Information pest: *Plum pox virus*

Plum pox virus is a regulated plant pathogen included in European legislation as a quarantine pest.
Sharka, caused by *Plum pox virus* is the most devastating viral disease worldwide in terms of agronomic impact and economic importance of stone fruits including peaches, apricots, plums, nectarines, almonds, sweet and sour cherries. The disease was first described in 1917 in Bulgaria; since then, it has progressively spread to a large part of Europe, India and America. The introduction of infected plant propagation material is considered the most important means of long-distance spread of *PPV* which is transmitted by many aphids species.

PPV consists of several strains; the most common are PPV-M and PPV-D.

Infected plants may not show symptoms for several months and symptoms are often transient in appearance. *PPV* symptoms on stone fruits depend on host species and cultivar and the strain of the virus. Symptoms may appear on leaves, petals, fruits and stones. They are particularly conspicuous on leaves in spring: chlorotic spots, bands or rings, vein clearing, or even leaf deformation in peaches. Some peach cultivars may also show flower breaking symptoms. Infected fruits show chlorotic spots or rings.

Introduction

The qPCR *Plum pox virus* set has been developed by Qualiplante based on Olmos et al., 2005. A verification was performed by Qualiplante (data not published) and the performance characteristics of the set are the same as the original publication.
The assay was developed for the detection of the main types of PPV: PPV-M and PPV-D, without any distinction. To design appropriate primers and probes, the nucleotide sequence flanked by the universal primers P1 and P2 (Wetzel et al., 1991) was selected. The specific amplification of the target sequence is a 76 bp DNA fragment. This sensitive method was applied successfully to plant material and to individual PPV vector.
The Real-Time RT-PCR test of Olmos et al., 2005 is recommended by ANSES (www.anses.fr) - ANSES/LSV/MA 043 - Version 2 - Décembre 2018 and by Progetto Aron-Arnadia - Armonizzazione Protocollo diagnostico per *Plum pox virus* (PPV), Progetto Strateco, Anno 2012.
This product should be used only for research purposes.

Intended use

The qPCR set is validated for the detection of *Plum pox virus (PPV)* in One-Step Real-Time RT-PCR. Suitable tissues are plant leaves, flowers and buds.

For leaves: the test portion is constituted by the basal part of each leaves of the sample. The sample preparation can be carried out as follow:
- overlap the leaves of the sample in the same direction from the basal part,
- cut and delete all the petioles,
- cut the leaf blade in order to obtain the same surface for each of the leaves constituting the sample,
- cut the basal part of the leaves, perpendicular to the central vein, to obtain a total weight of sample equal to 1 gram.

For flowers: does not require particular preparation:
- cut the flowers constituting the sample to obtain a total weight of the sample equal to 1 gram.

If the sample weighs more than 1 gramme of flowers, collect a fragment of each flower as representative as possible until the total weight required for the sample is obtained.

For buds: does not require particular preparation. If the sample consists of several branches, it is necessary to take buds of each branch, as representative as possible, until the total weight required of 1 gram is reached. Buds can be removed from twigs with a scalpel.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7PPV--q2	qPCR *Plum pox virus* set 24
7PPV--q9	qPCR *Plum pox virus* set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7PPV--q2-DM-	2x48 tests 7PPV--q9-DM-
RT-Enzyme	24 tests 7PPV--q2-RT-	96 tests 7PPV--q9-RT-
Positive Control	3 tests 7PPV--q2-PC-	8 tests 7PPV--q9-PC-
Negative Control	3 tests 7PPV--q2-NC-	8 tests 7PPV--q9-NC-

Storage conditions

This set can be shipped at room temperature but upon receipt it should be stored immediately at the recommended storage temperature: **from -30 ° C to -10 ° C**.
Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the set is correctly stored, at constant-temperature freezer, its performance is guaranteed until the expiration date indicated on the tubes label.

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Materials and equipment (not provided)

- RNA extraction tools and reagents
- Nuclease-free filter tips and micropipettes
- Optical grade nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- Thermal-cycler for Real-Time PCR with filters calibrated for FAM®

Nucleic acids extraction

Extract RNA from samples according to your usual protocol. Upon request, Qualiplante can recommend you an extraction method.

Preparation of the PPV 1-Step master mix

a) Slowly thaw Direct Master Mix and RT-Enzyme by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and RT-Enzyme and spin down the liquid.
c) In a new tube called **PPV 1-Step master mix**, mix 19,375 µl of Direct Master Mix and 0,625 µl of RT-Enzyme per reaction. Do not forget to count the Positive Control and the Negative Control in the number of reactions to prepare.

