

PathoSEEK[®] Virus/Viroid Detection Assays

User Guide v5

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) Assays for the detection of virus/viroid in cannabis plants

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Introduction

The PathoSEEK® Cannabis Virus Detection kits combine a reverse transcriptase with a quantitative PCR (qPCR) assay to detect the presence of viroids and/or viruses in addition to an internal plant DNA reaction control. Unlike other techniques, this multiplexing strategy verifies the performance of the assay when detecting plant pathogens, resulting in the minimization of false negatives due to reaction set-up errors or failing experimental conditions.

Process Overview

The reverse transcriptase enzyme creates complementary DNA (cDNA) from a messenger RNA (mRNA) template, which then becomes the template DNA for qPCR. PathoSEEK® qPCR Detection Assays use a multiplex system of primers to detect both cannabis DNA and the targets of interest. The figure below shows a simplified depiction of the process.

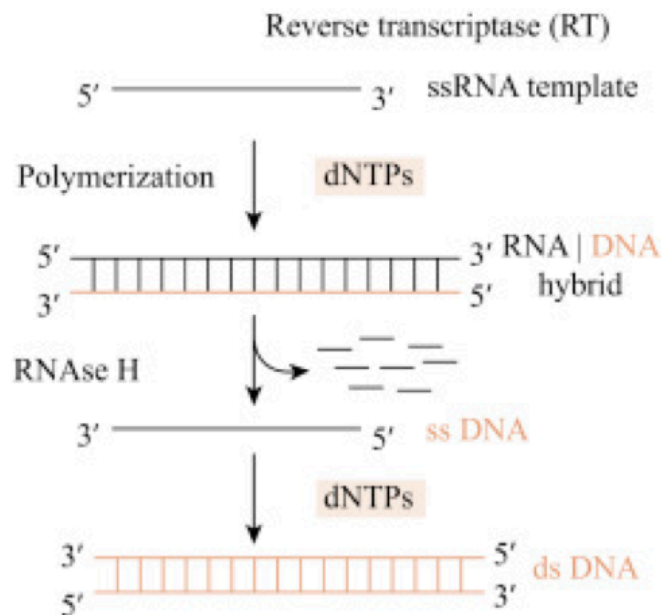


Figure 1: Overview of Reverse Transcription

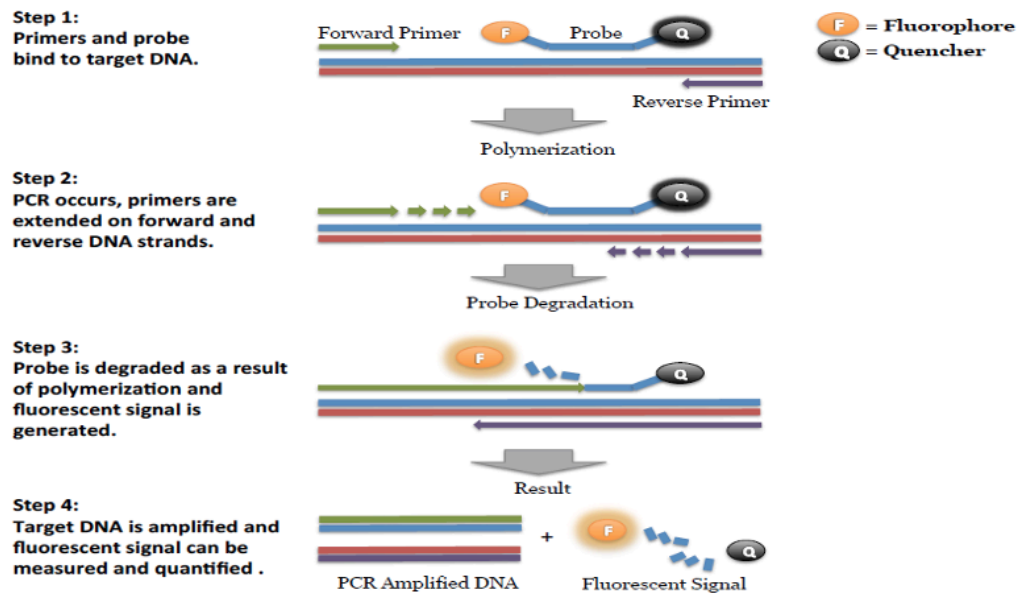


Figure 2: Overview of qPCR

Test Kit Information

Virus Assays Kits:

1. **PathoSEEK® Hop Latent / Lettuce Chlorosis / Cannabis Cryptic Virus Multiplex Detection Assay Kit - P/N 420513**
 - a. Assay — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.
 - b. PathoSEEK® Amplification Mix - 4 Vials - store lyophilized at room temperature until ready to use. Before first use rehydrate in 550 µL of Nuclease Free Water and swirl or gently tip mix. Any unused rehydrated master mix can be stored at -20 °C for up to 3 months.
2. **PathoSEEK® Hop Latent Viroid Detection Assay v2 Kit- P/N 420512**
 - a. Assay — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.
 - b. PathoSEEK® Amplification Mix - 4 Vials - store lyophilized at room temperature until ready to use. Before first use rehydrate in 550 µL of Nuclease Free Water and swirl or gently tip mix. Any unused rehydrated master mix can be stored at -20 °C for up to 3 months.
3. **PathoSEEK® Tobacco Mosaic Virus Detection Assay v2 Kit - P/N 420530**
 - a. Assay — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.
 - b. PathoSEEK® Amplification Mix - 4 Vials - store lyophilized at room temperature until ready to use. Before first use rehydrate in 550 µL of Nuclease Free Water and swirl or gently tip mix. Any unused rehydrated master mix can be stored at -20 °C for up to 3 months.
4. **PathoSEEK® Beet Curly Top Virus Detection Assay v2 Kit - P/N 420531**
 - a. Assay — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.
 - b. PathoSEEK® Amplification Mix - 4 Vials - store lyophilized at room temperature until ready to use. Before first use rehydrate in 550 µL of Nuclease Free Water and swirl or gently tip mix. Any unused rehydrated master mix can be stored at -20 °C for up to 3 months.

Virus Assay Controls:

1. PathoSEEK® Virus Multiplex HLV, CCV, LCV Positive Control - P/N 420126.
 - a. Control — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.

2. PathoSEEK® Hop Latent Viroid Detection Assay Positive Control - P/N 420123.
 - a. Control — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.
3. PathoSEEK® Tobacco Mosaic Virus Assay Positive Control - P/N 420132.
 - a. Control — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.
4. PathoSEEK® Beet Curly Top Virus Assay Positive Control - P/N 420131.
 - a. Control — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.

