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**FemINDICator™ Analysis Quick Table**

FemINDICator™ Assay	Cq Value	Fluor	Negative Control (Cq)	Cq threshold
Male	< 35	FAM	> 35	Presence/Absence
Female	> 35	FAM	> 35	Presence/Absence
Internal Control*	<35	HEX	*Internal control verifies the presence or absence of plant DNA	
Assay Positive Controls	<35	FAM		

## Detailed Assay Data Analysis

### 1. Presence / Absence Assays

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

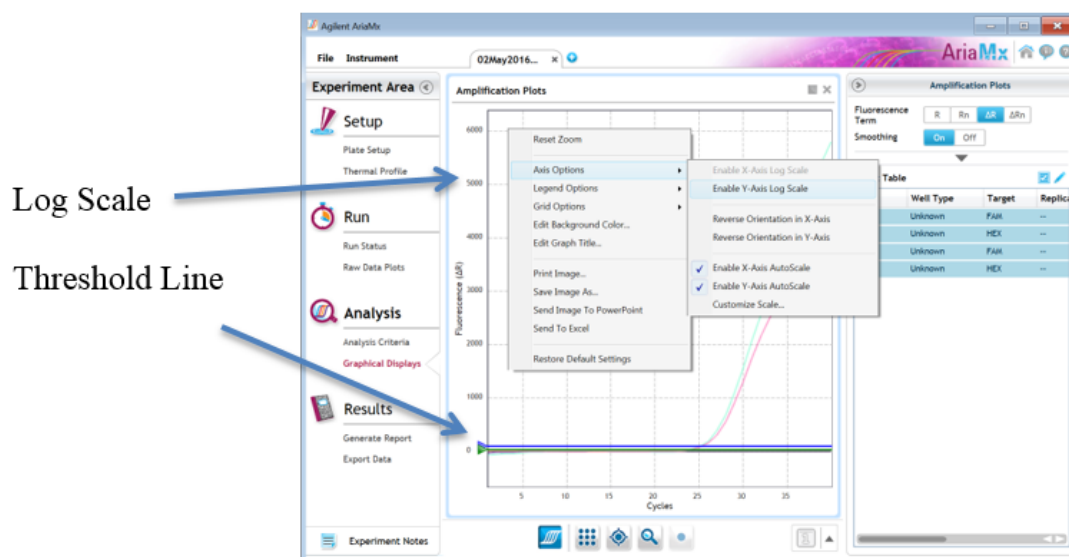
- Amplification plots will be available for viewing
- The Cq values will appear to the right in the table

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 set thresholds by typing the threshold to 100 RFU for the FAM flourophore and 450 RFU for the HEX flourophore.

