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	Potato spindle tuber viroid Tomato chlorotic dwarf viroid	
Short description	Real-time RT-PCR (TaqMan RT-PCR) for Potato spindle tuber viroid (PSTVd) and/or Tomato chlorotic dwarf viroid (TCDVd) in leaf material of horticultural crops	
Laboratory contact details	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands	
Date and reference of the validation report	28-08-2012 - v1.2	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 N. Boonham, L. González-Pérez, M.S. Mendez, E. Lilia Peralta, A. Blockley, K. Walsh, I. Barker,R.A. Mumford, 2004. Development of a real-time RT-PCR assay for the detection of Potato spindle tuber viroid. Journal of Virological Methods 116:139-146.	
Is the test the same as described in the EPPO DP?	No IPPC protocol is in preparation	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Solanum lycopersicum; Ornamentals like: Brugmansia, Calibrachoa, Dahlia (spiked greenhouse material), Lycianthes rantonettii, Petunia, Solanum jasminoides, Streptosolen.	
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 KingFisher and SBeadex maxi plant kit (LGC) or 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?	Solanum lycopersicon: up to 10^6 - 10^7 dilution in sap of healthy tomato leaves. Ornamentals: Relative sensitivity dependent on initial viroid concentration and host plant species. Validated for bulking rates up to 25 for Brugmansia, Calibrachoa, Dahlia (greenhouse), Petunia, Solanum jasminoides and Streptosolen jamesonii, but test is more sensitive. For some crops like field Dahlia, only the summer period seems suitable for (reliable) testing	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98		
Specify the standard test		
Analytical specificity		
Specificity value	100%	
Number of strains/populations of target organisms tested	9 PSTVd isolates, 5 TCDVd isolates	
Number of non-target organisms tested	8 other pospiviroids: CLVd, CSVd, CEVd, IrVd-1, MPVd, PCFVd, TASVd, TPMVd 4 Pospiviroidae: ASSVd, HLVd, HSVd, DLVd 2 avsunviroids: ASBVd, CChMVd 8 viruses (tomato): AMV, CMV, PepMV, PVY, ToMV, TMV, ToCV, TYLCV	
Cross reacts with (specify the species)	MPVd, PSTVd and TCDVd are detected equally well (up to 10^6-10^7 dilution in sap of healthy tomato leaves); TMPVd is also detected, but not as well as PSTVd, TCDVd or MPVd (up to 10-10^2 dilution).	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test		
Specify the standard test		
Reproducibility		

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
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.**	