Supplies, Reagents, and Equipment

1. Agilent AriaMx Real-Time PCR System G8830A—Containing the following Optical Channels:
FAM and HEX. ROX (Can be used to detect Texas Red fluorophore) and Cy5 if running
multiplex assay
 - a. Agilent HP Notebook PC option 650
 - b. Optical Strip Caps.—Agilent #401425
Note: If using adhesive seals instead of strip caps, use Applied Biosystems MicroAmp Optical Film Compression Pad, Fisher Scientific, #43-126-39 to prevent evaporation and cross contamination between wells.
2. Bio-Rad CFX96 Touch™ Real-Time System
 - a. Bio-Rad Personal PC
3. 96-Well Optical qPCR plate - Medicinal Genomics #100164
4. Adhesive optical seal for qPCR plates - Medicinal Genomics #100177
5. PCR Grade Nuclease Free Water - Medicinal Genomics #420184
6. Multi-channel pipette P20, P50, P200 (optional)
7. Single channel pipette P10, P20, P200, and P1000
8. Filtered pipette tips for P10, P20, P50, and P200
9. Compact PCR Tube Rack (USA Scientific, #2300-9602 or similar)
10. 1.5 mL tubes (USA Scientific #1415-2600)
11. Crushed ice or cold racks (1.5ul Tube Bench-top Cryogenic Racks, VWR #89004-558 or similar)
12. Tabletop mini plate centrifuge (Fisher Scientific #14-100-143 or similar)

13. Dual rotor personal microcentrifuge, (USA Scientific #2641-0016)
14. Tabletop Vortex Genie (Scientific Industries #SI-0236 or Similar)
15. Lab Refrigerator, 4 °C
16. Lab Freezer, -20 °C
17. Laboratory Gloves (USA Scientific, # 4904-3300 or similar)
18. 10% bleach

Safety Precautions and Recommendations for Best Results

Environment

1. The quality of results depends on strict compliance with Good Laboratory Practices (for example, the EN ISO 7218 standard), especially concerning PCR:
 - a. Never circulate lab equipment from one workstation to another.
 - b. Always use a positive and negative control for each series of amplification reactions.
 - c. Periodically verify the accuracy and precision of pipettes, as well as correct functioning of the instruments.
 - d. Change gloves often, especially if you suspect contamination.
 - e. Clean workspaces periodically with 10% bleach and other decontaminating agents.
 - f. Use powder-free gloves and avoid fingerprints and writing on tube caps because both can interfere with data acquisition.

Safety Precautions

1. Assay users should observe good lab practices and safety precautions when performing this assay. Wear protective gloves, lab coats, eye/face protection as indicated by your quality system.
2. 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[®] Virus Detection Assays and all components required to perform the method are intended for use by trained personnel familiar with laboratory techniques associated with viral detection.

Plant Tissue Sampling

Please refer to the [Plant Sampling Guide](#) located at medicinalgenomics.com > Resources > Protocols

RT- qPCR Protocol

1. Remove RT-qPCR Reagents including Amplification Mix, Viral Detection Assay tube, and assay positive control from the -20 °C freezer.
 - a. If Lyophilized PathoSEEK[®] Amplification Mix enzyme has not been rehydrated, rehydrate in 550 ul of Nuclease Free water. Swirl or Pipette mix. After resuspension, store at -15 to -20 °C when not in use.
 - b. Allow all frozen reagents to defrost at room temperature. Once defrosted, place tubes on ice.
 - c. Keep PathoSEEK[®] Amplification Mix cool on ice or in the storage freezer while frozen reagents thaw.
2. Before preparing the master mix, invert or vortex and spin down the reagents.
 - a. Detection Assay – Vortex quickly followed by a pulse spin down in a microcentrifuge.
 - b. Assay Positive Control tube – Vortex quickly followed by a pulse spin down in a microcentrifuge.
 - c. PathoSEEK[®] Amplification Mix – Invert the bottle 5-10 times to mix.
 - d. Return all reagents to the ice.
3. Prepare master mix in a 1.5mL tube (the detection assay also contains the internal plant control, ICC probe mix). Label tube “RT-qPCR MM”. Always prepare enough master mix for an additional 1 or 2 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 plants, you

would need to make enough master mix for 13 or 14 reactions, which would account for 1 or 2 excess.

Note: *It is best to add Amplification Mix first and Virus Detection Assay last.*

Reagent	Volume for 1 Reaction
PathoSEEK [®] Amplification Mix	10 μ L
Virus Detection Assay	1 μ L
Total	11 μ L

- a. Once combined, carefully tip mix or invert the tube 5 times to combine.
 - b. Pulse spin down in microcentrifuge.
 - c. Place RT-qPCR MM tube on ice until used in step 5.
4. Make positive control.
 - a. For the positive control(s), transfer 1 μ L of Assay Positive Control into 9 μ L of water, vortex to mix well and pulse spin down in a microcentrifuge. Change gloves after performing this dilution.

Note: *It is best to add the largest volume reagent first, in this case water.*
 - b. For the negative control, use water (found in the kit).
5. Transfer Prepared Master Mix to qPCR Plate.
 - a. Add 11 μ L of prepared RT-qPCR MM to each well of qPCR plate that will be used for reactions (e.g., sample wells, positive control well, and negative control well).
6. Transfer the lysed samples, positive control, and negative control to their corresponding reaction wells.
 - a. Open caps of tubes containing lysed samples carefully (pay attention not to spill any liquid). Transfer 9 μ L of each lysed sample into a separate well of the qPCR plate and slowly tip mix. Avoid adding bubbles to the mixture.
 - b. Transfer 9 μ L of the positive control into the designated positive control well.
 - c. Transfer 9 μ L of PCR Grade Nuclease Free Water water into the designated negative control well.

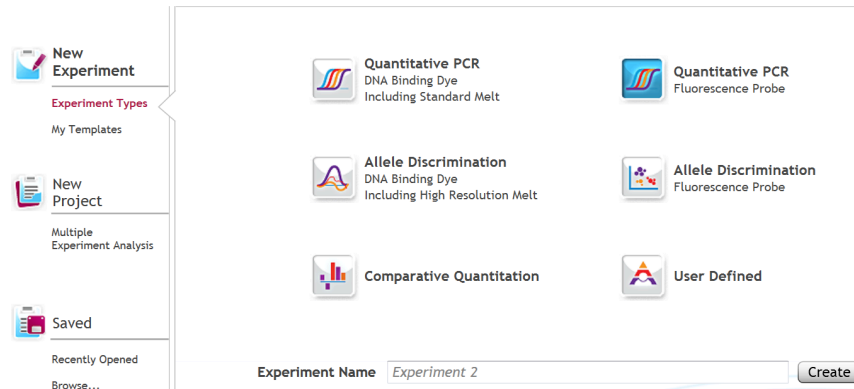
Note: *Be sure to use a new filtered tip for each transfer!*
7. Seal the plate with strip caps or adhesive seal. If using an adhesive seal make sure to completely seal the plate wells and plate edges using a pen or flat object to slide back and forth along the seal.

