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	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	

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 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 10-10^2 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%
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

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