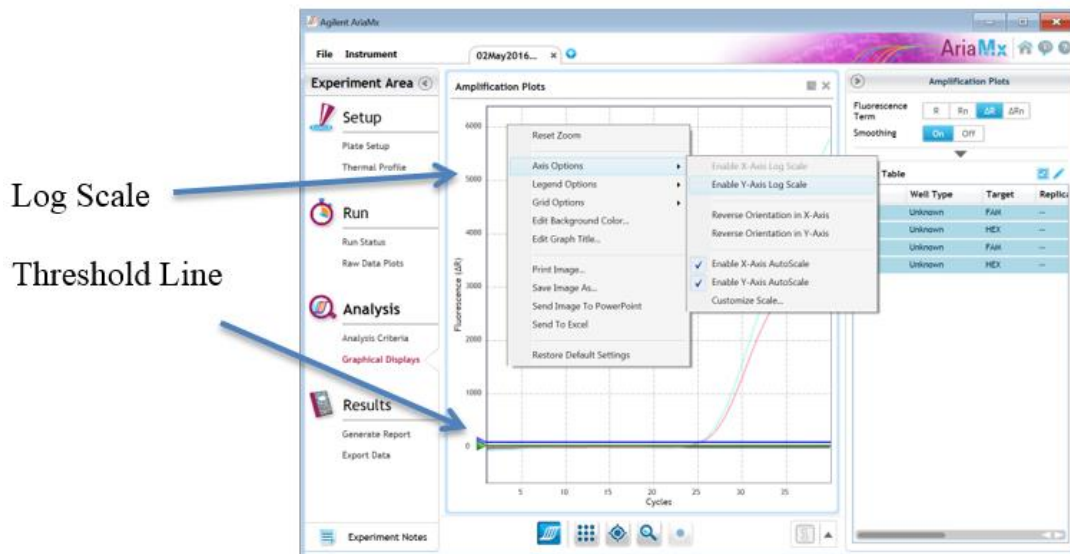
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

3.2. 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, ROX and Cy5 fluorophores.



Controls

- Assay specific Positive Control, on the FAM Fluorophore, should have a Cq value ≤ 40.
 - Visually confirm with the curve on the graph.
- Assay specific Negative Control, on the FAM Fluorophore, should have no Cq value.
 - Visually confirm with the curve on the graph.
- Unknown Pseudomonas or Staphylococcus Target (**FAM Fluorophore detects both pathogens**)
 - Internal Control, on the HEX Fluorophore, 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 Pseudomonas or Staphylococcus target.
 - Any Cq value for the FAM Fluorophore ≤ 40.
 - Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a presence result occurs. 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 Pseudomonas or Staphylococcus target.
 - No Cq value for the FAM Fluorophore.
 - Visually confirm no curve on the graph.)

4. Threshold Assay: Total Yeast and Mold

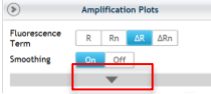
4.1. Open the Data Analysis window when the run is complete.

