

**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>	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
<b>Short description of the test</b>	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
<b>Date, reference of the validation report</b>	2012-08-28 - v1.2
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	
<b>If yes, please specify</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Potato spindle tuber viroid(PSTVD0) Tomato chlorotic dwarf viroid(TCDVD0)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Leaves leaves of Solanum lycopersicum; Ornamentals like: Brugmansia, Calibrachoa, Dahlia (spiked greenhouse material), Lycianthes rantonettii, Petunia, Solanum jasminoides, Streptosolen.
<b>Plant species tested</b>	Brugmansia sp., Calibrachoa sp., Dahlia sp., Lycianthes sp., Petunia sp., Solanum laxum, Solanum lycopersicum, Streptosolen sp.
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular real time RT PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	

<b>As or adapted from an IPPC diagnostic protocol</b>	
<b>Is the test modified compared to the reference test</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>Other details on the test</b>	
<b>Method: Molecular Extraction DNA RNA (2)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	
<b>As or adapted from an IPPC diagnostic protocol</b>	
<b>Is the test modified compared to the reference test</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	LGC
<b>Specify the kit used</b>	sbeadex maxi plant
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	
<b>Method: Molecular real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</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 2016)
<b>Name of the test</b>	Real-time RT-PCR using the primers of Boonham et al. (2004)
<b>Is the test modified compared to the</b>	

reference test	
<b>Kit</b>	
Is a kit used	
<b>Other information</b>	
Reaction type	
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	no
<b>Performance Criteria :</b>	
Organism 1.:	<b>Potato spindle tuber viroid(PSTVD0)</b>
<b>Analytical sensitivity</b>	
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
<b>Diagnostic sensitivity</b>	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	
Standard test(s)	
<b>Analytical specificity - inclusivity</b>	
Number of strains/populations of target organisms tested	9 PSTVd isolates, 5 TCDVd isolates
Specificity value	100%
<b>Analytical specificity - exclusivity</b>	
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 $10^1$ - $10^2$ dilution).
Cross reacts with	
<b>Diagnostic Specificity</b>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	

<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Organism 2.:</b>	<b>Tomato chlorotic dwarf viroid(TCDVD0)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	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
<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>	
<b>Standard test(s)</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	9 PSTVd isolates, 5 TCDVd isolates
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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
<b>Specificity value</b>	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).
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%

<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful</b>	

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