- a. Spin plate down for at least 1 minute in the plate microcentrifuge.
Note: *Check for bubbles at the bottom of the wells (a few bubbles on the surface edge of the liquid is acceptable). If bubbles remain in the bottom of the wells or cover the top, spin down for another minute.*
- b. Label the plate as RT-qPCR Plate_[date].
- c. 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 holes in the plate.
- d. Place the sealed plate onto the qPCR instrument, positioning the A1 well in the top left corner.
- e. Close the lid.
- f. Follow the software-specific instructions to initiate the run.

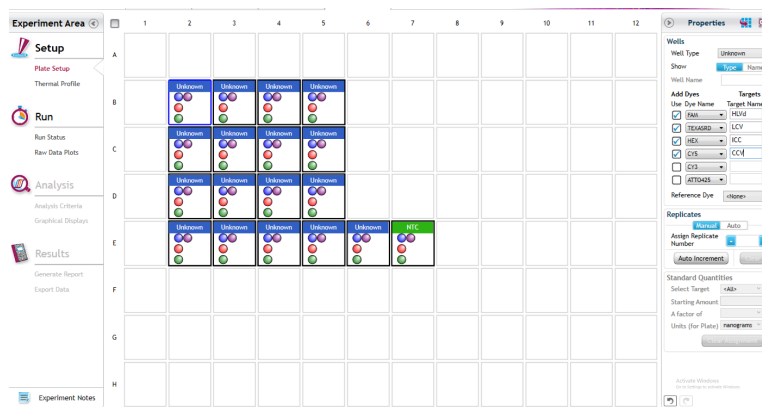
Agilent AriaMX

Setting up the run:

1. Create a New Experiment on the Agilent AriaMX qPCR instrument.

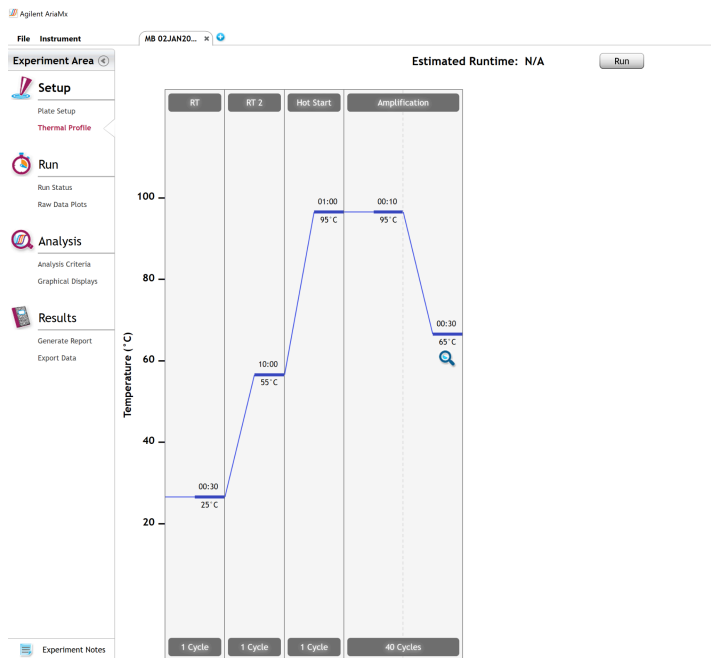


- a. Select “Quantitative PCR: Fluorescence Probe” from Experiment Types.



- b. Turn on wells that have samples, and assign the targets to the appropriate dye.
 - i. PathoSEEK[®] Virus Multiplex Detection Assay A
 1. FAM: HLVd
 2. Texas Red: LCV
 3. Cy5: CCV
 4. HEX: Internal Cannabis Control (ICC)
 - ii. PathoSEEK[®] Hop Latent Viroid, Tobacco Mosaic or Beet Curly top Detection Assays

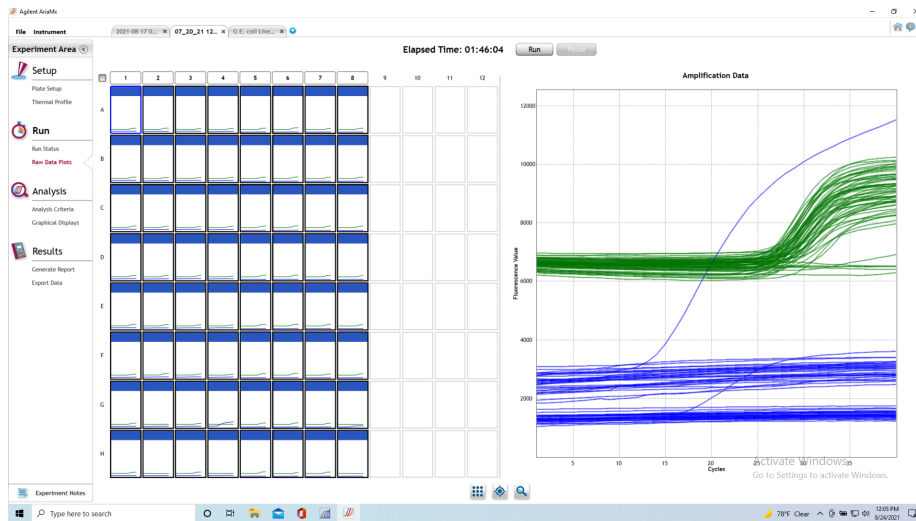
1. FAM: HLVd, TMV, or BCTV
 2. HEX: Internal Cannabis Control (ICC)
2. Set RT-qPCR Cycling Conditions.
- a. Under Setup > Thermal Profile, create the following PCR thermal profile.
 - i. 25 °C for 30 seconds.
 - ii. 55 °C for 10 minutes
 - iii. 95 °C for 1 minute
 Followed by **40** cycles of:
 - iv. 95 °C, 10 seconds
 - v. 65 °C, 30 seconds
 - vi. Plate read



