

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**  
**ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES**  
(11-17239)

**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>Target Organism</b>	Pospiviroid	
<b>Short description</b>	Real-time RT-PCR (TaqMan RT-PCR) for pospiviroids in leaves of horticultural crops	
<b>Laboratory contact details</b>	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands	
<b>Date and reference of the validation report</b>	28-09-2012 - V1.2	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	N/R M. Botermans, B.T.L.H. van de Vossenbergh, J.Th.J. Verhoeven, J.W. Roenhorst, M. Hooftman, R. Dekter, E.T.M. Meekes (2013). Development and validation of a real-time RT-PCR assay for generic detection of pospiviroids. <i>Journal of Virological Methods</i> 187: 43-50	
<b>Is the test the same as described in the EPPO DP?</b>		
<b>Is the lab accredited for this test?</b>	Yes	
<b>Plant species tested (if relevant)</b>	Solanum lycopersicum, ornamentals like: Brugmansia, Calibrachoa, Cestrum, Dahlia (spiked greenhouse material), Lycianthes rantonettii, Nematanthus, Petunia, Solanum jasminoides, Streptosolen, Vinca.	
<b>Matrices tested (if relevant)</b>	leaves	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>		
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	X	RNA isolation using KingFisher and SBeadex maxi plant kit (LGC) or RNeasy Plant mini kit (Qiagen), followed by real-time RT-PCR
<b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>		
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b>		
<b>Pathogenicity test</b>		

<b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA profiling</b>		
<b>Morphological and morphometrical methods intended for identification</b>		
<b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>		
<b>Other</b>		
<b>Analytical sensitivity (= limit of detection)</b>		
<b>What is 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>Specify the standard test</b>	PCR sequencing of the complete viroid genome is considered the standard test.	
<b>Analytical specificity</b>		
<b>Specificity value</b>	100%	
<b>Number of strains/populations of target organisms tested</b>	28 pospiviroid isolates of 10 species (Botermans et al., 2013)	
<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>Cross reacts with (specify the species)</b>	no	
<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 standard test</b>		
<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><u>Test performance study</u></b>	
<b>Test performance study?</b>	Yes
<b>Include brief details of the test performance study and its output. It available, provide a 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><u>Other information</u></b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	Choice of PCR mix is important (Botermans et al., 2013)