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	
Short description	detection of PSTVd by RT-PCR and real-time RT-PCR	
Laboratory contact details	Bavarian State Research Center for Agriculture, Institute for Plant Protection - Phytopathology and Diagnosis Lange Point 10, 85354 Freising, Germany	
Date and reference of the validation report	2016-12-07 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/033 IPPC Standard INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES - ISPM 27 DIAGNOSTIC PROTOCOLS - DP 7: Potato spindle tuber viroid“	
Is the test the same as described in the EPPO DP?	Modified RNA extraction: same as EPPO DP; RT-PCR: different from EPPO DP; Real time RT-PCR: unclear, primer/probe not given in EPPO DP Internal Control RT-PCR and Real time RT-PCR: different from EPPO DP	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Tobacco, tomato, Calibrachoa sp., Solanum jasminoides	
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 extraction: RNeasy Plant Mini Kit (QIAGEN) Conventional RT-PCR: Pospiviroids: Verhoeven et al. 2004 (Pospi and Vid primers), IPC: Menzel et al. 2002 (nad5 mRNA primers) Real time RT-PCR: PSTVd: Boonham et al. 2004 (PSTVd primers), IPC: Weller et al. 2000 (COX primers and probe), Botermans et al. 2004 (nad5 mRNA primers and probe)
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?	Conventional RT-PCR: Without testing RNA extraction (i.e. dilution of extracted PSTVd-RNA in RNA from negative tomato): Pospi primers: with dilution 1:10, 100% of positives, with dilutions 1:100 and 1:1000 in 94% of positives and with dilution 1:10000, 63% of positives Vid primers: with dilution 1:10, 100% of positives and with dilution 1:100, 44% of positives. With testing of RNA extraction: popsi primers: with dilutions 1:100 and 1:1000, 100% of positives Vid primers: with dilution 1:100, 100% of positives and with dilution 1:1000, 31 to 50% of positives. Real-time RT-PCR: Without testing RNA extraction (i.e. dilution of extracted PSTVd-RNA in RNA from negative tomato): with dilutions 1:10 to 1:100000, 100% of positives With testing of RNA extraction: with dilutions 1:100 to 1:10000 (highest dilution tested) in 100% of positives
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	Not evaluated	
Specify the standard test		
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested	1 PSTVd PV-0950	
Number of non-target organisms tested	3 for real-time RT-PCR (Boonham primers/probe) CEVd PV-0942, TASVd PV-1151, TCDVd PV-1148 (DSMZ) 5 for RT-PCR (Pospi and Vid primers): CEVd, PCFVd, TASVd, TCDVd (all Wageningen, Verhoven), CSVd (DSMZ)	

Cross reacts with (specify the species)	Boonham primers/probe: TASVd Pospi primers: CEVd, PCFVd, TASVd, TCDVd Vid primers: TCDVd
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Not evaluated
Specify the standard test	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Real-time RT-PCR and RT-PCR: fresh material and storage for 3d and 14 d at app. 8 °C and app. minus 80 °C: 100 %
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	see analytical senstivitiy
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.**	Selectivity is determined using the results from various experiments with different matrices (tobacco, tomato, Calibrachoa sp. and Solanum jasminoides). Inhibitory effects of the plant matrixon RT-PCR/real-time RT-PCR were not found in the experiments.
The following complementary files are available online:	• Bericht_Validierung_PSTVd_Pospiviroide_RNA-Extraktion_RT-PCR_Realtime RT-PCR_Ergänzung 07.12.2016