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PathoSEEK™ Analysis Quick Reference Tables:

Table 1: FLOWER, with Decontamination Step

PathoSEEK™ Assay	Cq Value (High CFU count)	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
<i>Aspergillus</i>	≤ 40	FAM	> 40	Presence/Absence
<i>E. coli</i>	≤ 40	FAM	> 40	Presence/Absence
STEC <i>E. coli</i>	≤ 40	FAM	> 40	Presence/Absence
Salmonella	≤ 40	TXR	> 40	Presence/Absence
Total Aerobic Count	≤ 21.2	FAM	> 30	100,000 (10 ⁵)
Total Coliform	≤ 30.1	FAM	> 40	1,000 (10 ³)
Total Enterobacteriaceae	≤ 28.7	TXR	> 40	1,000 (10 ³)
Total Yeast and Mold	≤ 27.4	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/TXR		

Detailed Assay Data Analysis

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

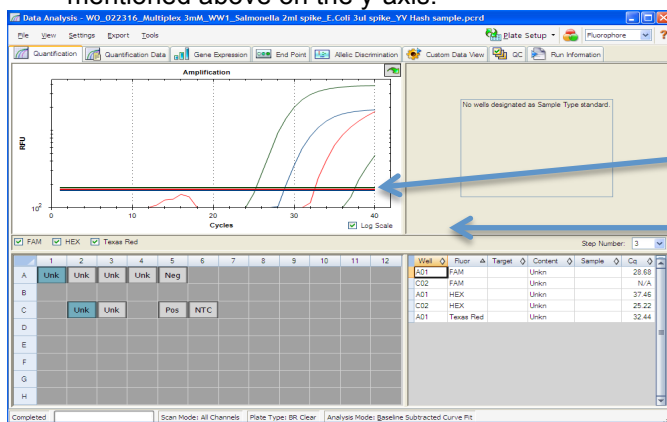
1.1. The Data Analysis window will open automatically when the run is complete.

1.2. Highlight well(s) of interest.

- The graph will appear above.
- The Cq values will appear to the right.

1.3. To analyze the results

- Start by turning the graph to Log Scale and manually moving the threshold lines to 10^2 for the FAM and TEXAS RED fluorophores. Also manually move the threshold line for the HEX fluorophore half way between 10^2 and 10^3 .
 - To turn the graph to Log Scale, click on the box at the bottom right of the graph.
 - To adjust the threshold, click on the horizontal lines, and move them to the specified value mentioned above on the y-axis.



Threshold Line

Log Scale

- Controls
 - Assay-specific Positive Controls, on the FAM and TEXAS RED fluorophores, has a Cq value ≤ 35 .
 - Visually confirm with the curve on the graph.
 - Assay-specific Negative Control, on the FAM and TEXAS RED fluorophores, has 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 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 fluorophore.
 - Visually confirm no curve on the graph.
- Unknown Salmonella Target (**TEXAS RED fluorophore detects Salmonella**)
 - 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 TEXAS RED fluorophore ≤ 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 TEXAS RED fluorophore.
 - Visually confirm no curve on the graph.

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

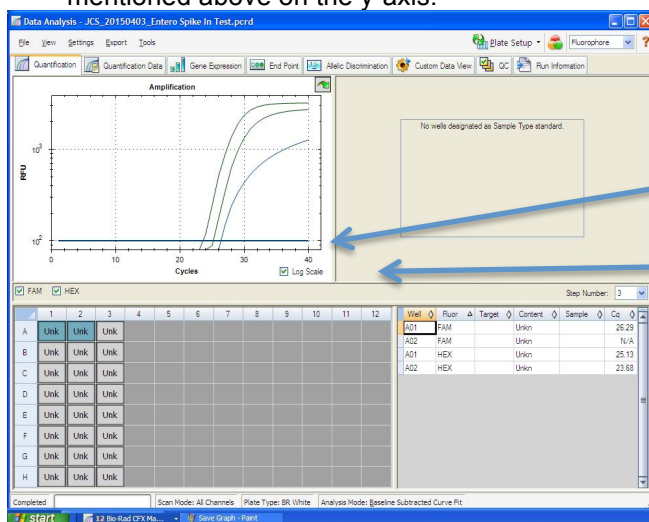
2.1. The Data Analysis window will open automatically when the run is complete.

