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.

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. Journal of Virological Methods 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		

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		
<u>Analytical sensitivity (= limit of detection)</u>		
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.	
<u>Diagnostic sensitivity</u>		
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	
<u>Analytical specificity</u>		
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).	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test		
Specify the standard test		
<u>Reproducibility</u>		
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.	
<u>Repeatability</u>		
Provide the calculated % of	100%:	

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.It 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 & Koenraadt HMS (2015) Detection of PSTVd and TCDVd in seeds of tomato using real-time RT-PCR. EPPO Bulletin 45: 14-21.