

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 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 | <ul style="list-style-type: none"> - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Serological detection of plum pox virus (PPV) in symptomatic and asymptomatic leaves of Prunus spp. - PPV1 Molecular 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 |
| Method(s) | Molecular Extraction DNA RNA 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 |
| New test being considered for inclusion in the next version of the EPPO diagnostic protocol? | yes |
| As or adapted from an IPPC diagnostic protocol | yes |

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| IPPC diagnostic Protocol name | ISPM 27 Annex 02 DP 02: Plum pox virus (version 2018) |
| Name of the test | Schneider WL, Sherman DJ, Stone AL, Damsteegt VD & Frederickt RD (2004) Specific detection and quantification of Plum pox virus by real-time fluorescent reverse transcription PCR. Journal of Virological Methods, 120, 97-105 |
| Is the test modified compared to the reference test | yes "TaqMan® RT-PCR Mix and TaqMan® RT Enzyme mix were used. The RT step was performed at 48 °C, the first denaturation step for 10 minutes. Further PCR cycling conditions: 40 cycles: 95°C - 15 sec 60°C - 1 min the measure of fluorescence is performed at the end of the annealing/elongation step (60°C)" |
| Kit | |
| Is a kit used | no |
| Other information | |
| Reaction type | Simplex - Probe |
| Performance Criteria : | |
| Organism 1.: | Potyvirus plumpoxi(PPV000) |
| Analytical sensitivity | |
| What is 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 | NA |
| Standard test(s) | NA |
| 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 | PNRSV0, CVA000, ACLSV0, LCHV10, PDV000, CGRMV0, NSPAV0, APMV00, CHALV0, PBNSPA, APV300 |
| Specificity value | 100% |
| Diagnostic Specificity | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | NA |
| Specify the test(s) | NA |
| Reproducibility | |

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| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | NA |
| <u>Repeatability</u> | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | NA |
| Test performance study | |
| Test performance study? | yes |
| Brief details of the test performance study and its output. It available, link to published article/report | Preliminary study to see if the test is suitable for the PPV test performance study organized in the framework of the VALITEST project |
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| The following complementary files are available online: | <ul style="list-style-type: none"> • VALITEST PPV TPS REPORT |

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