

**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.

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| <b>Laboratory contact details</b>               | Netherlands Institute for Vectors, Invasive plants and Plant health<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands  |
| <b>Short description of the test</b>            | Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.  |
| <b>Date, reference of the validation report</b> | 2020-06-30 - PPV1  |
| <b>Link to other validation data</b>            | <ul style="list-style-type: none"> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.</li> <li>- PPV1 Molecular detection of plum pox virus (PPV)</li> </ul> |

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|   | in symptomatic and asymptomatic leaves of Prunus spp.   |
| <b>Validation process according to EPPO Standard PM7/98?</b>  | yes   |
| <b>Is the lab accredited for this test?</b>   | no  |
| <b>Was the validated data generated in the framework of a project?</b>                              | Other_project   |
| <b>If yes, please specify</b>   | VALITEST  |
| <b>Description of the test</b>  |   |
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| <b>Organism(s)</b>  | Potyvirus plumpoxi(PPV000)  |
| <b>Detection / identification</b>   | detection   |
| <b>Method(s)</b>  | Molecular Extraction DNA RNA<br>Molecular Conventional RT PCR   |
| <b>Method: Molecular Extraction DNA RNA</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>As or adapted from an IPPC diagnostic protocol</b>   | no  |
| <b>Is the test modified compared to the reference test</b>  | no  |
| <b>Kit</b>  |   |
| <b>Is a kit used</b>  | yes   |
| <b>Manufacturer name</b>  | QIAGEN  |
| <b>Specify the kit used</b>   | RNeasy Plant Mini Kit   |
| Kit used following the manufacturer's instructions?   | no Followed RNA extraction protocol as described in Botermans et al., 2013 (Journal of Virological Methods, 187: 43-50) |
| <b>Other information</b>  |   |
| <b>Method: Molecular Conventional RT PCR</b>  |   |
| <b>Reference of the test description</b>  |   |
| <b>As or adapted from an EPPO diagnostic protocol</b>   | yes   |
| <b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b> | yes   |
| <b>EPPO Diagnostic Protocol name</b>  | PM 7/032 Plum pox potyvirus (version 1)   |
| <b>As or adapted from an IPPC diagnostic</b>  | yes   |

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| <b>protocol</b>  |  |
| <b>IPPC diagnostic Protocol name</b>   | ISPM 27 Annex 02 DP 02: Plum pox virus (version 2018)  |
| <b>Name of the test</b>  | Wetzel T, Candresse T, Ravelonandro M & Dunez J (1991) A polymerase chain reaction assay adapted to Plum pox potyvirus detection. Journal of Virological Methods 33, 355-365   |
| <b>Is the test modified compared to the reference test</b>   | yes "Higher concentration of dNTPs (0.4mM), OneStep RT-PCR buffer and Enzyme mix were used. The following PCR cycling conditions were used: RT-step: 50°C - 30 min Denaturation: 95°C - 15 min 40 cycles: 94°C - 30 sec 60°C - 30 sec 72°C - 1 min final extension: 72°C - 10 min" |
| <b>Kit</b>   |  |
| <b>Is a kit used</b>   | no   |
| <b>Other information</b>   |  |
| <b>Reaction type</b>   | Simplex  |
| <b>Performance Criteria :</b>  |  |
| <b>Organism 1.:</b>  | <b>Potyvirus plumpoxi(PPV000)</b>  |
| <b>Analytical sensitivity</b>  |  |
| <b>What is smallest amount of target that can be detected reliably?</b>  | PPV-infected Nicotiana benthamiana extracts could be diluted up to at least 10 <sup>4</sup> times in PPV free Prunus sp. extract and still show a positive signal  |
| <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> | NA   |
| <b>Standard test(s)</b>  | NA   |
| <b>Analytical specificity - inclusivity</b>  |  |
| <b>Number of strains/populations of target organisms tested</b>  | PPV strain An, C, CR, D, EA, M, Rec, T   |
| <b>Specificity value</b>   | 100%   |
| <b>Analytical specificity - exclusivity</b>  |  |
| <b>Number of non-target organisms tested</b>   | PNRSV0, CVA000, ACLSV0, LCHV10, PDV000, CGRMV0, NSPAV0, APMV00, CHALV0, PBNSPA, APV300   |
| <b>Specificity value</b>   | 100%   |
| <b>Diagnostic Specificity</b>  |  |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | NA   |
| <b>Specify the test(s)</b>   | NA   |
| <b>Reproducibility</b>   |  |
| <b>Provide the calculated % of agreement for a</b>   | NA   |

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| <b>given level of the pest (see PM 7/98)</b>  |  |
| <b>Repeatability</b>  |  |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                          | NA   |
| <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> | Preliminary study to see if the test is suitable for the PPV test performance study organized in the framework of the VALITEST project |
| The following complementary files are available online:   |  |
|   | <ul style="list-style-type: none"> <li>• <a href="#">VALITEST PPV TPS REPORT</a></li> </ul>  |

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