

**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 | Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands |
| Short description of the test | Real-time RT-PCR (TaqMan RT-PCR) for Potato spindle tuber viroid (PSTVd) and/or Tomato chlorotic dwarf viroid (TCDVd) in leaf material of horticultural crops |
| Date, reference of the validation report | 2012-08-28 - v1.2 |
| Validation process according to EPPO Standard PM7/98? | yes |
| Is the lab accredited for this test? | yes |
| Was the validated data generated in the framework of a project? | |
| Description of the test | |
| Organism(s) | Potato spindle tuber viroid / Pospiviroid fusituberis (PSTVD0) Tomato chlorotic dwarf viroid / Pospiviroid chloronani (TCDVD0) |
| Detection / identification | detection |
| Method(s) | Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular real time RT PCR |
| Method: Molecular Extraction DNA RNA | |
| Reference of the test description | |
| Kit | |
| Is a kit used | yes |
| Manufacturer name | QIAGEN |
| Specify the kit used | RNeasy Plant Mini Kit |
| Kit used following the manufacturer's instructions? | |
| Other information | |
| Method: Molecular Extraction DNA RNA (2) | |
| Reference of the test description | |
| Kit | |

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| Is a kit used | yes |
| Manufacturer name | LGC |
| Specify the kit used | sbeadex maxi plant |
| Kit used following the manufacturer's instructions? | |
| Other information | |
| Method: Molecular real time RT PCR | |
| Reference of the test description | |
| As or adapted from an EPPD diagnostic protocol | no |
| As or adapted from an IPPC diagnostic protocol | yes |
| IPPC diagnostic Protocol name | ISPM 27 Annex 07 DP 07: Potato spindle tuber viroid (version 2016) |
| Name of the test | Real-time RT-PCR using the primers of Boonham et al. (2004) |
| Other information | |
| Are the performance characteristics included in the EPPD diagnostic protocol? | no |
| Performance Criteria : | |
| Organism 1.: | Pospiviroid fusituberis(PSTVD0) |
| Analytical sensitivity | |
| What is smallest amount of target that can be detected reliably? | Solanum lycopersicon: up to 10^6 - 10^7 dilution in sap of healthy tomato leaves. Ornamentals: Relative sensitivity dependent on initial viroid concentration and host plant species. Validated for bulking rates up to 25 for Brugmansia, Calibrachoa, Dahlia (greenhouse), Petunia, Solanum jasminoides and Streptosolen jamesonii, but test is more sensitive. For some crops like field Dahlia, only the summer period seems suitable for (reliable) testing |
| Analytical specificity - inclusivity | |
| Number of strains/populations of target organisms tested | 9 PSTVd isolates, 5 TCDVd isolates |
| Specificity value | 100% |
| Analytical specificity - exclusivity | |
| Number of non-target organisms tested | 8 other pospiviroids: CLVd, CSVd, CEVd, IrVd-1, MPVd, PCFVd, TASVd, TPMVd 4 Pospiviroidae: ASSVd, HLVd, HSVd, DLVd 2 avsunviroids: ASBVd, CChMVd 8 viruses (tomato): AMV, CMV, PepMV, PVY, ToMV, TMV, ToCV, TYLCV |
| Specificity value | MPVd, PSTVd and TCDVd are detected equally well (up to 10^6 - 10^7 dilution in sap of healthy tomato leaves); TMPVd is also detected, but not as well as PSTVd, TCDVd or MPVd (up to 10^1 - 10^2 dilution). |
| Reproducibility | |

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| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 100% |
| Repeatability | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 100% |
| Organism 2.: | Pospiviroid chloronani(TCDVD0) |
| Analytical sensitivity | |
| What is smallest amount of target that can be detected reliably? | Solanum lycopersicon: up to 10 ⁶ - 10 ⁷ dilution in sap of healthy tomato leaves. Ornamentals: Relative sensitivity dependent on initial viroid concentration and host plant species. Validated for bulking rates up to 25 for Brugmansia, Calibrachoa, Dahlia (greenhouse), Petunia, Solanum jasminoides and Streptosolen jamesonii, but test is more sensitive. For some crops like field Dahlia, only the summer period seems suitable for (reliable) testing |
| Analytical specificity - inclusivity | |
| Number of strains/populations of target organisms tested | 9 PSTVd isolates, 5 TCDVd isolates |
| Specificity value | 100% |
| Analytical specificity - exclusivity | |
| Number of non-target organisms tested | 8 other pospiviroids: CLVd, CSVd, CEVd, IrVd-1, MPVd, PCFVd, TASVd, TPMVd 4 Pospiviroidae: ASSVd, HLVd, HSVd, DLVd 2 avsunviroids: ASBVd, CChMVd 8 viruses (tomato): AMV, CMV, PepMV, PVY, ToMV, TMV, ToCV, TYLCV |
| Specificity value | MPVd, PSTVd and TCDVd are detected equally well (up to 10 ⁶ -10 ⁷ dilution in sap of healthy tomato leaves); TMPVd is also detected, but not as well as PSTVd, TCDVd or MPVd (up to 10-10 ² dilution). |
| Reproducibility | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 100% |
| Repeatability | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 100% |
| Test performance study | |
| Test performance study? | no |

Creation date: 2014-06-12 00:00:00 - Last update: 2021-05-14 17:01:51