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.

<table>
<thead>
<tr>
<th>Target Organism</th>
<th>Plum pox virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short description</td>
<td>Detection of Plum pox virus by direct and indirect ELISA</td>
</tr>
<tr>
<td>Laboratory contact details</td>
<td>Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy</td>
</tr>
<tr>
<td>Date and reference of the validation report</td>
<td>2013 - Pasquini et al., 2013. Petria 23 (2), 2013: 351-394</td>
</tr>
<tr>
<td>Validation process according to EPPO Standard PM 7/98:</td>
<td>Yes</td>
</tr>
<tr>
<td>Reference of the test description</td>
<td>0</td>
</tr>
<tr>
<td>Is the test the same as described in the EPPO DP?</td>
<td>Yes</td>
</tr>
<tr>
<td>Is the lab accredited for this test?</td>
<td>Yes</td>
</tr>
<tr>
<td>Plant species tested (if relevant)</td>
<td>apricot, plum, peach, myrabolan, Nicotiana benthamiana</td>
</tr>
<tr>
<td>Matrices tested (if relevant)</td>
<td>leaves and bark</td>
</tr>
</tbody>
</table>

**List of methods used**

<p>| 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 &amp; DNA | |</p>
<table>
<thead>
<tr>
<th><strong>Profiling</strong></th>
</tr>
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<tbody>
<tr>
<td>Morphological and morphometrical methods intended for identification</td>
</tr>
<tr>
<td>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

### 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 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).

### 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: \( SE = 100 \times PA / (ND + PA) \)

### 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, myraban, 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, myraban, 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;
<table>
<thead>
<tr>
<th>Cross reacts with (specify the species)</th>
<th>No cross reaction with the non-target organisms tested</th>
</tr>
</thead>
</table>

### 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 % |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Specify the standard test</td>
<td>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: $SP = 100 \times \frac{NA}{(NA + PD)}$</td>
</tr>
</tbody>
</table>

### 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

<table>
<thead>
<tr>
<th>Test performance study?</th>
<th>Yes</th>
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| 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.

Other information

| Any other information considered useful e.g. robustness, ease of performing the test, etc. |  |