

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	Pospiviroid	
Short description	Development and validation of a real-time RT-PCR assay for generic detection of Pospiviroids	
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	2012-04-04 - NRC-ref: 2010.molbio.015	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	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. Journal of Virological Methods 187 43- 50	
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 using the RNeasy Plant Mini Kit (Qiagen), followed by real-time RT-PCR
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 pospiviroids species were detected up to a relative infection rate of 0.13% (=770 times diluted)	
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 Posp1-FW/Posp1-RE are used to sequence the partial viroid genome.	
Analytical specificity		
Specificity value	100 %	
Number of strains/populations of target organisms tested	28 pospiviroid strains, see table 1 Botermans et al. (2013)	
Number of non-target organisms tested	8 non -ospiviroid strains and 8 tomato viruses, see table 1 Botermans et al. (2013)	
Cross reacts with (specify the species)		
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	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 Posp1-FW/Posp1-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% at undiluted, 500 x, and 1000x diluted	

Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% at undiluted, 500 x, and 1000x diluted
Test performance study	
Test performance study?	Yes
Include brief details of the test performance study and its output. It available, provide a link to published article/report	Three laboratories participated in the inter-laboratory 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 Tomato chlorotic dwarf viroid (TCDVd) at different relative infection rates were tested at the three laboratories (Table 6, Botermans et al. 2013)
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	For tomato leaves the assay is not influenced by the matrix (selectivity). To determine the robustness of the test an alternative RNA extraction kit was used: the Sbeadex maxi plant kit on a Kingfisher KF96 system, and Real-time RT-PCRs were carried out on different real-time PCR machines. The test results were similar for all samples tested.
The following complementary files are available online:	<ul style="list-style-type: none"> • Development and validation of a real-time RT-PCR assay for generic detection of pospiviroids