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.

Laboratory contact details	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
Short description of the test	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).
Date, reference of the validation report	2012-05-02 - V1.0
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)	Tomato chlorotic dwarf viroid / Pospiviroid chloronani (TCDVD0) Potato spindle tuber viroid / Pospiviroid fusituberis (PSTVD0)
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	no
As or adapted from an IPPC diagnostic protocol	no
Kit	
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 EPPO 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 EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Pospiviroid chloronani(TCDVD0)**
Analytical sensitivity	
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.
Standard test(s)	Grow out and sequence analysis
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	6 PSTVd isolates (5 tested on seed), 5 TCDVd isolates (4 tested on seed)
Specificity value	1
Analytical specificity - exclusivity	
Number of non-target organisms tested	Pospiviroids: CEVd, CSVd, IrVd-1, MPVd, PCFVd, TASVd, TPMVd
Specificity value	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).
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 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.
Organism 2.:	**Pospiviroid fusituberis(PSTVD0)**
Analytical sensitivity	
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.
Standard test(s)	Grow out and sequence analysis
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	6 PSTVd isolates (5 tested on seed), 5 TCDVd isolates (4 tested on seed)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Pospiviroids: CEVd, CSVd, IrVd-1, MPVd, PCFVd, TASVd, TPMVd
Specificity value	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).
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 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.
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, 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	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.

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