

# Development of a high throughput PPV detection method based on unique nucleic acid isolation system

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## ABSTRACT

Plum pox virus (PPV) is a serious plant disease affecting tender fruit including peaches, plums, apricots and nectarines, causing significant yield losses. Evidence suggests that the Dideron strain of PPV was first discovered in Ontario in 2000. Despite the rigid PPV eradication program that has been in place in Canada over the last decade, PPV still remains a threat to tender fruit growers in the Niagara region. PPV is transmissible from infected trees by aphids and it can take several years before virus titers are high enough or present in enough of the tree to be detectable by ELISA. This test is currently used by the Canadian Food Inspection Agency (CFIA) in the eradication program because of the assays specificity, low cost, high throughput and relatively simple methodology. Nevertheless, cross-reactivity, strain variability, as well as uneven and low virus titers in plant tissue have been associated with detection inconsistencies. Nucleic acid (NA) based detection has been proven to be more sensitive than immunological diagnostic methods; however, NA-based detection is more expensive and not as simple to implement. At Norgen Biotek Corp we have developed a NA-based PPV detection kit that is rapid, sensitive, inexpensive and simple to adopt high throughput robotic system. To improve the current PPV detection system, we have focused on the optimization of RNA purification, as sample preparation is the most critical step in any reliable diagnostic kit. Here we present In-house evaluation data on this NA-based PPV detection kit. We also anticipate to evaluate the PPV detection kit further with a large volume of field samples through a collaboration with a third party where regulatory pathogens (e.g. PPV) can be handled and analyzed.

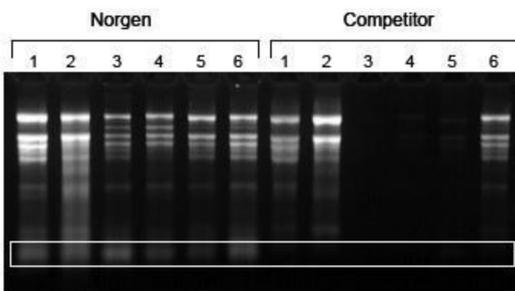
## INTRODUCTION

Plum pox virus (PPV) is a member of the genus *Potyvirus*, and the virus is considered the most serious pathogen affecting stone fruits including apricots, cherry, nectarine, peaches, and plums. The symptoms of plum pox disease or Sharka include fruit abnormality, loss of taste, and fruits that drop prematurely. These symptoms may cause losses as high as 80–100% of a crop and consequently it is a disease of economic significance in every country where stone fruits are grown. To achieve successful eradication or effective management of the associated disease, detection of the virus in infected plants is required.

As sample preparation is the most critical step in any reliable diagnostic analysis, Norgen's proprietary resin showed outstanding affinity to purify all sizes of RNA from large mRNA and microRNA (miRNA) including small interfering RNA (siRNA) in comparison with a current market leader. This distinctive advantage has become a powerful tool contributing to a sensitive detection of virus and viroids. Single and 96 well plate RNA purification format has been developed with a compatibility with a robotic automation system for a high throughput analysis.

In order to provide a reliable, fast and cost effective PPV detection method, PPV RT-PCR Detection Kit was developed and evaluated in house with PPV transcripts expressed from the universal region of PPV RNA.

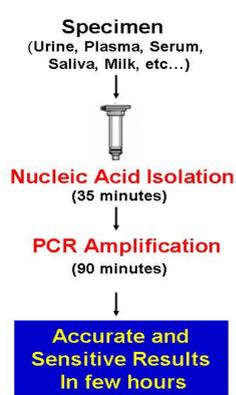
## RESULTS



**Figure 1. Isolation of High Quality RNA, even from Difficult Samples.** Total RNA was isolated from 50 mg samples of apple (Lanes 1), peach (Lanes 2), grape (Lanes 3), pine needle (Lanes 4), strawberry (Lanes 5) and pear (Lanes 6) using Norgen's kit and a competitor's kit. Five  $\mu$ L of total RNA from the 50  $\mu$ L of elution was loaded on 1X MOPS 1.0 % Formaldehyde-Agarose RNA gel for analysis. Norgen's kit allowed for the isolation of high quality RNA from all the samples, including the difficult samples, while the competitor failed to isolate RNA from grape, pine needles and strawberry. Furthermore, only Norgen's kit was able to isolate the small RNA species (white box).