2.2. Highlight the well of interest.

- The graph will appear above.
- The Cq values will appear to the right.

2.3. To analyze the results

- Start by turning the graph to Log Scale and manually moving the threshold to 10^2 for the FAM fluorophore. Also manually move the threshold for the HEX fluorophore half way between 10^2 and 10^3 .
 - To turn the graph to Log Scale, click on the box at the bottom right of the graph.
 - To adjust the threshold, click on the horizontal lines, and move them to the specified value mentioned above on the y-axis.



Threshold Line

Log Scale

- 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.
- 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.
 - 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 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 Aspergillus target.

- No Cq value for the FAM fluorophore.
- Visually confirm no curve on the graph.

3. CFU Threshold Assay: Total Aerobic Count

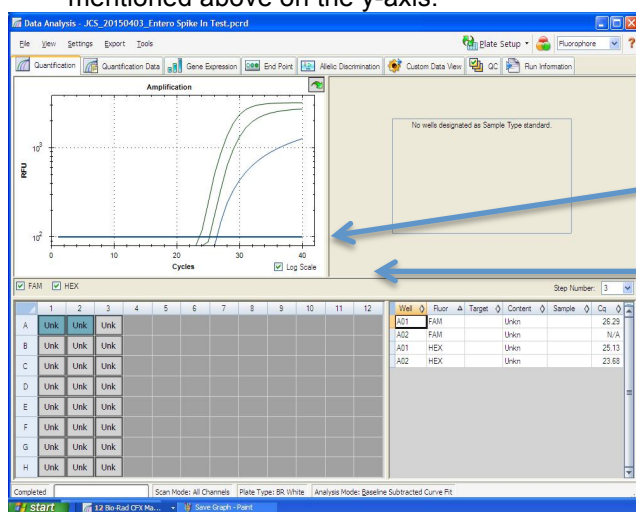
3.1. The Data Analysis window will open automatically when the run is complete.

3.2. Highlight the well of interest.

- The graph will appear above.
- The Cq values will appear to the right.

3.3. To analyze the results.

- Start by turning the graph to Log Scale and manually moving the threshold to 10^2 for the FAM fluorophore. Also manually move the threshold for the HEX fluorophore half way between 10^2 and 10^3 .
 - To turn the graph to Log Scale, click on the box to the bottom right of the graph.
 - To adjust the threshold, click on the horizontal lines, and move them to the specified value mentioned above on the y-axis.



- 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 Table 1 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 Fluorophore. See Table 1 for Cq cutoff value depending on matrix being tested.
 - Visually confirm with the curve on the graph.

4. CFU Threshold Assay: Total Yeast & Mold

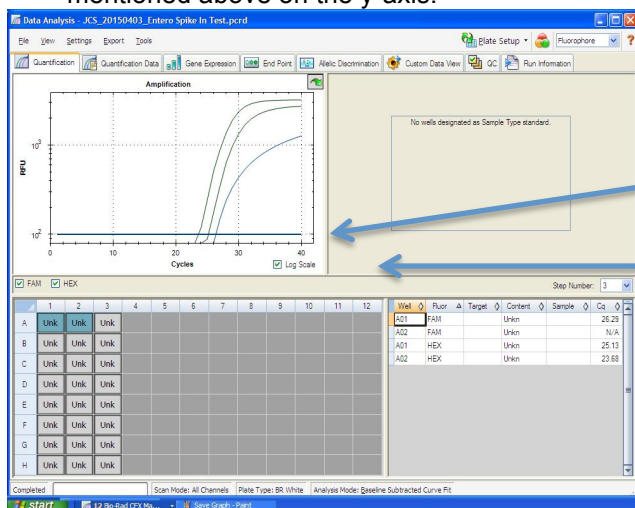
4.1. The Data Analysis window will open automatically when the run is complete.

