

**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 pospiviroids in leaves of horticultural crops
<b>Date, reference of the validation report</b>	2012-09-28 - 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>Description of the test</b>	
<b>Organism(s)</b>	Pospiviroid (1POSPG)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Leaves leaves of Solanum lycopersicum, ornamentals like: Brugmansia, Calibrachoa, Cestrum, Dahlia (spiked greenhouse material), Lycianthes rantonettii, Nematanthus, Petunia, Solanum jasminoides, Streptosolen, Vinca.
<b>Plant species tested</b>	Brugmansia sp., Calibrachoa sp., Cestrum sp., Dahlia sp., Lycianthes sp., Nematanthus sp., Petunia sp., Solanum laxum, Solanum lycopersicum, Streptosolen sp., Vinca 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>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>Method: Molecular Extraction DNA RNA (2)</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 real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/138 Pospiviroids (genus Pospiviroid) (version 1)
<b>Name of the test</b>	Real-time RT-PCR (Botermans et al., 2013)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Other information</b>	
<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(1POSPG)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	Solanum lycopersicon: Botermans et al., 2013. Ornamentals: Relative sensitivity dependent on initial viroid concentration and host plant species. Validated for bulking rates up to 25 for Brugmansia, Calibrachoa, Cestrum, Dahlia (greenhouse)*, Nematanthus, Petunia, Solanum jasminoides and Streptosolen jamesonii, but test is more sensitive. For Calibrachoa, Solanum jasminoides and Streptosolen jamesonii matrix effects have been observed at dilutions over 100x. 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>	100%
<b>Standard test(s)</b>	PCR sequencing of the complete viroid genome is considered the standard test.
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	28 pospiviroid isolates of 10 species (Botermans et al., 2013)

<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Avsunviroidae: ASBVd, CChMVd, ELVd Pospiviroidae: ASSVd, CbVd-1, HpLVd, HpSVd, DLVd Viruses: AMV, CMV, PepMV, PVY, ToMV, TMV, ToCV, TYLCV
<b>Specificity value</b>	no cross reactions
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% Inter and intralaboratory testing
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% Inter and intralaboratory testing
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Three laboratories participated in the interlaboratory comparison: Dutch General Inspection Service for Agricultural Seed and Seed Potatoes (NAK, Emmeloord), Naktuinbouw (Roelofarendsveen) and the National Reference Centre of the National Plant Protection Organization (Wageningen). Sixteen samples of tomato leaves infected with PSTVd, TASVd or TCDVd at different relative infection rates were tested at the three laboratories (Botermans et al. 2013)
<b>Other information</b>	
<b>Any other information considered useful</b>	Choice of PCR mix is important (Botermans et al., 2013)

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