## 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.

Laboratory contact details	Netherlands Institute for Vectors, Invasive plants and Plant health
	P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.
Date, reference of the validation report	2020-06-30 - PPV1
Link to other validation data	<ul> <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 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 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> </ul>

	in symptomatic and asymptomatic leaves of Prunus spp.	
Validation process according to EPPO Standard PM7/98?	yes	
Is the lab accredited for this test?	no	
Was the validated data generated in the framework of a project?	Other_project	
If yes, please specify	VALITEST	
Description of the test		
Organism(s)	Plum pox virus / Potyvirus plumpoxi (PPV000)	
Detection / identification	detection	
Method(s)	Molecular Extraction DNA RNA Molecular Conventional RT PCR	
Method: Molecular Extraction DNA RNA		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	yes	
EPPO Diagnostic Protocol name	PM 7/032 Plum pox potyvirus (version 1)	
As or adapted from an IPPC diagnostic protocol	no	
Is the test modified compared to the reference test	no	
Kit		
Is a kit used	yes	
Manufacturer name	QIAGEN	
Specify the kit used	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)	
Other information		
Method: Molecular Conventional RT PCR		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
As or adapted from an IPPC diagnostic protocol	yes	
IPPC diagnostic Protocol name	ISPM 27 Annex 02 DP 02: Plum pox virus (version 2018)	

Name of the test	Levy, L. & Hadidi, A. 1994. A simple and rapid method for processing tissue infected with Plum pox potyvirus for use with specific 3' non-coding region RT-PCR assays. EPPO Bulletin, 24: 595–604.	
Is the test modified compared to the reference test	yes "Higher concentration of dNTPs (0.4mM), OneStep RT-PCR buffer and Enzyme mix were used. The following PCR cycling conditions were used: RT- step: 50°C – 30 min Denaturation: 95°C – 15 min 40 cycles: 94°C – 30 sec 62°C – 30 sec 72°C – 1 min final extension: 72°C – 10 min"	
Kit		
Is a kit used	no	
Other information		
Reaction type	Simplex	
Performance Criteria :		
Organism 1.:	Potyvirus plumpoxi(PPV000)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	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	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100	
Standard test(s)	Known status of of samples. Positive samples with known Ct values were diluted in PPV free Prunus extract.	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	PPV strain An, C, CR, D, EA, M, Rec, T	
Specificity value	100%	
Analytical specificity - exclusivity		
Number of non-target organisms tested	NA	
Specificity value	NA	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	90%	
Specify the test(s)	Known status of of samples. All specimens were sequenced using NGS to verify viral content (PPV and other viruses)	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	99%	

Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% evaluated with 2 replicate samples	
Test performance study		
Test performance study?	yes	
Brief details of the test performance study and its output.It available, link to published article/report	Test performance study organized in the framework of the VALITEST project involving 12 laboratories from 10 countries	
The following complementary files are available online:	VALITEST PPV TPS REPORT	

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