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	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Validation of a conventional RT-PCR assay for detection and identification of CLVd, PSTVd and TCDVd using primers Vid-FW/RE (verhoeven et al. 2004)
Date, reference of the validation report	2013-09-17 - NRC-ref: 2010.molbio.032
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Columnea latent viroid(CLVD00) Potato spindle tuber viroid(PSTVD0) Tomato chlorotic dwarf viroid(TCDVD0)
Detection / identification	detection and identification
Method(s)	Molecular Extraction DNA RNA Molecular Conventional RT PCR Molecular other
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 Conventional RT PCR	
Reference of the test description	
As or adapted from an IPPC diagnostic protocol	yes

IPPC diagnostic Protocol name	ISPM 27 Annex 07 DP 07: Potato spindle tuber viroid (version 2006)
Name of the test	Conventional RT-PCR using the primers of Verhoeven et al. (2004)
Other information	
Other details on the test	section 3.3.3.3 Conventional RT-PCR using the primers of Verhoeven et al. (2004) J.Th.J. Verhoeven, C.C.C. Jansen, T.M. Willemen, L.F.F. Kox, R.A. Owens and J.W. Roenhorst (2004) Natural infections of tomato by Citrus exocortis viroid, Columnea latent viroid, Potato spindle tuber viroid and Tomato chlorotic dwarf viroid. European Journal of Plant Pathology 110: 823–831 RT-PCR by Vid-FW/Vid-RE primers and sequencing of the amplicon
Method: Molecular other	
Reference of the test description	
Other information	
Other details on the test	Sequencing
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Columnea latent viroid(CLVD00)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Undiluted infected leaf sap was considered 100% infected. Starting with undiluted plant sap from infected hosts up to 10^7 times dilutes in sap of healthy tomato leaves, all CLVd species were detected up to a relative infection rate of 10 % (=10 times diluted). PSTVd and TCDVd were detected to a relative infection rate of 100 % (undiluted).
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	1
Standard test(s)	PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and. TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	6, see table 4 of validation report
Specificity value	100 % The test was able to detect CLVd, TCDVd and PSTVd and no reactions were observed for the

	other viroids and viruses included in the validation study. During routine testing it was observed that some PSTVd strains could not be amplified using primers Vid-FW/Vid-RE.
Analytical specificity - exclusivity	
Number of non-target organisms tested	10 (4 Avsunviroidae and 6 Pospiviroidae), see table 6 of validation report
Specificity value	The test was able to detect CLVd, TCDVd and PSTVd and no reactions were observed for the other viroids and viruses included in the validation study. During routine testing it was observed that some PSTVd strains could not be amplified using primers Vid-FW/Vid-RE.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
Specify the test(s)	PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and. TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% with undiluted, 2x, and 4x diluted samples
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% with undiluted, 2x, and 4x diluted samples
Organism 2.:	**Potato spindle tuber viroid(PSTVD0)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Undiluted infected leaf sap was considered 100% infected. Starting with undiluted plant sap from infected hosts up to 10^7 times dilutes in sap of healthy tomato leaves, all CLVd species were detected up to a relative infection rate of 10 % (=10 times diluted). PSTVd and TCDVd were detected to a relative infection rate of 100 % (undiluted).
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	1
Standard test(s)	PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and. TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the

	partial viroid genome.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	6, see table 4 of validation report
Specificity value	100 % The test was able to detect CLVd, TCDVd and PSTVd and no reactions were observed for the other viroids and viruses included in the validation study. During routine testing it was observed that some PSTVd strains could not be amplified using primers Vid-FW/Vid-RE.
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Number of non-target organisms tested	10 (4 Avsunviroidae and 6 Pospiviroidae), see table 6 of validation report
Specificity value	The test was able to detect CLVd, TCDVd and PSTVd and no reactions were observed for the other viroids and viruses included in the validation study. During routine testing it was observed that some PSTVd strains could not be amplified using primers Vid-FW/Vid-RE.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
Specify the test(s)	PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and. TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.
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Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% with undiluted, 2x, and 4x diluted samples
Organism 3.:	**Tomato chlorotic dwarf viroid(TCDVD0)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Undiluted infected leaf sap was considered 100% infected. Starting with undiluted plant sap from infected hosts up to 10^7 times dilutes in sap of healthy tomato leaves, all CLVd species were detected up to a relative infection rate of 10 % (=10 times diluted). PSTVd and TCDVd were detected to a relative infection rate of 100 % (undiluted).
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the	1

standard test, see appendix 2 of PM 7/98	
Standard test(s)	PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and. TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	6, see table 4 of validation report
Specificity value	100 % The test was able to detect CLVd, TCDVd and PSTVd and no reactions were observed for the other viroids and viruses included in the validation study. During routine testing it was observed that some PSTVd strains could not be amplified using primers Vid-FW/Vid-RE.
Analytical specificity - exclusivity	
Number of non-target organisms tested	10 (4 Avsunviroidae and 6 Pospiviroidae), see table 6 of validation report
Specificity value	The test was able to detect CLVd, TCDVd and PSTVd and no reactions were observed for the other viroids and viruses included in the validation study. During routine testing it was observed that some PSTVd strains could not be amplified using primers Vid-FW/Vid-RE.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
Specify the test(s)	PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and. TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% with undiluted, 2x, and 4x diluted samples
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% with undiluted, 2x, and 4x diluted samples
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	To determine the robustness of the test two different reaction mixes were used to generate PCR products: OneStep RT-PCR kit (Qiagen) and

	SuperScript One-Step RT-PCR System with Platinum Taq (Invitrogen). The latter yielded the best results. Different extraction buffers were used. Using a GH plus buffer with a heating step yielded the best results. Freezing the samples prior to analysis did not influence the qualitative results obtained.
The following complementary files are available online:	• Validation of a conventional RT-PCR assay for detection and identification of CLVd, PSTVd and TCDVd using primers Vid-FW/RE (verhoeven et al. 2004). (In Dutch)

Creation date: 2014-05-28 00:00:00 - Last update: 2021-05-10 17:01:48