EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)

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.

Target Organism	Plum pox virus	
Short description	Detection of Plum pox virus by reverse transcription loop-mediated isothermal amplification (RT-LAMP)	
Laboratory contact details	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy	
Date and reference of the validation report	2014 - Pasquini et al., 2014. Journal of Plant Pathology (2014), 96 (4, Supplement), S4.37	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0	
Is the test the same as described in the EPPO DP?	No Molecular test based Loop-mediated isothermal amplification (LAMP) technique.	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Peach, plum, apricot	
Matrices tested (if relevant)	Leaves	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	1) Total RNA (TRNA) extracted from leaves by RNeasy Plant Mini kit (Qiagen). 2) Fresh sap from leaves obtained with ELISA extraction buffer
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay employing a 'ready-to-use' Master Mix developed by Hyris Ltd./Qualiplante SAS.
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		

Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
<u>Analytical sensitivity (= limit of detection)</u>		
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	
<u>Diagnostic sensitivity</u>		
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 %	
Specify the standard test	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)	
<u>Analytical specificity</u>		
Specificity value	Total RNA: 100 % Fresh sap: 100 %	
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	
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.	
Cross reacts with (specify the	No cross reaction with the non-target organisms tested	

species)	
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 standard test	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
Include brief details of the test performance study and its output.It available, provide a 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
<u>Other information</u>	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	