

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)

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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| Target Organism | Potato spindle tuber viroid Tomato chlorotic dwarf viroid | |
| Short description | Detection of Potato spindle tuber viroid (PSTVd) and/or Tomato chlorotic dwarf viroid (TCDVd) in tomato seed with real-time RT-PCR (TaqMan RT-PCR). | |
| Laboratory contact details | Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands | |
| Date and reference of the validation report | 2012-05-02 - V1.0 | |
| Validation process according to EPPO Standard PM 7/98: | Yes | |
| Reference of the test description | 0 N. Boonham, L. González-Pérez, M.S. Mendez, E. Lilia Peralta, A. Blockley, K. Walsh, I. Barker, R.A. Mumford, 2004. Development of a real-time RT-PCR assay for the detection of Potato spindle tuber viroid. <i>Journal of Virological Methods</i> 116:139-146. | |
| Is the test the same as described in the EPPO DP? | No IPPC protocol is in preparation | |
| Is the lab accredited for this test? | Yes | |
| Plant species tested (if relevant) | Solanum lycopersicum | |
| Matrices tested (if relevant) | seeds | |
| List of methods used | | |
| Method for extraction / isolation / baiting of target organism from matrix | | |
| Molecular methods, e.g. hybridization, PCR and real time PCR | X | RNA isolation using KingFisher and SBeadex maxi plant kit (LGC) followed by real-time RT-PCR |
| Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay | | |
| Plating methods: selective isolation | | |
| Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting. | | |
| Pathogenicity test | | |

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| Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling | | |
| Morphological and morphometrical methods intended for identification | | |
| Biochemical methods: e.g. enzyme electrophoresis, protein profiling | | |
| Other | | |
| Analytical sensitivity (= limit of detection) | | |
| What is smallest amount of target that can be detected reliably? | Probability of detection of 1 infested seed in a sample of 1000 is >95% when testing 3 sub samples of each 1000 seeds. A comparative study using two naturally contaminated seed lots showed that increasing the sample size to 20,000 seeds combined with decreasing the size of the subsamples to 400, did not influence the overall outcome of the test. | |
| Diagnostic sensitivity | | |
| Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98 | No “standard” assay is available. Sequence analysis proved that PSTVd / TCDVd was present on seeds, but grow out assay negative for all tested samples. No seed transmission observed in grow out. | |
| Specify the standard test | Grow out and sequence analysis | |
| Analytical specificity | | |
| Specificity value | 100% | |
| Number of strains/populations of target organisms tested | 6 PSTVd isolates (5 tested on seed), 5 TCDVd isolates (4 tested on seed) | |
| Number of non-target organisms tested | Pospiviroids: CEVd, CSVd, IrVd-1, MPVd, PCFVd, TASVd, TPMVd | |
| Cross reacts with (specify the species) | MPVd is also detected (which is a desirable trait). TMPVd is detected when concentration is high enough, which is not likely to occur on seeds (detection of TPMVd would also be a desirable trait). | |
| Diagnostic Specificity | | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | | |
| Specify the standard test | | |
| Reproducibility | | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 100%: 100 infested seed in 1000 seeds, 10 infested seeds in 1000, 5 infested seeds in 1000 and 1 infested seed in 1000 tomato seeds. | |
| Repeatability | | |
| Provide the calculated % of | 100%: | |

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| agreement for a given level of the pest (see PM 7/98) | 100 infested seed in 1000 seeds, 10 infested seeds in 1000, 5 infested seeds in 1000 and 1 infested seed in 1000 tomato seeds. |
| Test performance study | |
| Test performance study? | Yes |
| Include brief details of the test performance study and its output. If available, provide a link to published article/report | Intra laboratory testing: Results were the same in the comparative test, and according to expectations. |
| Other information | |
| Any other information considered useful e.g. robustness, ease of performing the test, etc. | The validation study has been published in EPPO Bulletin: Bakker D, Bruinsma M, Dekter RW, Toonen MAJ, Verhoeven JThj & Koenraad HMS (2015) Detection of PSTVd and TCDVd in seeds of tomato using real-time RT-PCR. EPPO Bulletin 45: 14-21. |