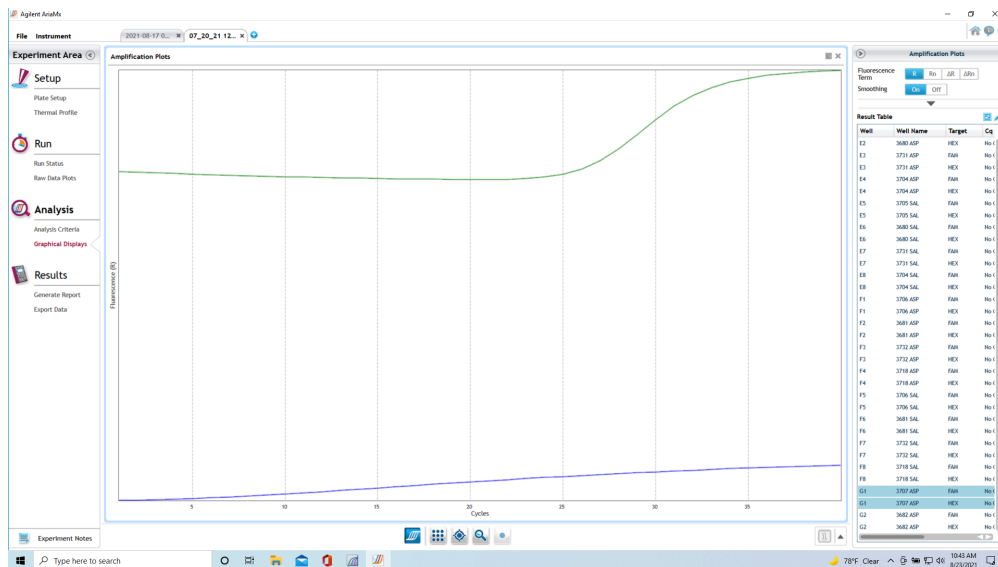
3. Save the experiment with the [User] and [date].
4. When the run is complete, immediately dispose of the plate. Do not open the plate seal after the run to avoid contamination in the lab.

Data Analysis:

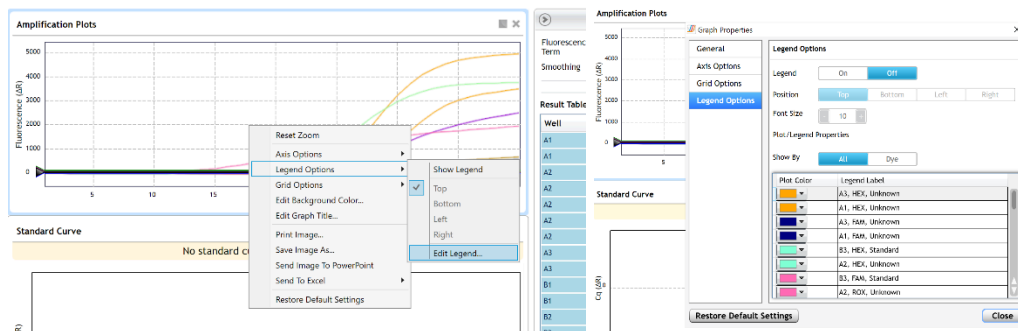
1. Review the raw data for each sample.
 - a. Use the plate view of the Raw Data Plots to scroll across the plate to confirm amplification in sample wells and positive control wells. This review also ensures that thermal cycling is uniform across the plate

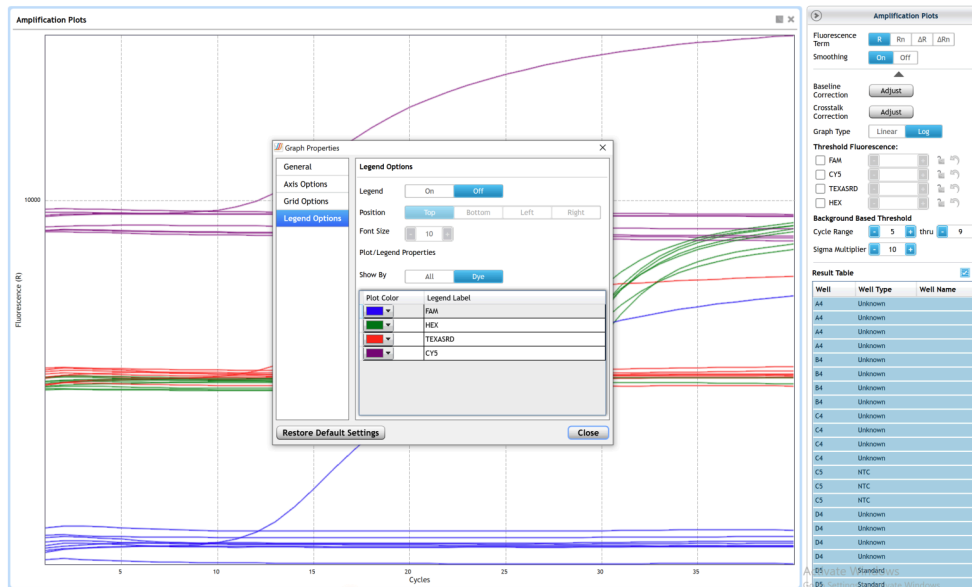
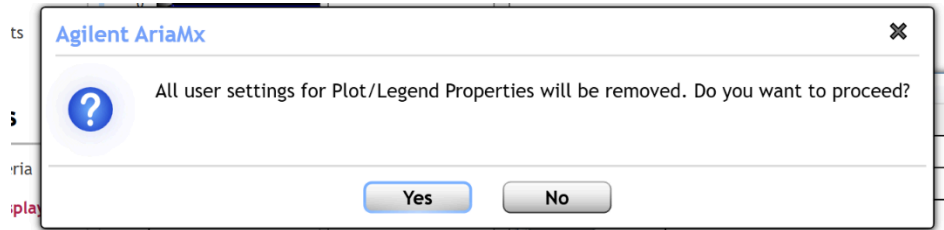


- b. Look at the raw data in the "Graphical Displays" view.
 - c. For each target make sure the baseline is flat and the fluorescence signal grows rapidly over 5-7 cycles as seen below. (To view the raw data select "R" next to "Fluorescence Term" at the top right-hand side of the screen.)