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

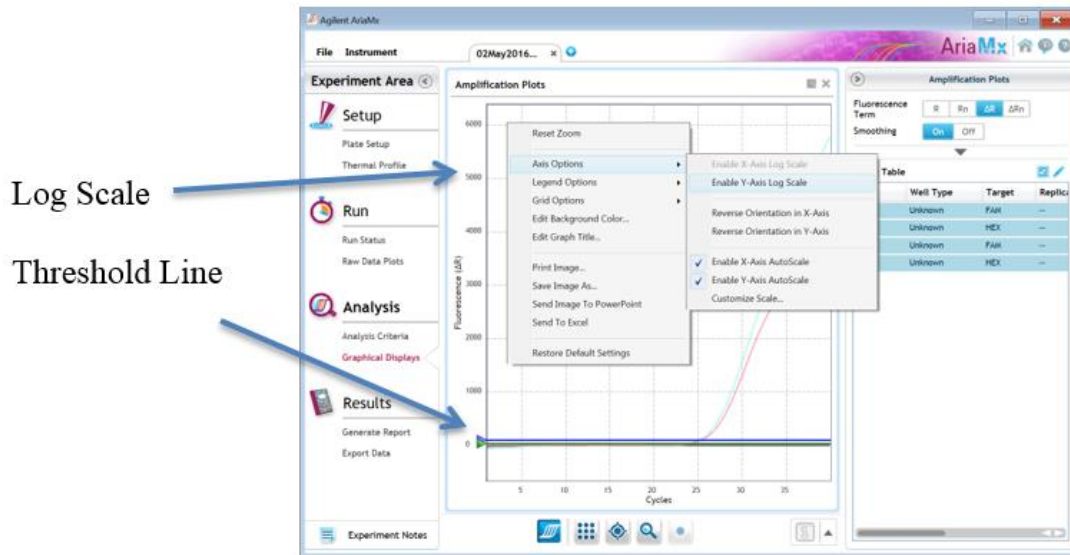
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

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 and FAM fluorophores.



- - Controls
 - Assay specific Positive Control, on the FAM Fluorophore, has a Cq value ≤ 35
 - Visually confirm with the curve on the graph.
 - Assay specific Negative Control, on the FAM Fluorophore, has a Cq value of > 35 or no Cq value.
 - Visually confirm with the curve on the graph.
 - Unknown TYM Target (**FAM Fluorophore detects TYM**)
 - Internal Control, on the HEX Fluorophore, 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 TYM target.
 - **Passing Sample Result:** Check Cq Value on the FAM Fluorophore. 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 occurs. 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 TYM target.
 - **Failing Sample Result:** Check Cq Value on the FAM Fluorophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
 - Visually confirm with the curve on the graph.

5. Threshold Assay: Total Aerobic Count

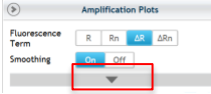
5.1. Open the Data Analysis window when the run is complete.

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

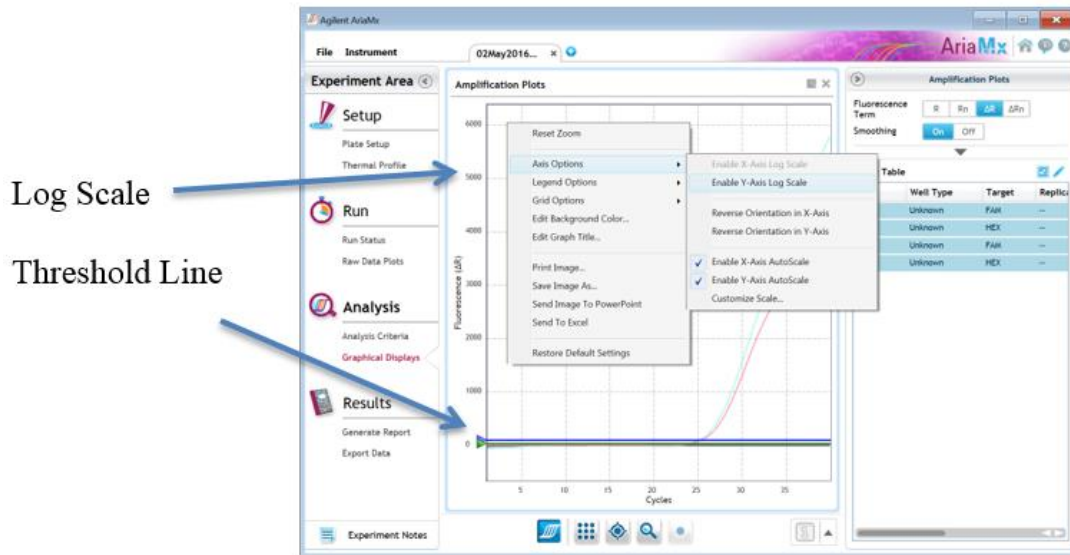
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

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, ROX and Cy5 fluorophores.



