

**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.

<b>Laboratory contact details</b>	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
<b>Short description of the test</b>	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)
<b>Date, reference of the validation report</b>	2013-09-17 - NRC-ref: 2010.molbio.032
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Columnnea latent viroid / Pospiviroid latenscolumnneae (CLVD00) Potato spindle tuber viroid / Pospiviroid fusicuberis (PSTVD0) Tomato chlorotic dwarf viroid / Pospiviroid chloronani (TCDVD0)
<b>Detection / identification</b>	detection and identification
<b>Matrix(ces) tested</b>	Leaves leaves
<b>Plant species tested</b>	Solanum lycopersicum
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Conventional RT PCR Molecular other
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	RNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
<b>Other information</b>	

<b>Method: Molecular Conventional RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an IPPC diagnostic protocol</b>	yes
<b>IPPC diagnostic Protocol name</b>	ISPM 27 Annex 07 DP 07: Potato spindle tuber viroid (version 2006)
<b>Name of the test</b>	Conventional RT-PCR using the primers of Verhoeven et al. (2004)
<b>Other information</b>	
<b>Other details on the test</b>	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
<b>Method: Molecular other</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	Sequencing
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Pospiviroid latenscolumneae(CLVD00)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	Undiluted infected leaf sap was considered 100% infected. Starting with undiluted plant sap from infected hosts up to 10 <sup>7</sup> 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).
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	1
<b>Standard test(s)</b>	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 Posp1-FW/Posp1-RE are used to sequence the partial viroid genome.

<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	6, see table 4 of validation report
<b>Specificity value</b>	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.
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	10 (4 Avsunviroidae and 6 Pospiviroidae), see table 6 of validation report
<b>Specificity value</b>	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.
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	1
<b>Specify the test(s)</b>	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 Posp1-FW/Posp1-RE are used to sequence the partial viroid genome.
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% with undiluted, 2x, and 4x diluted samples
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% with undiluted, 2x, and 4x diluted samples
<b>Organism 2.:</b>	<b>Pospiviroid fusituberis(PSTVD0)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	Undiluted infected leaf sap was considered 100% infected. Starting with undiluted plant sap from infected hosts up to 10 <sup>7</sup> 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).
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	1

<b>Standard test(s)</b>	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 Posp1-FW/Posp1-RE are used to sequence the partial viroid genome.
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<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	10 (4 Avsunviroidae and 6 Pospiviroidae), see table 6 of validation report
<b>Specificity value</b>	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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<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% with undiluted, 2x, and 4x diluted samples
<b>Organism 3.:</b>	<b>Pospiviroid chloronani(TCDVD0)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	Undiluted infected leaf sap was considered 100% infected. Starting with undiluted plant sap from infected hosts up to 10 <sup>7</sup> 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).
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	1
<b>Standard test(s)</b>	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 Posp1-FW/Posp1-RE are used to sequence the partial viroid genome.
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<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	10 (4 Avsunviroidae and 6 Pospiviroidae), see table 6 of validation report
<b>Specificity value</b>	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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<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	1
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<b>Reproducibility</b>	
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<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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:	<ul style="list-style-type: none"> <li>• <a href="#">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)</a></li> </ul>

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