

**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.

<b>Target Organism</b>	Plum pox virus	
<b>Short description</b>	Detection of Plum pox virus by direct and indirect ELISA	
<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>Date and reference of the validation report</b>	2013 - Pasquini et al., 2013. <i>Petria</i> 23 (2), 2013: 351-394	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	0	
<b>Is the test the same as described in the EPPO DP?</b>	Yes	
<b>Is the lab accredited for this test?</b>	Yes	
<b>Plant species tested (if relevant)</b>	apricot, plum, peach, myrabolan, <i>Nicotiana benthamiana</i>	
<b>Matrices tested (if relevant)</b>	leaves and bark	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>	X	as described in EPPO PM 7/032
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>		
<b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>	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).
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b>		
<b>Pathogenicity test</b>		
<b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA</b>		

<b>profiling</b>		
<b>Morphological and morphometrical methods intended for identification</b>		
<b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>		
<b>Other</b>		
<b>Analytical sensitivity (= limit of detection)</b>		
<b>What is 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>Specify the standard test</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</b>		
<b>Specificity value</b>	Leaf samples: 100 % Woody samples: 100 %	
<b>Number of strains/populations of target organisms tested</b>	<p>A) Leaf samples:</p> <ul style="list-style-type: none"> <li>- 6 PPV-D isolates from apricot, plum, myrabolan, peach GF305;</li> <li>- 9 PPV-M isolates from apricot, plum, peach, peach GF305;</li> <li>- 1 PPV-EI Amar isolate from peach;</li> <li>- 1 PPV-Rec isolate from plum;</li> <li>- 1 PPV-C isolate from N. benthamiana</li> </ul> <p>B) Woody samples:</p> <ul style="list-style-type: none"> <li>- 7 PPV-D isolates from apricot, plum, myrabolan, peach GF305;</li> <li>- 10 PPV-M isolates from apricot, plum, peach, peach GF305;</li> <li>- 1 PPV-EI Amar isolate from peach;</li> <li>- 1 PPV-Rec isolate from plum.</li> </ul>	
<b>Number of non-target organisms tested</b>	<p>A) Leaf samples:</p> <ul style="list-style-type: none"> <li>- 1 isolate of Potato virus Y (PVY) (Potyvirus) from potato;</li> <li>- 1 isolate of Apple chlorotic leaf spot virus (ACLSV) from peach GF305;</li> <li>- 1 isolate of Prunus necrotic ring spot virus (PNRSV) from peach GF305;</li> <li>- 1 isolate of Prune dwarf virus (PDV) from peach GF305</li> </ul> <p>B) Woody samples:</p> <ul style="list-style-type: none"> <li>- 1 isolate of Apple chlorotic leaf spot virus (ACLSV) from peach GF305;</li> <li>- 1 isolate of Prunus necrotic ring spot virus (PNRSV) from peach GF305;</li> </ul>	

	- 1 isolate of Prune dwarf virus (PDV) from peach GF305
<b>Cross reacts with (specify the species)</b>	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 standard test</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>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b>	<p>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:</p> <ol style="list-style-type: none"> <li>1) leaf tissue from symptomatic and asymptomatic plants, during spring;</li> <li>2) woody tissue (bark), during winter.</li> </ol> <p>A panel of target and non-target samples was specifically used for each considered sampling period, as following detailed.</p> <ol style="list-style-type: none"> <li>1) Spring sampling: 39 target and 7 non-target samples. <ol style="list-style-type: none"> <li>a) Target: <ul style="list-style-type: none"> <li>- 1 symptomatic fruit sample (apricot) infected by PPV-D</li> <li>- 5 symptomatic leaf samples (apricot, plum, myrabolan, peach GF305) infected by PPV-D;</li> <li>- 9 symptomatic leaf samples (apricot, plum, peach, peach GF305) infected by PPV-M;</li> <li>- 1 symptomatic leaf sample (peach) infected by PPV-El Amar;</li> <li>- 1 symptomatic leaf sample (plum) infected by PPV-Rec;</li> <li>- 1 symptomatic leaf sample (N. benthamiana) infected by PPV-C;</li> <li>- 21 symptomless leaf samples (peach) infected by PPV-M.</li> </ul> </li> <li>b) Non-target: <ul style="list-style-type: none"> <li>- 1 sample (potato) infected by Potato virus Y (PVY) (Potyvirus);</li> <li>- 1 sample (peach GF305) infected by Apple chlorotic leaf spot virus (ACLSV);</li> <li>- 1 sample (peach GF305) infected by Prunus necrotic ring</li> </ul> </li> </ol> </li> </ol>

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-EI 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.

<b>Other information</b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	