

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 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 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 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 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 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 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) |

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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 |
| Description of the test | |
| Organism(s) | Plum pox virus(PPV000) |
| Detection / identification | detection |
| Method(s) | Molecular Extraction DNA RNA Molecular Conventional 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 Conventional 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 | Levy, L. & Hadidi, A. 1994. A simple and rapid method for processing tissue infected with Plum pox potyvirus for use with specific 3' non-coding region RT-PCR assays. EPPO Bulletin, 24: 595- 604. |
| Is the test modified compared to the reference test | 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 62°C - 30 sec 72°C - 1 min final extension: 72°C - 10 min" |
| Kit | |
| Is a kit used | no |
| Other information | |
| Reaction type | Simplex |
| Performance Criteria : | |
| Organism 1.: | Plum pox virus(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 | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | NA |

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| Repeatability | |
| 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 |

Creation date: 2020-09-29 11:08:43 - Last update: 2020-09-30 12:30:36