4.2. Highlight the well of interest.

- The graph will appear above.
- The Cq values will appear to the right.

4.3. To analyze the results.

- Start by turning the graph to Log Scale and manually moving the threshold to 10^2 for the FAM fluorophore. Also manually move the threshold for the HEX fluorophore half way between 10^2 and 10^3 .
 - To turn the graph to Log Scale, click on the box to the bottom right of the graph.
 - To adjust the threshold, click on the horizontal lines, and move them to the specified value mentioned above on the y-axis.



- 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.
- Unknown Yeast and Mold Target (**FAM fluorophore detects Total Yeast & Mold**)
 - 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 Y&M target.
 - **Passing Sample Result:** Check Cq value on the FAM Fluorophore. See Table 1 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 Fluorophore. See Table 1 for Cq cutoff value depending on matrix being tested.
 - Visually confirm with the curve on the graph.

5. CFU Threshold Multiplex Assay: Total Coliform and Enterobacteriaceae

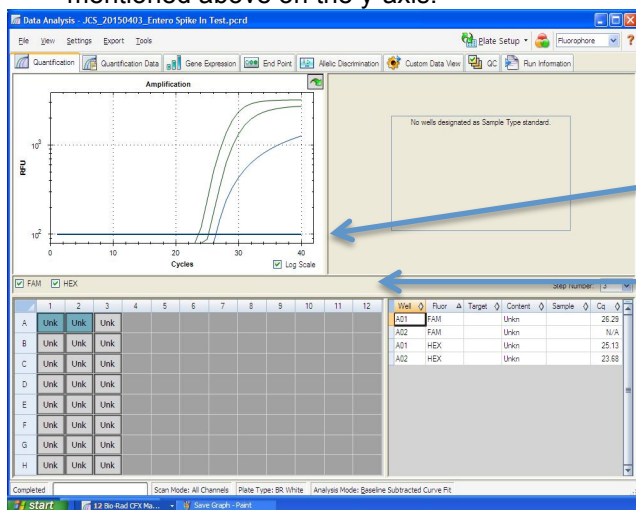
5.1. The Data Analysis window will open automatically when the run is complete.

5.2. Highlight the well of interest.

- The graph will appear above.
- The Cq values will appear to the right.

5.3. To analyze the results.

- Start by turning the graph to Log Scale and manually moving the threshold to 10² for the FAM and TEXAS RED fluorophore. Also manually move the threshold for the HEX fluorophore half way between 10² and 10³.
 - To turn the graph to Log Scale, click on the box to the bottom right of the graph.
 - To adjust the threshold, click on the horizontal lines, and move them to the specified value mentioned above on the y-axis.



Threshold Line

Log Scale

- 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.
- Unknown 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 coliform target.
 - **Passing Sample Result:** Check Cq Value on the FAM Fluorophore. See Table 1 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 Fluorophore. See Table 1 for Cq cutoff value depending on matrix being tested.
 - Visually confirm with the curve on the graph.

- **Unknown Entero Target (TEXAS RED fluorophore detects Entero)**
 - Internal Control, on the HEX fluorophore, 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 TEXAS RED Fluorophore. See Table 1 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 TEXAS RED Fluorophore. See Table 1 for Cq cutoff value depending on matrix being tested.
 - Visually confirm with the curve on the graph.