Example:	1 rxn	10 rxns
Direct Master Mix	19,375 µl	193,75 µl
RT-enzyme	0,625 µl	6,25 µl

d) Store the **PPV 1-Step master mix** by placing it on ice or at 4°C.

Reaction set-up

a) Shake briefly **PPV 1-Step master mix** and spin down the liquid.
b) Add 20 µl of **PPV 1-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
c) Add 5 µl of RNA template to the **PPV 1-Step master mix**. Do not forget to prepare a PCR tube or well of an optical-grade PCR plate for the Positive Control and the Negative Control.

Components	Volume/PCR tube or well
RNA template or Positive control or Negative control	5 µl
PPV 1-Step master mix	20 µl
Total Volume / PCR tube or well	25 µl

In order to confirm the absence of any reagent's contamination, we strongly recommend including a no-template control (e.g. DEPC water) in the assay.

Run and thermal cycling

a) Seal carefully the PCR tubes or PCR plate. Centrifuge briefly to collect components at the bottom of the PCR tubes or wells of the plate. Protect from light before thermocycling.
b) Load the PCR tubes or plate into the thermal-cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
Reverse transcription	55°C	15 min	1
Enzyme activation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing and elongation	60°C	60 sec	

Results analysis

The reaction for *Plum pox virus* will generate a specific FAM®-labeled amplification curve.

Fig.1: Example of an amplification curve relative to a *PPV* positive sample.

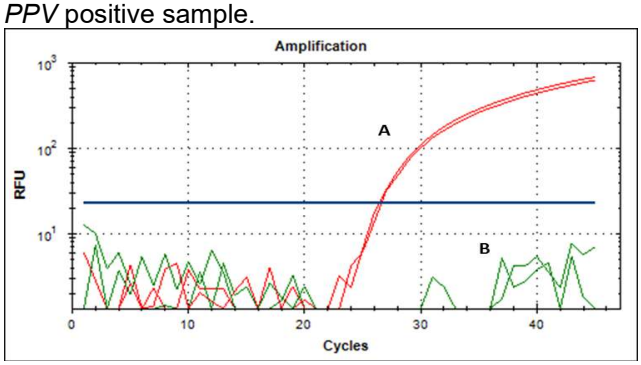

fig.1 shows the amplification curves associated to a *PPV*-infected sample or Positive Control (red curve) and to a healthy sample or Negative Control (green curve)

ANALYSIS VALIDATION

The PCR plate is validated only when:

- ✓ the Positive Control generates an amplification curve for FAM® fluorophore. The Cycle threshold (Ct) value of the FAM®-labeled amplification curve should be below or equal to 38 (***fig.1***).
- ✓ the Negative Control does not generate any amplification curve associated to FAM® fluorophore or a curve is generated for FAM® fluorophore but the Ct is higher than 38 (***fig.1***).

RESULTS INTERPRETATION

When all the previous conditions are performed, the amplification results are interpreted as indicated in the **tab. 1**, by considering for each sample the Ct of the curve generated by FAM® fluorochrome specific to *PPV*.

Ct	Interpretation
Ct ≤38	Positive
Ct > 38	Negative

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Special handling instructions

This set was designed to be used by laboratory staff trained to follow the usual molecular biology precautions. Always perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing DNA/RNA and the components of the set. Do not touch any set components with an ungloved hand. Use appropriate laboratory disposable parts. Use nuclease-free tubes and filter tips to avoid degradation and cross-contamination. Do not use components from sets with different batch numbers in the same test procedure. Do not interchange reagents with other sets. To avoid cross-contamination, use separate rooms for (a) nucleic acids extraction, (b) preparation of the Master Mix and (c) amplification. To avoid cross-contamination and obtain reliable results, it is essential to strictly follow the protocol in this manual. Avoid unnecessary freeze-thaw cycles of the set components. Do not use reagents after their expiration date.

Troubleshooting

Post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal:

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate	Repeat the test using the appropriate tools to seal correctly the plate
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the thermal cycler supplier
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In case of doubt, contact us
Abnormal amplification	Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles

No amplification reaction is observed in the positive control well, while other samples are positive:

Possible causes	Corrective actions
The positive control provided with the set was not added into the reaction well	Repeat the test. If the problem persists, contact us

An amplification plot is observed in the negative control well:

Possible causes	Corrective actions
Contamination of the negative control or the Master Mix with target-positive nucleic acid	Repeat the test by applying appropriate quality procedures to prevent contamination. Seal the plate correctly

Warranty and Responsibilities

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