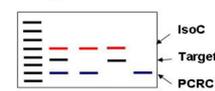


## Single Column system

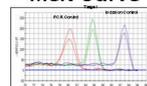


### Two Options for Detection:

#### 1) Agarose Gel

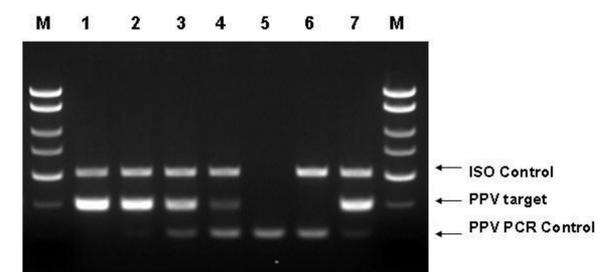
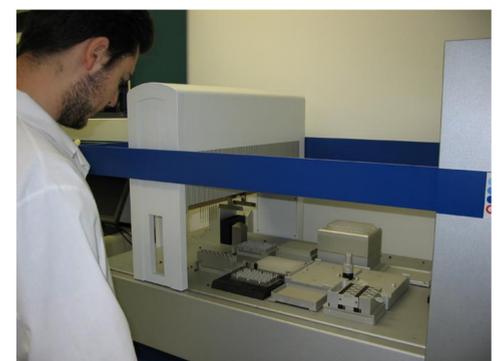


#### 2) Real-Time PCR Melt Curve



**Figure 2. Norgen's PPV RT-PCR Detection Kit is a ready-to-use system for the isolation and detection of PPV from plant samples.** First, the kit contains components for the rapid isolation of total RNA, including viroid RNA, from the samples using spin-column chromatography based on Norgen's proprietary resin. Second, the kit contains PPV Master Mix and controls to allow for PCR Amplification. The amplified PCR products are then detected using agarose gel electrophoresis. Alternatively, detection can be performed based on real-time PCR using melt curves.

## 96 well plate & Robotic system



**Figure 4. Interpretation of the Plum Pox RT-PCR Detection Kit.** A representative 1X TAE 1.7% agarose gel showing the amplification of Plum Pox Virus at different concentrations (PPV Target). The size of the PPV target amplicon corresponds to 295 bp as represented by the provided RNA Marker (M). The size of the PPV Isolation Control (Iso C) corresponds to 498bp as represented by the provided RNA Marker (M). The PPV 2X PCR Master Mix contains a PPV PCR Control (PCRC). The PPV PCRC controls for PCR inhibition. The size of the PPV PCRC corresponds to 150bp as represented by the provided RNA Marker (M). The amplification from each lane is interpreted as shown below. Lane 1 to 4: Plum Pox Virus detected: Lanes showed the detection of all three PCR amplicons. Lane 5: No IsoC and Template- Only PPV PCRC was detected. Lane 6: PPV not detected: Detection of PPV IsoC and PPV PCRC, suggesting that the RNA isolation was successful but no PPV RNA was present in the sample. Lane 7: Positive: All three PCR amplicons were detected



**Figure 3. The kit is a ready-to-use system for the isolation and detection of Plum Pox Virus from PPV host samples.** The PPV Master Mix contains reagents and enzymes for the specific amplification of a 295 bp region of the viral genome. In addition, PPV PCR Detection Kit contains a second heterologous amplification system to identify possible PCR inhibition and/or inadequate isolations thereby eliminating false negatives.

## Summary & Discussion

The results of this study indicate that RNA extraction from plant samples with Norgen's proprietary resin yields high quality, inhibitor-free RNA, which can be successfully amplified by RT-PCR for the detection of PPV. To detect PPV using PCR method it is important that the samples of nucleic acids be as pure as possible. This is especially true when a reverse transcription phase is necessary, as the reverse transcriptase is highly susceptible to interfering or inhibitory substances. Norgen's PPV RT-PCR Detection Kit is an efficient, simple and reproducible method for the isolation of virus RNA from infected plant tissues. The rapid procedure allows the isolation and detection of PPV in under 3 hours. The isolated RNA was of high quality and could serve as a robust template for reverse transcription. Under conditions of the PPV RNA isolation procedure, Norgen's PPV RT-PCR Detection Kit can detect viroid in the linear range of 100 copies up to  $1 \times 10^6$  copies, indicating the high sensitivity and specificity of the method. As the resulting amplification bands are clearly distinct it also offers accuracy and ease of interpreting results.