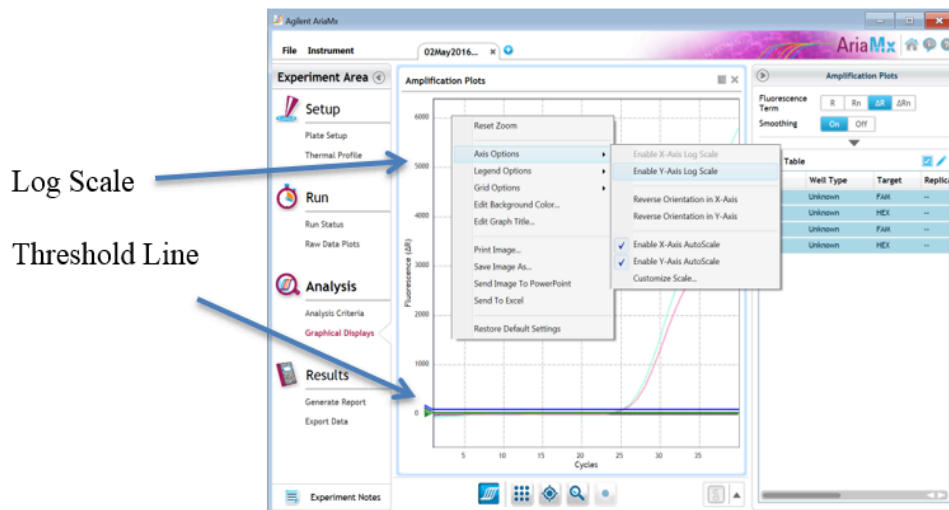


2. Presence / Absence Assay:
 - a. Open the Data Analysis window when the run is complete.
 - b. Highlight the wells of interest in the Analysis Criteria under Analysis, then select Graphical Display.
3. Amplification plots will be available for viewing.
4. The Cq values will appear to the right in the table.
5. Right-click inside the graph, select Edit Legend under Legend Options.
6. Change “All” to “Dye”.
7. All user settings for Plot/Legend Properties will be removed. Do you want to proceed? Select “Yes”.
8. This will assign a single color to each fluorophore.





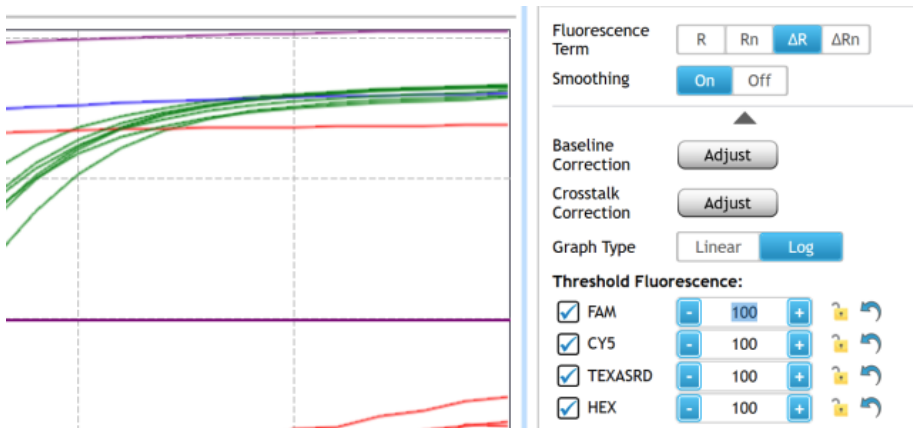
9. To analyze the results, start by turning the graph to Log Scale with a right-click on the chart, select Axis options, and enable y-axis log scale.



10. Expand the amplification plots settings by clicking on the triangle.

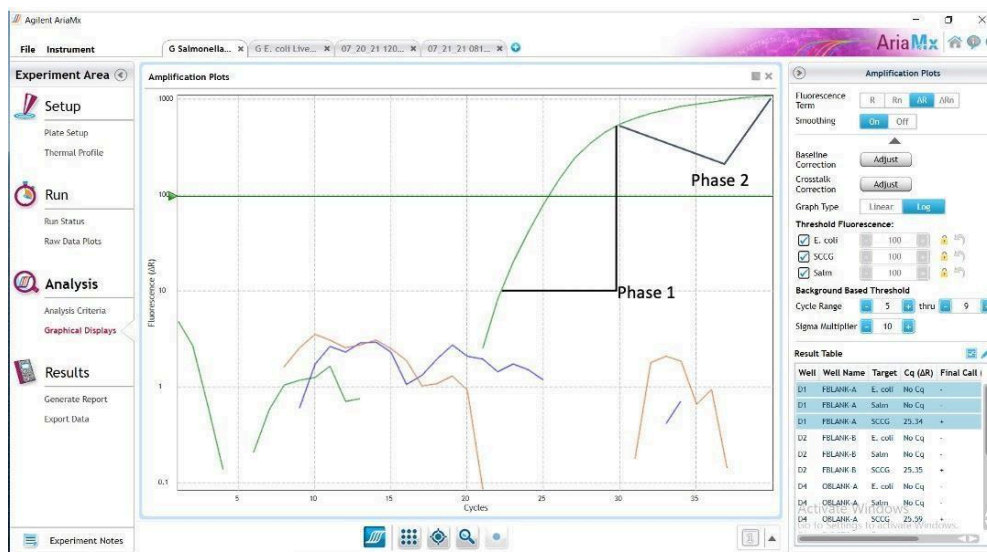


11. Manually adjust and lock thresholds to 100 RFU for all fluorophores.



12. Check the amplification plots for a semi-logarithmic curve with two distinct phases that crosses the threshold

- Phase 1 shows exponential growth in the fluorescence, in a span of around 5 cycles.
- Phase 2 is a plateau where the amplification signal growth ends but remains level.



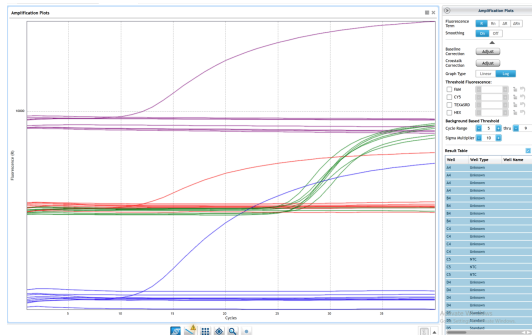
13. Assay Controls

- a. PathoSEEK® Virus Multiplex HLVD, CCV, LCV Positive Control
 - i. FAM, Texas Red and Cy5 fluorophores, Cq values < 35.
 - 1. Visually confirm with the curve on the graph.
- b. PathoSEEK® Hop Latent Viroid, Tobacco Mosaic Virus or Beet Curly Top Virus Positive Control
 - i. FAM fluorophore, Cq value < 35.
 - 1. Visually confirm with the curve on the graph.
- c. PathoSEEK® Virus Multiplex HLVD, CCV, LCV Negative Control
 - i. FAM, Texas Red and Cy5 fluorophores, No Cq value
 - 1. Visually confirm with the curve on the graph.
- d. PathoSEEK® Hop Latent Viroid, Tobacco Mosaic Virus or Beet Curly Top Virus Negative Control
 - i. FAM fluorophore, No Cq value
 - a. Visually confirm with the curve on the graph.

