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 direct and indirect ELISA	
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	2013 - Pasquini et al., 2013. Petria 23 (2), 2013: 351-394	
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?	Yes	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	apricot, plum, peach, myrabolan, Nicotiana benthamiana	
Matrices tested (if relevant)	leaves and bark	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	as described in EPPO PM 7/032
Molecular methods, e.g. hybridization, PCR and real time PCR		
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay	X	DASI-ELISA (Cambra et al., 1994) by using universal monoclonal antibodies 5B-IVIA DAS-ELISA (validation data obtained with the serological kit Bioreba cod. n. 150565).
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		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	The analytical sensitivity was calculated analyzing three samples at twelve diluition levels (1/1-1/1.000.000000.000). The dilutions were in leaf or bark tissue from an healthy plant. Last dilution level with 100% positive results: 1/1000 (both for leaf and bark samples)	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	Symptomatic leaf samples: 100 % Asymptomatic leaf samples: 57 % Woody samples: 70 %	
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)	
Analytical specificity		
Specificity value	Leaf samples: 100 % Woody samples: 100 %	
Number of strains/populations of target organisms tested	A) Leaf samples: - 6 PPV-D isolates from apricot, plum, myrabolan, peach GF305; - 9 PPV-M isolates from apricot, plum, peach, peach GF305; - 1 PPV-El Amar isolate from peach; - 1 PPV-Rec isolate from plum; - 1 PPV-C isolate from N. benthamiana B) Woody samples: - 7 PPV-D isolates from apricot, plum, myrabolan, peach GF305; - 10 PPV-M isolates from apricot, plum, peach, peach GF305; - 1 PPV-El Amar isolate from peach; - 1 PPV-Rec isolate from plum.	
Number of non-target organisms tested	A) Leaf samples: - 1 isolate of Potato virus Y (PVY) (Potyvirus) from potato; - 1 isolate of Apple chlorotic leaf spot virus (ACLSV) from peach GF305; - 1 isolate of Prunus necrotic ring spot virus (PNRSV) from peach GF305; - 1 isolate of Prune dwarf virus (PDV) from peach GF305 B) Woody samples: - 1 isolate of Apple chlorotic leaf spot virus (ACLSV) from peach GF305; - 1 isolate of Prunus necrotic ring spot virus (PNRSV) from peach GF305;	

	- 1 isolate of Prune dwarf virus (PDV) from peach GF305
Cross reacts with (specify the species)	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	Symptomatic leaf samples: 100 % Asymptomatic leaf samples: 100 % Woody 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)	Symptomatic leaf samples: 88.89 % Asymptomatic leaf samples: not calculated Woody samples: not calculated
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Symptomatic leaf samples: 100 % Asymptomatic leaf samples: not calculated Woody samples: not calculated
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 carried out among 11 Italian laboratories. Performance of the ELISA test was evaluated starting from two different plant matrices according with the considered sampling season: 1) leaf tissue from symptomatic and asymptomatic plants, during spring; 2) woody tissue (bark), during winter. A panel of target and non-target samples was specifically used for each considered sampling period, as following detailed. 1) Spring sampling: 39 target and 7 non-target samples. a) Target: - 1 symptomatic fruit sample (apricot) infected by PPV-D - 5 symptomatic leaf samples (apricot, plum, myrabolan, peach GF305) infected by PPV-D; - 9 symptomatic leaf samples (apricot, plum, peach, peach GF305) infected by PPV-M; - 1 symptomatic leaf sample (peach) infected by PPV-El Amar; - 1 symptomatic leaf sample (plum) infected by PPV-Rec; - 1 symptomatic leaf sample (N. benthamiana) infected by PPV-C; - 21 symptomless leaf samples (peach) infected by PPV-M. b) Non-target: - 1 sample (potato) infected by Potato virus Y (PVY) (Potyvirus); - 1 sample (peach GF305) infected by Apple chlorotic leaf spot virus (ACLSV); - 1 sample (peach GF305) infected by Prunus necrotic ring

	spot virus (PNRSV); - 1 sample (peach GF305) infected by Prune dwarf virus (PDV); - 3 samples from healthy plants (apricot, plum, peach). 2) Winter sampling: 19 target and 6 non-target samples. a) Target: - 7 samples (apricot, plum, myrabolan, peach GF305) infected by PPV-D; - 10 samples (apricot, plum, peach, peach GF305) infected by PPV-M; - 1 sample (peach) infected by PPV-El Amar; - 1 sample (plum) infected by PPV-Rec. b) Non-target: - 1 sample (peach GF305) infected by Apple chlorotic leaf spot virus (ACLSV); - 1 sample (peach GF305) infected by Prunus necrotic ring spot virus (PNRSV); - 1 sample (peach GF305) infected by Prune dwarf virus (PDV); - 3 samples from healthy plants (apricot, plum, peach). TPS allowed to validate two ELISA methods (DAS- and -DASI-ELISA) for the serological detection of PPV. For both methods identical values of the performance parameters (analytical sensitivity and specificity, diagnostic sensitivity and specificity, repeatability and reproducibility) were recorded.
<u>Other information</u>	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	