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	Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	Detection of Plum pox virus by reverse transcription loop-mediated isothermal amplification (RT-LAMP)
Date, reference of the validation report	2014-01-01 - Pasquini et al., 2014. Journal of Plant Pathology (2014), 96 (4, Supplement), S4.37
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?	
Description of the test	
Organism(s)	Plum pox virus / Potyvirus plumpoxi (PPV000)
Detection / identification	detection
Method(s)	Extraction Molecular Extraction DNA RNA Molecular LAMP
Method: Extraction	
Reference of the test description	
Other information	
Other details on the test	Fresh sap from leaves obtained with ELISA extraction buffer
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	RNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
Other information	

Other details on the test	Total RNA (TRNA) extracted from leaves by RNeasy Plant Mini kit (Qiagen)
Method: Molecular LAMP	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Kit	
Is a kit used	yes
Manufacturer name	QUALIPLANTE
Specify the kit used	Isothermal PCR kit (ref bK.1/PPV)
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	Reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay employing a 'readyto-use' Master Mix developed by Hyris Ltd./Qualiplante SAS
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Potyvirus plumpoxi(PPV000)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Analytical sensitivity was calculated analyzing eight serial dilutions (ten fold) of three samples naturally infected by PPV-M isolate. Dilutions were made in TRNA extracted from healthy plants. Total RNA - Last level with 100 % positive results: 1/100000 - Last dilution with positive results: 1/10000000 Fresh sap - Last level with 100 % positive results: 1/1000 - Last dilution with positive results: 1/10000
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Total RNA: - symptomatic leaf samples: 100 % - asymptomatic leaf samples: 66.67 % Fresh sap: - symptomatic leaf samples: 100 % - asymptomatic leaf samples: 55.56 %
Standard test(s)	Data obtained analyzing a panel of target (symptomatic and asymptomatic) and non-target samples. Parameter calculation was performed according to the PM7/98 recommendations, as follow: SE = 100 x PA / (ND + PA)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	11 target organisms, represented by: - 5 PPV-D isolates from peach and peach GF305; - 4 PPV-M isolates from peach and peach GF305; - 1 PPV-El Am isolate from peach GF305; - 1 PPV-Rec isolate

	from peach GF305
Specificity value	Total RNA: 100 % Fresh sap: 100 %
Analytical specificity - exclusivity	
Number of non-target organisms tested	5 non-target organisms, represented by: - 1 isolate of Zucchini yellow mosaic virus (ZYMV) from zucchini; - 1 isolate of Apple chlorotic leaf spot virus (ACLSV) from peach GF305; - 1 isolate of Apple mosaic virus (ApMV) from peach GF305; - 1 isolate of Prune dwarf virus (PDV) from peach GF305; - 1 isolate of Prunus necrotic ring spot virus (PNRSV) from peach GF305.
Specificity value	No cross reaction with the non-target organisms tested
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Total RNA: - symptomatic leaf samples: 100 % - asymptomatic leaf samples: 100 % Fresh sap: - symptomatic leaf samples: 100 % - asymptomatic leaf samples: 100 %
Specify the test(s)	Data obtained analyzing a panel of target (symptomatic and asymptomatic) and non-target samples. Parameter calculation was performed according to the PM7/98 recommendations, as follow: SP = 100 x NA / (NA + PD)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Total RNA: - symptomatic leaf samples: 100 % - asymptomatic leaf samples: 86.67 % Fresh sap: - symptomatic leaf samples: 100 % - asymptomatic leaf samples: 86.67 %
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Total RNA: - symptomatic leaf samples: 100 % - asymptomatic leaf samples: 80 % Fresh sap: - symptomatic leaf samples: 100 % - asymptomatic leaf samples: 73.33 %
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	A TPS was performed among four Italian laboratories. A panel of 15 target (symptomatic and asymptomatic) and 6 non-target leaf samples was used for the calculation of validation parameters. A) Target samples: - 1 symptomatic peach infected by PPV-M - 2 symptomatic apricot infected by PPV-M - 1 symptomatic plum infected by PPV-D - 1 symptomatic plum infected by PPV-Rec - 1 symptomatic peach GF305 infected by PPV-El Am - 1 asymptomatic peach infected by PPV-M - 1 asymptomatic apricot infected by PPV-M - 1 asymptomatic plum infected by PPV-Rec - 2 spiked peach samples (asymptomatic leaves from PPV-M infected tree mixed with leaves from healthy plants at the ratio of 1/2 and 1/4) - 2 spiked apricot samples (asymptomatic leaves from PPV-M infected

	tree mixed with leaves from healthy plants at the ratio of 1/2 and 1/4) - 2 spiked plum samples (asymptomatic leaves from PPV-Rec infected tree mixed with leaves from healthy plants at the ratio of 1/2 and 1/4) B) Non-target samples: - 1 healthy peach - 1 healthy apricot - 2 healthy plums - 2 water samples

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