Table 2, Cq to CFU Conversion Equation Table

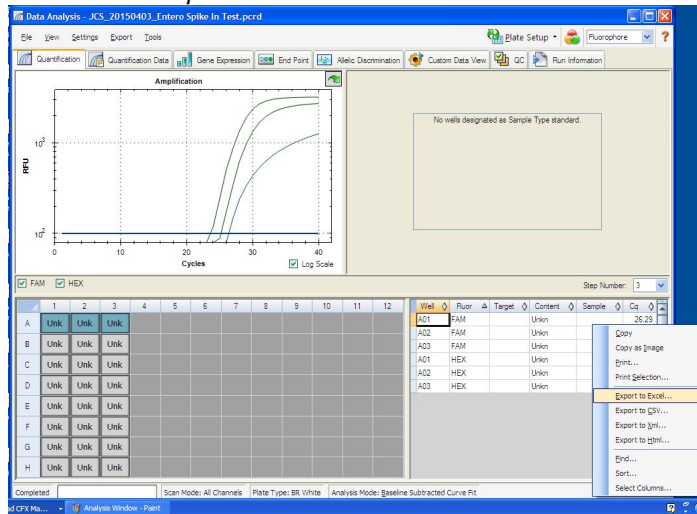
Matrix	Microbial Test	Cq to CFU/g Conversion Equation
Flower	Total Yeast and Mold	$CFU/g = 10^{[(42.185 - Cq\ Value)/3.6916]}$
Flower	Total Aerobic Count	$CFU/g = 10^{[(36.465 - Cq\ Value)/3.044]}$
Flower	Total Coliform	$CFU/g = 10^{[(38.476 - Cq\ Value)/2.7849]}$
Flower	Total Enterobacteriaceae	$CFU/g = 10^{[(37.834 - Cq\ Value)/3.0432]}$

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

6. Export the Data

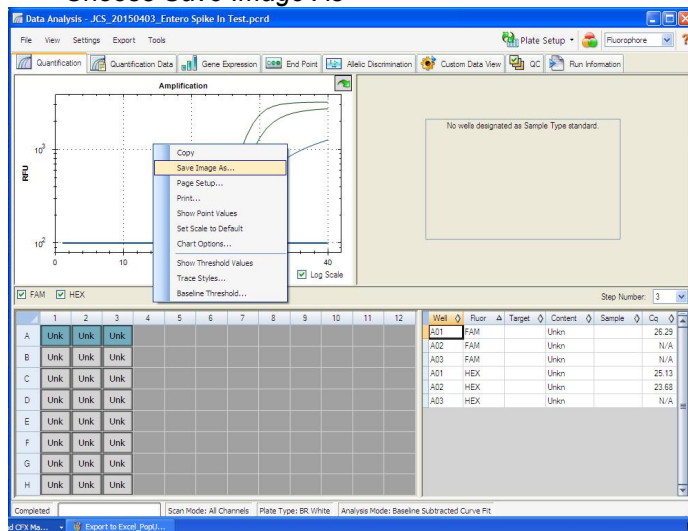
6.1. Exporting the Cq values into an Excel spreadsheet.

- 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...*

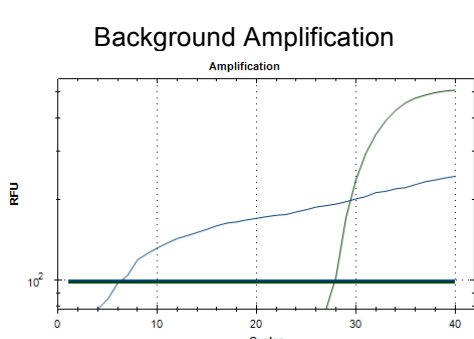


6.2. Saving a visual of the graph

- To save a picture of the graph, right-click the graph on the top left of the screen.
- Choose *Save Image As*



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 “View/Edit Plate Setup” from the Settings drop down. All wells should have both FAM and HEX, Multiplex Samples should also have Texas Red. Once completed and the 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.
<p>Background Amplification</p> 	Unclear	<p>This is usually seen with a very low Cq reading (<15). The curve is usually missing an exponential growth phase, but rather appears as a gradual increase of fluorescent signal. Visual analysis of the graph is necessary to determine if signal is real or background. This is usually a negative result. If still unclear after visual analysis, it is suggested to re-run the assay.</p>

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 consumable that has cannabis plant material of concentrate mixed in.

DISCLAIMER

This test was developed and its performance characteristics determined by Medicinal Genomics Corporation, 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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