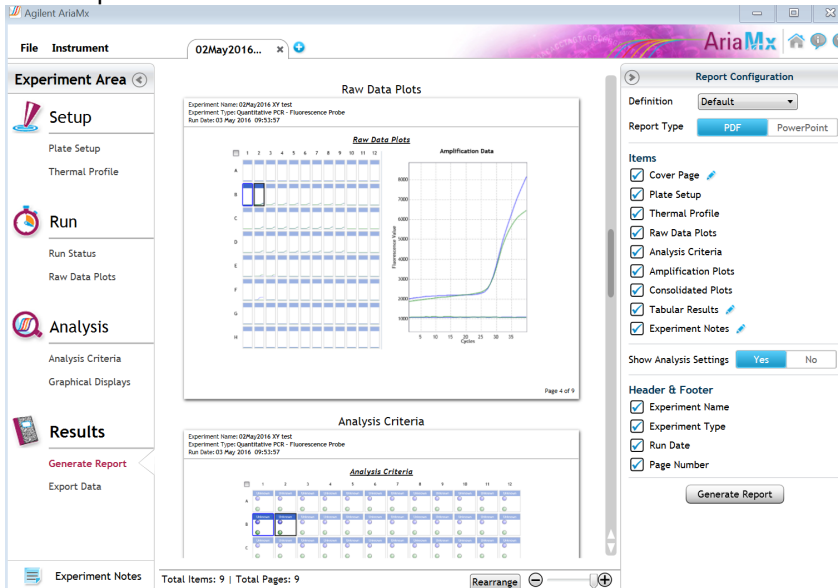


- Controls
  - Male specific Positive Control, on the FAM flourophore, has a Cq value < 35.
    - Visually confirm with the curve on the graph.
  - Male specific Negative Control, on the FAM flourophore, has a Cq value > 35 or no Cq value.
    - Visually confirm with the curve on the graph.
- Unknown Samples
  - Internal Control, on the HEX flourophore, has a Cq value < 35.
    - Visually confirm with the curve on the graph
  - A “presence” or “male” result for the unknown sample.
    - Any Cq value for the FAM flourophore < 35.
    - 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.)
  - An “absence” or “female” result for the unknown sample.
    - No Cq value for the FAM flourophore or a Cq value >35.
    - Visually confirm no curve on the graph.

## 2. Export the Data

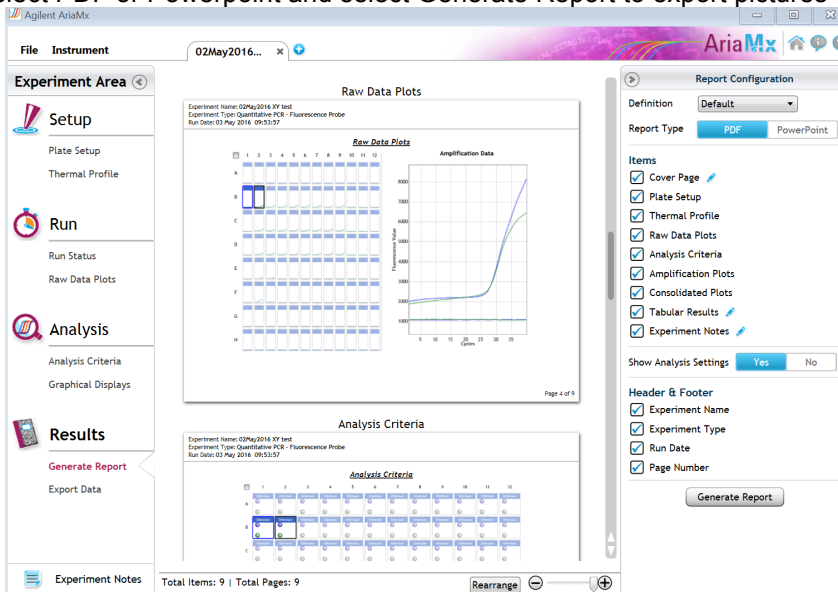
### 2.1. Exporting the Cq values into an Excel spreadsheet.

- 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...

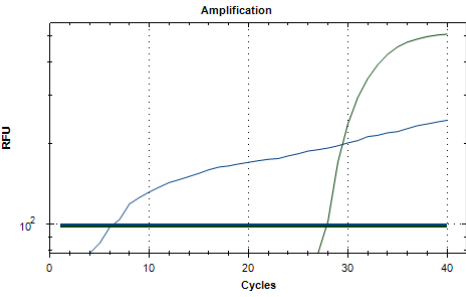


### 2.2. Saving a visual of the graph

- 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 FemINDICATOR™ 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 Decontamination and 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	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 Decontamination and qPCR by following the protocol.
	Insufficient pre-setup bleaching	Wipe down the lab workspace and all equipment with 10% Bleach. Repeat Decontamination and qPCR Protocols.
Negative Positive Control	Mix up in Reaction Setup	Repeat the Decontamination and qPCR by following the protocol.
Total run failure	Excessive vortex of the qPCR Master Mix	Repeat the Decontamination and 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 fluorescent signal. This is usually a negative result, but should be repeated if the trace cannot be confirmed as background.

## **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 samples where no or a very high Cq is expected. It helps to ensure that all Assay specific reactions are clean of contaminants.

The assay specific **Positive Controls** are the samples 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 where a Cq is expected. It ensures the effectiveness and efficiency of each reaction. The internal control is targeting a Single Copy Control Gene or SCCG, using the HEX fluorophore.

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