-
- Controls
 - Assay specific Positive Control, on the FAM Fluorophore, has a Cq value ≤ 35
 - Visually confirm with the curve on the graph.
 - Assay specific Negative Control, on the FAM Fluorophore, has a Cq value of > 30 or no Cq value.
 - Visually confirm with the curve on the graph.
- Unknown Aerobic Count Target (**FAM Fluorophore detects Total Aerobic Count Bacteria**)
 - Internal Control, on the HEX Fluorophore, 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 Fluorophore. 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 occurs. 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 Fluorophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
 - Visually confirm with the curve on the graph.

6. Threshold Assay: BTGN – Bile Tolerant Gram-Negative Bacteria

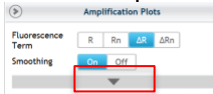
6.1. Open the Data Analysis window when the run is complete.

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

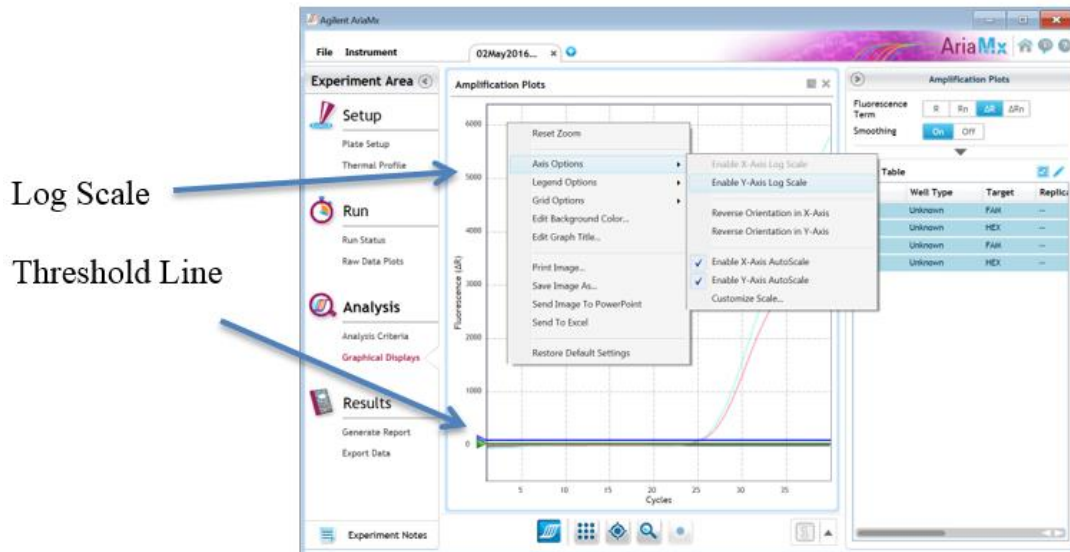
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

6.3. 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, ROX and Cy5 fluorophores.



- **Controls**

- Assay specific Positive Control, on the FAM Fluorophore, has a Cq value ≤ 35
 - Visually confirm with the curve on the graph.
- Assay specific Negative Control, on the FAM Fluorophore, has a Cq value of > 35 or no Cq value.
 - Visually confirm with the curve on the graph.

- **Unknown BTGN Target (FAM Fluorophore detects BTGN)**

- Internal Control, on the HEX Fluorophore, 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 BTGN target.
 - **Passing Sample Result:** Check Cq Value on the FAM Fluorophore. 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 occurs. 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 BTGN target.
 - **Failing Sample Result:** Check Cq Value on the FAM Fluorophore. See Tables 1-3 for Cq cutoff value depending on matrix being tested.
 - Visually confirm with the curve on the graph.

7. Threshold Assay: Total Coliform

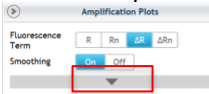
7.1. Open the Data Analysis window when the run is complete.