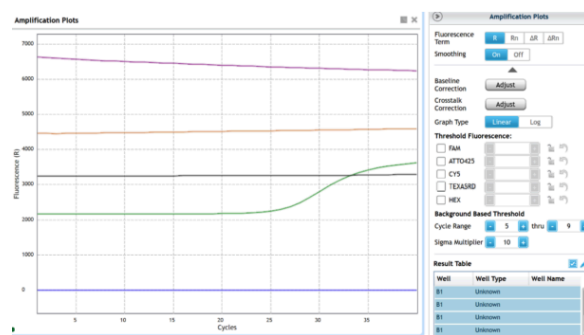
14. Unknown Leaf or Root Samples

- a. A “presence” result for the unknown sample.
 - i. Any Cq value for the FAM, Texas Red or Cy5 fluorophores ≤ 35
 - 1. If the Cq value is between 35 and 40: Retest sample with fresh root if available. If root is not available, re-test a fresh leaf sample. If the retest value results in a Cq of less than 35 the sample should be considered positive. If the Cq value is still between 35 and 40 the result is inconclusive.
 - ii. 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.)
- b. An “absence” result for the unknown sample.
 - i. Internal Cannabis Control, on the HEX fluorophore, has a Cq value ≤ 35 .
 - 1. Visually confirm with the curve on the graph
 - ii. No Cq value for the FAM, Texas Red or Cy5 fluorophores.

- iii. Visually confirm no curve on the graph.
- iv. Review the Raw, Linear data for all samples. No channel should have “0” baseline value for any channels being assessed (see below):



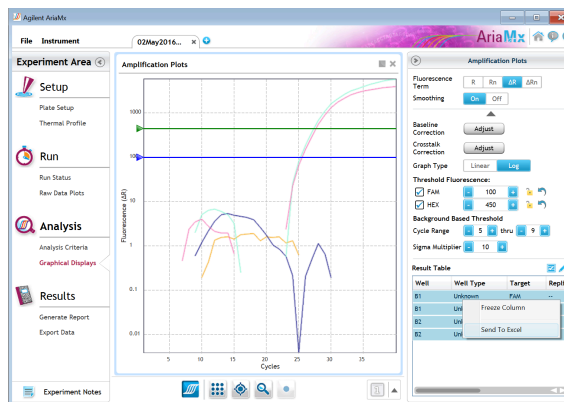
Example of acceptable Raw, Linear data.



Example of unacceptable Raw, Linear data

15. Export the Data

- a. Exporting the Cq values into an Excel spreadsheet:
 - i. To export the Cq values to an Excel spreadsheet, right-click on the chart on the bottom right of the screen.
 - ii. Choose Export To Excel.



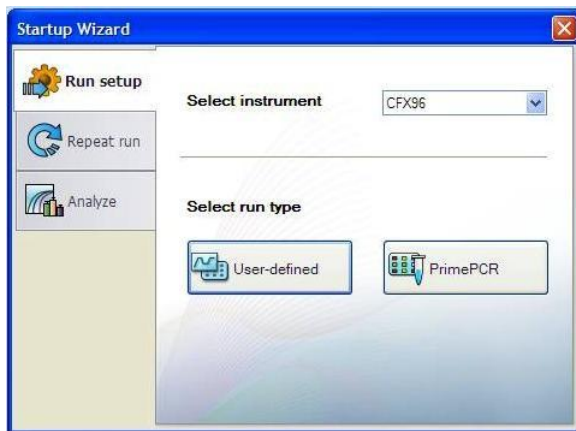
16. Saving a visual of the graph

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

Bio-Rad CFX 96

Setting up the run

1. Create a New Experiment on the Bio-Rad qPCR instrument.
 - a. Select “User Defined” under run type, then select "Create New".

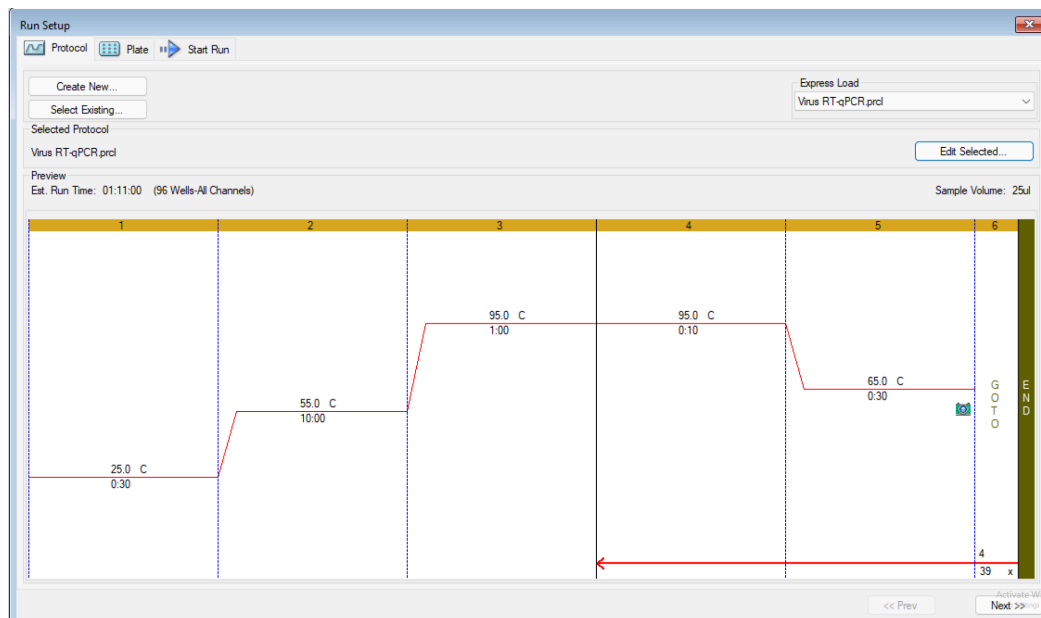


- b. Set RT-qPCR Cycling Conditions as follows:

- i. 25 °C for 30 seconds.
 - ii. 55 °C for 10 minutes
 - iii. 95 °C for 1 minute

Followed by **40** cycles of:

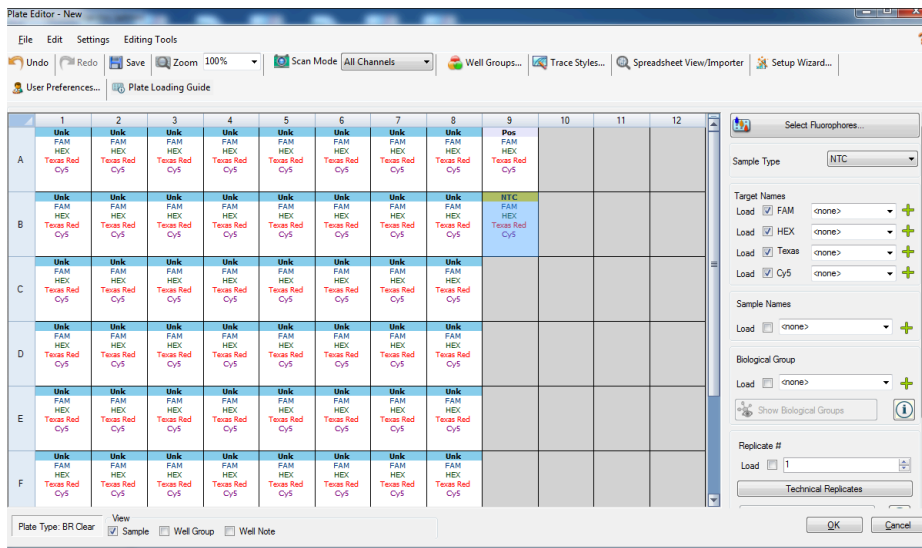
- iv. 95 °C, 10 seconds
 - v. 65 °C, 30 seconds
 - vi. Plate read



- c. Save as RT-qPCR in the 'Express Load' folder.
- d. Click next.
- e. On the Plate Editor Screen, change the Sample Type to correlate with your 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:

1. Unknown
2. Positive Control
3. Negative Control



f. Choose correct fluorophores, see below. Save the plate as ‘RT-qPCR’ in the ‘express load’ folder.

i. PathoSEEK® Virus Multiplex Detection Assay A

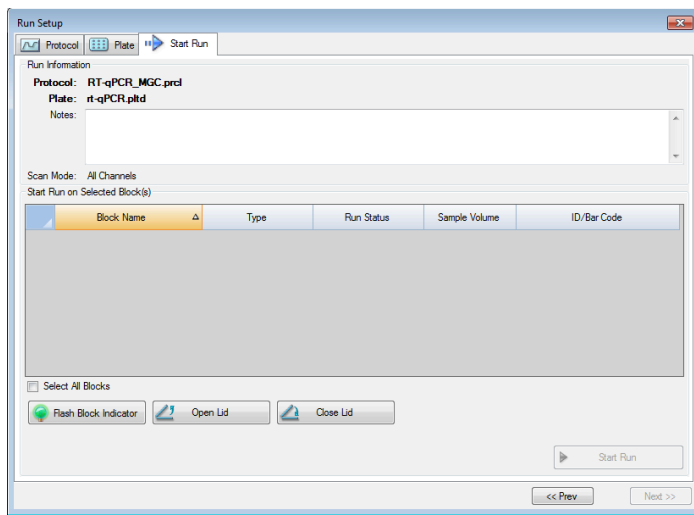
1. FAM: HLVd
2. Texas Red: LCV
3. Cy5: CCV
4. HEX: Internal Cannabis Control (ICC)

ii. PathoSEEK® Hop Latent Viroid, Tobacco Mosaic, or Beet Curly top Detection Assays

1. FAM: HLVd, TMV, or BCTV
2. HEX: Internal Cannabis Control (ICC)

g. Click Ok, then next.

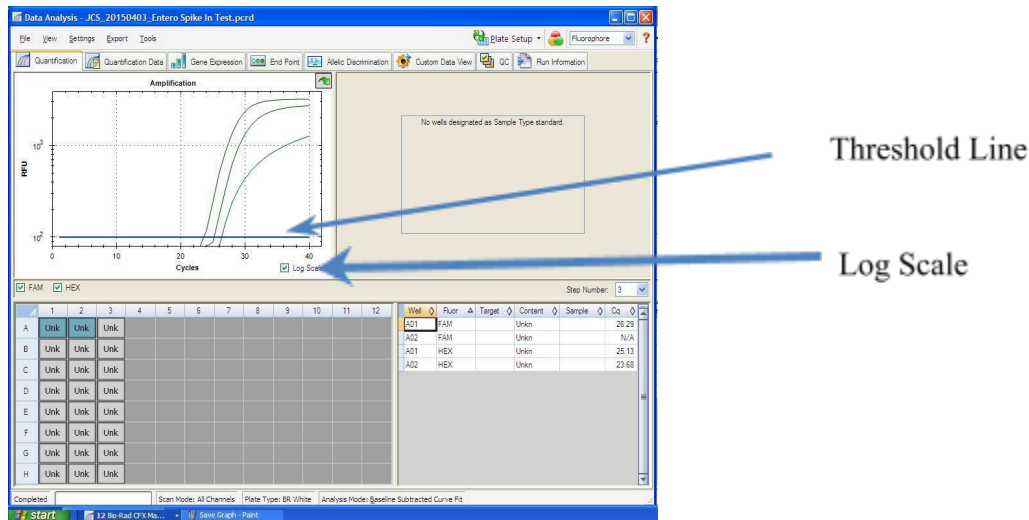
2. Place the sealed plate into CFX 96, close the lid, and click ‘start run’.



3. When the 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

1. The Data Analysis window will open automatically when the run is complete.
2. Highlight the well 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. 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.

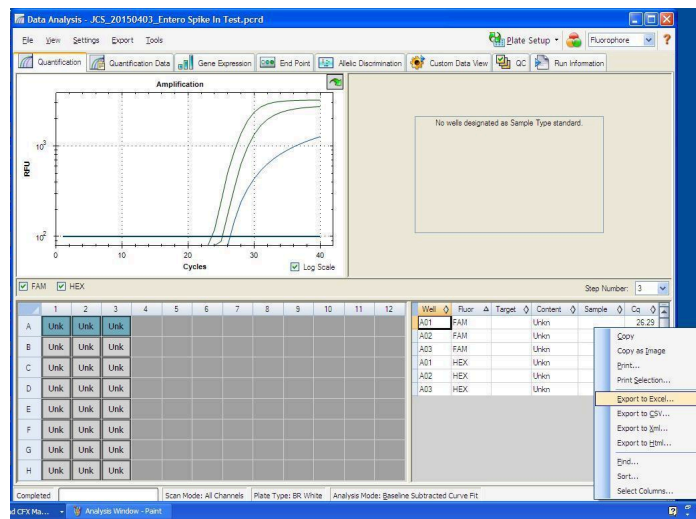


4. Assay Controls
 - a. PathoSEEK[®] Virus Multiplex HLVD, CCV, LCV Positive Control
 - i. FAM, Texas Red, and Cy5 fluorophores, Cq values < 35.
 1. Visually confirm with the curve on the graph.

