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	- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV)

	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	yes
EPPO Diagnostic Protocol name	PM 7/032 Plum pox potyvirus (version 1)
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	Wetzel T, Candresse T, Ravelonandro M & Dunez J (1991) A polymerase chain reaction assay adapted to Plum pox potyvirus detection. Journal of Virological Methods 33, 355-365
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 60°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 10^4 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	87.5%
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)	98.75%

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 9 countries
The following complementary files are available online:	• VALITEST PPV TPS REPORT

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