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

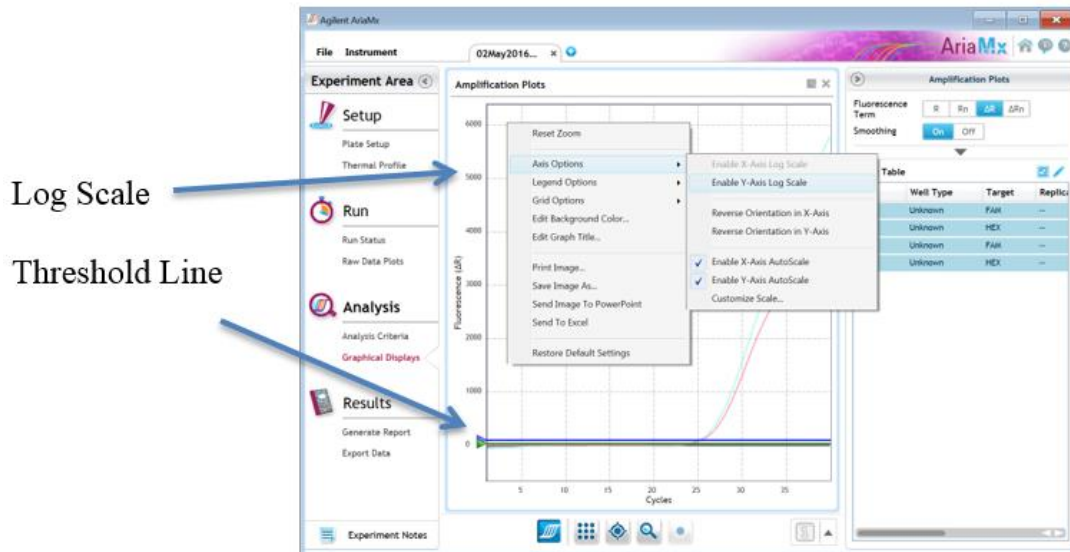
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

7.3. 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 Cy5 fluorophores.



- **Controls**

- Assay specific Positive Control, on the FAM Fluorophore, has a Cq value ≤ 35
 - Visually confirm with the curve on the graph.
- Assay specific Negative Control, on the FAM Fluorophore, has a Cq value of > 35 or no Cq value.
 - Visually confirm with the curve on the graph.

- **Unknown Total Coliform Target (FAM Fluorophore detects Total Coliform)**

- Internal Control, on the HEX Fluorophore, 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 Total Coliform target.
 - **Passing Sample Result:** Check Cq Value on the FAM Fluorophore. 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 occurs. 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 Total Coliform target.
 - **Failing Sample Result:** Check Cq Value on the FAM Fluorophore. 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

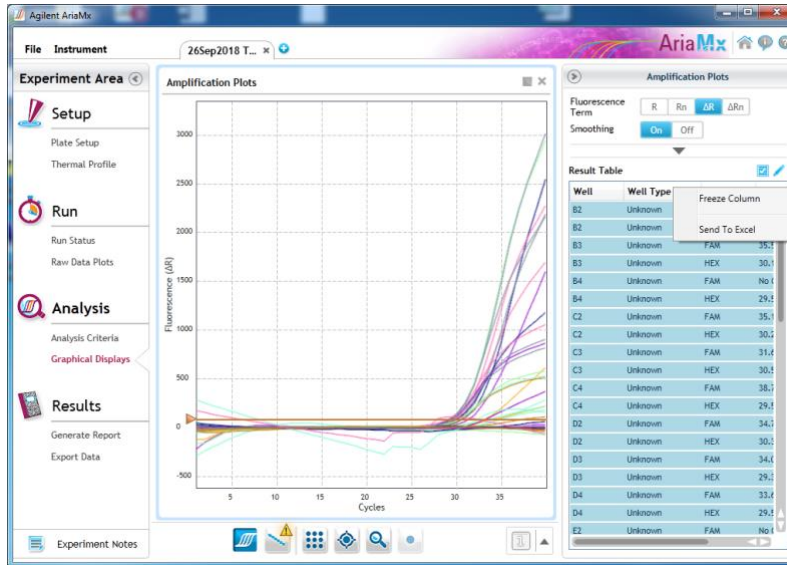
Matrix	Microbial Test	Cq to CFU/g Conversion Equation
Flower	Total Yeast and Mold (spin prep, v3MM)	$CFU/g = 10^{[-0.1896 * Cq Value + 8.5798]}$
Flower	Total Aerobic Count	$CFU/g = 10^{[(35.111 - Cq Value)/2.8883]}$
Flower	Total Coliform (Singleplex)	$CFU/g = 10^{[(40.073 - Cq Value)/3.3417]}$
Flower	BTGN (singleplex)	$CFU/g = 10^{[(38.665 - Cq Value)/3.2417]}$
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 (Singleplex)	$CFU/g = 10^{[(41.935 - Cq Value)/3.6274]}$
MIP/Extract	BTGN (singleplex)	$CFU/g = 10^{[(40.58 - Cq Value)/3.3403]}$
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 (Singleplex)	$CFU/g = 10^{[(52.888 - Cq Value)/5.9643]}$
Gummy	BTGN (singleplex)	$CFU/g = 10^{[(40.458 - Cq Value)/3.4035]}$

