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.

Target Organism	Columnea latent viroid Potato spindle tuber viroid Tomato chlorotic dwarf viroid	
Short description	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)	
Laboratory contact details	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands	
Date and reference of the validation report	2013-09-17 - NRC-ref: 2010.molbio.032	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	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	
Is the test the same as described in the EPPO DP?		
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Solanum lycopersicum	
Matrices tested (if relevant)	leaves	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	RNA isolation by the RNeasy Plant Mini Kit (Qiagen), followed by RT-PCR by Vid-FW/Vid-RE primers and sequencing of the amplicon
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective		

enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	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 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).	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100 %	
Specify the standard test	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 Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.	
Analytical specificity		
Specificity value	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.	
Number of strains/populations of target organisms tested	6, see table 4 of validation report	
Number of non-target organisms tested	10 (4 Avsunviroidae and 6 Pospiviroidae), see table 6 of validation report	
Cross reacts with (specify the species)	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.	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing	100 %	

negative compared to results from a standard test	
Specify the standard test	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 Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% with undiluted, 2x, and 4x diluted samples
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% with undiluted, 2x, and 4x diluted samples
Test performance study	
Test performance study?	No
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	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:	• 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)