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 direct and indirect ELISA
Date, reference of the validation report	2013-01-01 - 100 ; Pasquini et al., 2013. Petria 23 (2), 2013: 351-394
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Plum pox virus / Potyvirus plumpoxi (PPV000)
Detection / identification	detection
Method(s)	Extraction Serological DAS-ELISA Serological DASI-ELISA
Method: Extraction	
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)
Is the test modified compared to the reference test	no
Other information	
Method: Serological DAS-ELISA	
Reference of the test description	
Kit	
Is a kit used	yes
Manufacturer name	BIOREBA
Specify the kit used	ELISA Plum pox virus

Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	PPV Reagent set 480 (cat. num. 150565) was used
Method: Serological DASI-ELISA	
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)
Name of the test	DASI-ELISA (Cambra et al., 1994)
Other information	
Other details on the test	DASI-ELISA (Cambra et al., 1994) by using universal monoclonal antibodies 5B-IVIA
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?	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 %
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	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.
Specificity value	Leaf samples: 100 % Woody samples: 100 %
Analytical specificity - exclusivity	

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
Specificity value	Leaf samples: 100 % Woody samples: 100 % 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 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)	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
Brief details of the test performance study and its output.It available, 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.

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