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

8. Export the Data

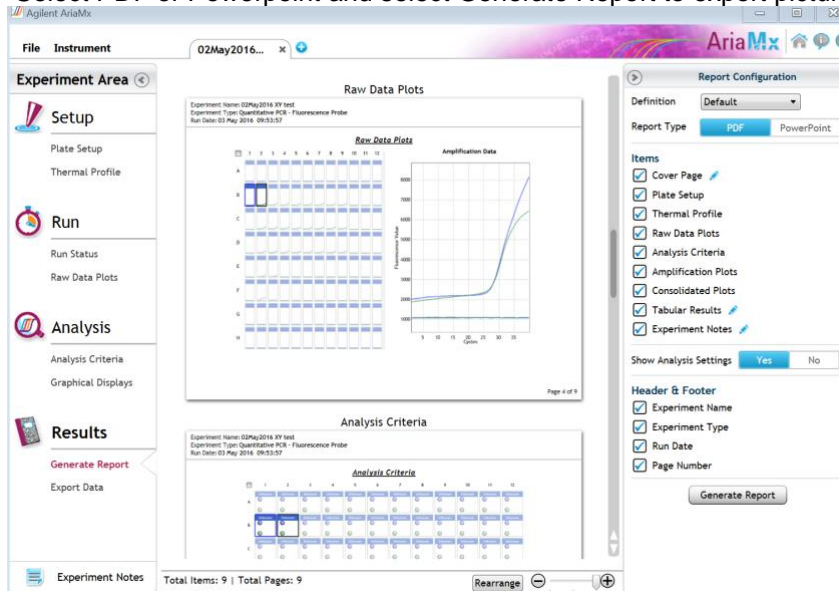
8.1. Exporting the Cq values into an Excel spreadsheet.

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

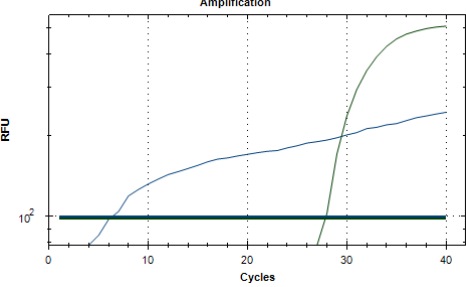


8.2. Saving a visual of the graph

- 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 Fluorophore 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 No Cq or a Cq of more than 35 is acceptable. Any Cq lower than 35 constitutes a rerun	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.
<p>Background Amplification</p> 	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.
No Cy5 Signal	Did not use Grim Reefer Positive control	Add Grim Reefer Positive Control to sample during SenSATIVAx DNA Extraction

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 Fluorophore.

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 Fluorophore.

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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