

**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.

<b>Laboratory contact details</b>	Council for Agricultural Research and Economics- Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
<b>Short description of the test</b>	Detection of Plum pox virus by direct and indirect ELISA
<b>Date, reference of the validation report</b>	2013-01-01 - 100 ; Pasquini et al., 2013. <i>Petria</i> 23 (2), 2013: 351-394
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	Plum pox virus / Potyvirus plumponxi (PPV000)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Bark, Leaves
<b>Plant species tested</b>	<i>Nicotiana benthamiana</i> , <i>Prunus armeniaca</i> , <i>Prunus domestica</i> , <i>Prunus persica</i> , <i>Terminalia catappa</i>
<b>Method(s)</b>	Extraction Serological DAS-ELISA Serological DAS-ELISA
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/032 Plum pox potyvirus (version 1)
<b>Is the test modified compared to the reference test</b>	no
<b>Other information</b>	
<b>Method: Serological DAS-ELISA</b>	
<b>Reference of the test description</b>	
<b>Kit</b>	

<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	BIOREBA
<b>Specify the kit used</b>	ELISA Plum pox virus
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	PPV Reagent set 480 (cat. num. 150565) was used
<b>Method: Serological DASI-ELISA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/032 Plum pox potyvirus (version 1)
<b>Name of the test</b>	DASI-ELISA (Cambra et al., 1994)
<b>Other information</b>	
<b>Other details on the test</b>	DASI-ELISA (Cambra et al., 1994) by using universal monoclonal antibodies 5B-IVIA
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Potyvirus plumpoxi(PPV000)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	The analytical sensitivity was calculated analyzing three samples at twelve dilution 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)
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	Symptomatic leaf samples: 100 % Asymptomatic leaf samples: 57 % Woody samples: 70 %
<b>Standard test(s)</b>	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: $SE = 100 \times PA / (ND + PA)$
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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.

<b>Specificity value</b>	Leaf samples: 100 % Woody samples: 100 %
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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
<b>Specificity value</b>	Leaf samples: 100 % Woody samples: 100 % No cross reaction with the non-target organisms tested
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Symptomatic leaf samples: 100 % Asymptomatic leaf samples: 100 % Woody samples: 100 %
<b>Specify the test(s)</b>	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: $SP = 100 \times NA / (NA + PD)$
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Symptomatic leaf samples: 88.89 % Asymptomatic leaf samples: not calculated Woody samples: not calculated
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Symptomatic leaf samples: 100 % Asymptomatic leaf samples: not calculated Woody samples: not calculated
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	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-EI 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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