- b. PathoSEEK® Hop Latent Viroid, Tobacco Mosaic Virus or Beet Curly Top Virus Positive Control
 - i. FAM fluorophore, Cq value < 35.
 - 1. Visually confirm with the curve on the graph.
 - c. PathoSEEK® Virus Multiplex HLVd, CCV, LCV Negative Control
 - i. FAM, Texas Red and Cy5 fluorophores, No Cq value
 - 1. Visually confirm with the curve on the graph.
 - d. PathoSEEK® Hop Latent Viroid, Tobacco Mosaic Virus, or Beet Curly Top Virus Negative Control
 - i. FAM fluorophore, No Cq value
 - 1. Visually confirm with the curve on the graph.
5. Unknown Leaf or Root Samples
- a. A “presence” result for the unknown sample.
 - i. Any Cq value for the FAM, Texas Red or Cy5 fluorophores ≤ 35
 - 1. If the Cq value is between 35 and 40: Retest sample with fresh root if available. If root is not available, re-test a fresh leaf sample. If the retest value results in a Cq of less than 35 the sample should be considered positive. If the Cq value is still between 35 and 40 the result is inconclusive.
 - ii. 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.)
 - b. An “absence” result for the unknown sample.
 - i. Internal Control, on the HEX fluorophore, has a Cq value ≤ 35 .
 - 1. Visually confirm with the curve on the graph
 - ii. PathoSEEK® Virus Multiplex HLVd, CCV, LCV
 - 1. No Cq value for the FAM, Texas Red, or Cy5 fluorophores
 - iii. PathoSEEK® Hop Latent Viroid, Tobacco Mosaic Virus or Beet Curly Top Virus
 - 1. No Cq value for the FAM fluorophore
 - iv. Visually confirm no curve on the graph.

6. Export the Data.

- a. Exporting the Cq values into an Excel spreadsheet:
 - i. To export the Cq values to an Excel spreadsheet, right-click on the chart on the bottom right of the screen.
 - ii. Choose Export To Excel.



- b. Saving a visual of the graph:
 - i. To save a picture of the graph, right click on the graph on the top left of the screen and choose “Save Image As”.

PathoSEEK® RT - qPCR Virus Assay Analysis Quick Reference Table

Assay	Cq Value	Fluor	Negative Control (Cq)	Cq threshold
PathoSEEK® Virus Multiplex Detection Assay A				
HLV – Hop Latent Viroid	≤ 35	FAM	No Value	Presence/Absence
LCV – Lettuce Chlorosis Virus	≤ 35	Texas Red	No Value	Presence/Absence
CCV – Cannabis Cryptic Virus	≤ 35	Cy5	No Value	Presence/Absence
PathoSEEK® Hop Latent Viroid Detection Assay				
HLVd – Hop Latent Viroid	≤ 35	FAM	No Value	Presence/Absence
PathoSEEK® Tobacco Mosaic Virus Detection Assay				
TMV – Tobacco Mosaic Virus	≤ 35	FAM	No Value	Presence/Absence
PathoSEEK® Beet Curly Top Virus Detection Assay				
BCT - Beet Curly Top Virus	≤ 35	FAM	No Value	Presence/Absence
Internal Control*	≤35	HEX	*Internal control verifies the presence or absence of plant DNA	
Assay Positive Control	<35	Virus MPX Assay = FAM/Texas Red/Cy5 HLVd, TMV and BCT Assays = FAM Only		

Troubleshooting Guide

Symptom	Reason	Solution
Internal cannabis control (ICC Primer) failure	Nucleic Acid Isolation Failure	Repeat Quick Lysis or PurePrep by following the protocol.
	If using PurePrep Residual ethanol in elution	Ethanol is an inhibitor to RT-qPCR. Return to the PurePrep protocol and repeat all steps.
	qPCR inhibition	Dilute extracted or lysed samples 1:10 with qPCR grade water and repeat the RT-qPCR.
	Mix up in Reaction Setup	Repeat the RT-qPCR by following the protocol.
	Missing Fluorophore on plate set up	Check Plate Setup to ensure the correct fluorophores were chosen on setup of run. They can be corrected post run.
Internal Cannabis Control (ICC) 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 RT-qPCR setup
Positive Negative Control	Small Cq value <15	Visually confirm that there is a true amplification curve. If not, this may be considered a background trace.
	Contamination	Repeat the RT-qPCR by following the protocol.
Positive Negative Control (Continued)	Insufficient pre-setup bleaching	Clean workspace and all equipment with 10% Bleach, repeat rt-qPCR.
Negative Positive Control	Mix-up in Reaction Setup	Repeat the RT-qPCR by following the protocol.
Total run failure	Excessive vortexing the RT-qPCR Master Mix	Repeat the RT-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 displays 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.

Reverse Transcription (RT): the process by which an enzyme, called reverse transcriptase, converts the viral RNA genome into a complementary DNA (cDNA) molecule.

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, Texas Red and Cy5 Fluorophores.

Amplification of the **Internal Cannabis Control (ICC)** is expected in every reaction containing cannabis DNA. It ensures the nucleic acid isolation procedure was successful. The internal control targets cannabis DNA, using the HEX Fluorophore.

REVISION HISTORY

Version	Date	Description
v1	February 2021	Product Launch
v2	March 2023	Additional Tissue Sampling Instructions
v3	November 2023	RT-qPCR Master Mix v2 and Cycling Conditions
v4	January 2025	<ol style="list-style-type: none"> 1. Name change - RT-qPCR Master Mix v2 changing to PathoSEEK[®] Amplification Mix. Additional vial included - 3 total for 200 reactions 2. Virus Triplex Assay and HLVdv2 Assay sold as kit with Amplification Mix 3. Updated Troubleshooting Guide
v5	February 2025	<ol style="list-style-type: none"> 1. Updating Amplification Mix to new format (4 vials @ 50 reactions each vial)

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 Agilent AriaMX or BIO-RAD CFX96 Touch[®] Real-Time PCR Detection System.

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