**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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| **Laboratory contact details** | Netherlands Institute for Vectors, Invasive plants and Plant health<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands |
| **Short description of the test** | Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. |
| **Date, reference of the validation report** | 2020-06-30 - PPV1 |
| **Link to other validation data** | - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp.<br>- PPV1 Serological detection of plum pox virus (PPV) |

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| | in symptomatic and asymptomatic leaves of Prunus spp. |
| **Validation process according to EPPO Standard PM7/98?** | yes |
| **Is the lab accredited for this test?** | no |
| **Was the validated data generated in the framework of a project?** | Other_project |
| **If yes, please specify** | VALITEST |
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| **Description of the test** | |
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| **Organism(s)** | Plum pox virus / Potyvirus plumpoxi (PPV000) |
| **Detection / identification** | detection |
| **Matrix(ces) tested** | Leaves PPV negative material was acquired from the NVWA (Wageningen, NL) collection and from Naktuinbouw (Horst, NL). |
| **Plant species tested** | Nicotiana benthamiana, Prunus, Prunus avium, Prunus domestica, Prunus persica |
| **Method(s)** | Molecular Extraction DNA RNA<br>Molecular real time RT PCR |
| **Method: Molecular Extraction DNA RNA** | |
| ***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) |
| **As or adapted from an IPPC diagnostic protocol** | no |
| **Is the test modified compared to the reference test** | no |
| ***Kit*** | |
| **Is a kit used** | yes |
| **Manufacturer name** | QIAGEN |
| **Specify the kit used** | 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) |
| ***Other information*** | |
| **Method: Molecular real time RT PCR** | |
| ***Reference of the test description*** | |
| **As or adapted from an EPPO diagnostic protocol** | no |

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| **As or adapted from an IPPC diagnostic protocol** | no |
| **Reference of the test** | Anonymous (2018) TaqMan RT-PCR voor pruimensharkavirus (Plum pox virus, PPV) in blad en twijg houtachtige Prunus soorten, Naktuinbouw protocol |
| **Is the test modified compared to the reference test** | no |
| ***Kit*** | |
| **Is a kit used** | no |
| ***Other information*** | |
| **Reaction type** | Duplex - Probe |
| **Performance Criteria :** | |
| **Organism 1.:** | **Potyvirus plumpoxi(PPV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | PPV-infected Nicotiana benthamiana extracts could be diluted up to at least 10^4 times in PPV free Prunus sp. extract and still show a positive signal |
| **Diagnostic sensitivity** | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98** | 98.6% |
| **Standard test(s)** | Known status of of samples. Positive samples with known Ct values were diluted in PPV free Prunus extract. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | PPV strain An, C, CR, D, EA, M, Rec, T |
| **Specificity value** | 100% |
| **Analytical specificity - exclusivity** | |
| **Number of non-target organisms tested** | NA |
| **Specificity value** | NA |
| **Diagnostic Specificity** | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | 87.5% |
| **Specify the test(s)** | Known status of of samples. All specimens were sequenced using NGS to verify viral content (PPV and other viruses) |
| **Reproducibility** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 97.50% |
| **Repeatability** | |

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| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 100% evaluated with 2 replicate samples |
| **Test performance study** | |
| **Test performance study?** | yes |
| **Brief details of the test performance study and its output.It available, link to published article/report** | Test performance study organized in the framework of the VALITEST project involving 13 laboratories from 10 countries |
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| The following complementary files are available online: | • VALITEST PPV TPS REPORT |

*Creation date: 2020-09-30 13:45:50 - Last update: 2023-06-13 16:54:42*