

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**  
**ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES**  
**Summary sheet of validation data for a diagnostic test**

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

<b>Laboratory contact details</b>	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
<b>Short description of the test</b>	Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.
<b>Date, reference of the validation report</b>	2020-06-30 - PPV1
<b>Link to other validation data</b>	<ul style="list-style-type: none"> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> </ul>

	in symptomatic and asymptomatic leaves of Prunus spp.
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	VALITEST
<b>Description of the test</b>	
<b>Organism(s)</b>	Potyvirus plumpoxi(PPV000)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time RT PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/032 Plum pox potyvirus (version 1)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	RNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	no Followed RNA extraction protocol as described in Botermans et al., 2013 (Journal of Virological Methods, 187: 43-50)
<b>Other information</b>	
<b>Method: Molecular real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	yes
<b>IPPC diagnostic Protocol name</b>	ISPM 27 Annex 02 DP 02: Plum pox virus (version 2018)

<b>Name of the test</b>	Schneider WL, Sherman DJ, Stone AL, Damsteegt VD & Frederickt RD (2004) Specific detection and quantification of Plum pox virus by real-time fluorescent reverse transcription PCR. Journal of Virological Methods, 120, 97-105
<b>Is the test modified compared to the reference test</b>	yes "TaqMan® RT-PCR Mix and TaqMan® RT Enzyme mix were used. The RT step was performed at 48 °C, the first denaturation step for 10 minutes. Further PCR cycling conditions: 40 cycles: 95°C - 15 sec 60°C - 1 min the measure of fluorescence is performed at the end of the annealing/elongation step (60°C)"
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Potyvirus plumpoxi(PPV000)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	PPV-infected Nicotiana benthamiana extracts could be diluted up to at least 10 <sup>4</sup> times in PPV free Prunus sp. extract and still show a positive signal
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	98.6%
<b>Standard test(s)</b>	Known status of of samples. Positive samples with known Ct values were diluted in PPV free Prunus extract.
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	PPV strain An, C, CR, D, EA, M, Rec, T
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	NA
<b>Specificity value</b>	NA
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	87.5%
<b>Specify the test(s)</b>	Known status of of samples. All specimens were sequenced using NGS to verify viral content (PPV and other viruses)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a</b>	97.50%

<b>given level of the pest (see PM 7/98)</b>	
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% evaluated with 2 replicate samples
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Test performance study organized in the framework of the VALITEST project involving 13 laboratories from 10 countries
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">VALITEST PPV TPS REPORT</a></li> </ul>

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