

**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>	Validation of a conventional RT-PCR assay for detection and preliminary identification of pospiviroids (expect CLVd) by Posp1-FW/Posp1-RE	
<b>Laboratory contact details</b>	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands	
<b>Date and reference of the validation report</b>	2013-09-17 - NRC-ref: 2010.molbio.033	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	N/R Update ISPM 27:2006 - Potato spindle tuber viroid (2006-022), 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	
<b>Is the test the same as described in the EPPO DP?</b>		
<b>Is the lab accredited for this test?</b>	No	
<b>Plant species tested (if relevant)</b>	Solanum lycopersicum Celosia cardiophyllum	
<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 by the RNeasy Plant Mini Kit (Qiagen), followed by RT-PCR by Posp1-FW/Posp1-RE primers and sequencing of the amplicon
<b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>		
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective</b>		

<b>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>	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 species were detected up to a relative infection rate of 2,5 % (=40 times diluted).	
<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. 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</b>		
<b>Specificity value</b>	100 %	
<b>Number of strains/populations of target organisms tested</b>	12 pospiviroid strains, see table 4 in validation report	
<b>Number of non-target organisms tested</b>	10 (4 Avsunviroidae and 6 Pospiviroidae), see table 8 in validation report	
<b>Cross reacts with (specify the species)</b>		
<b>Diagnostic Specificity</b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100 %	
<b>Specify the standard test</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% at undiluted, 2x, 4x, 10x and 100 diluted
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% at undiluted, 2x, 4x, 10x and 100 diluted
<b>Test performance study</b>	
<b>Test performance study?</b>	No
<b>Include brief details of the test performance study and its output. It available, provide a link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	<p>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.</p> <p>Freezing the samples prior to analysis did not influence the qualitative results obtained.</p>
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 preliminary identification of pospiviroids (expect CLVd) by Posp1-FW/RE. (In Dutch)</a></li> </ul>