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	Real-time RT-PCR (TaqMan RT-PCR) for pospivirods in leaves of horticultural crops	
Laboratory contact details	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands	
Date and reference of the validation report	28-09-2012 - V1.2	
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 Vossenberg, 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?	Yes	
Plant species tested (if relevant)	Solanum lycopersicum, ornamentals like: Brugmansia, Calibrachoa, Cestrum, Dahlia (spiked greenhouse material), Lycianthes rantonettii, Nematanthus, Petunia, Solanum jasminoides, Streptosolen, Vinca.	
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: 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.	
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.	
Analytical specificity		
Specificity value	100%	
Number of strains/populations of target organisms tested	28 pospiviroid isolates of 10 species (Botermans et al., 2013)	
Number of non-target organisms tested	Avsunviroidae: ASBVd, CChMVd, ELVd Pospiviroidae: ASSVd, CbVd-1, HpLVd, HpSVd, DLVd Viruses: AMV, CMV, PepMV, PVY, ToMV, TMV, ToCV, TYLCV	
Cross reacts with (specify the species)	no	
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% Inter and intralaboratory testing	
Repeatability		

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Inter and intralaboratory testing
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 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)
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	Choice of PCR mix is important (Botermans et al., 2013)