

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 in potato leaf using Boonham real-time RT-PCR. version 1	
Laboratory contact details	Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands	
Date and reference of the validation report	January 2015 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/033(1)	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Solanum tuberosum	
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	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?	For routine samples grown in a greenhouse: 1 infected leave in a pool of 2500 leaves can be detected. Relative infection rate: 0.04%. Test have been validated for bulking rates up to 100 (100% detection in sample composed of 1 infected and 99 healthy leaves)	
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		
Number of strains/populations of target organisms tested	Potato spindle viroid: isolates PD5627996, M16826, N, PD3077695, Howell, W202, W223, W224.	
Number of non-target organisms tested	Potato virus Y, Potato virus Y, Potato virus Y, Potato virus A, Potato virus X, Potato virus S, Potato leaf roll virus, Potato virus M, Potato virus V, Pepino mosaic virus, Andean potato latent virus, Andean potato mottle virus, Potato black ringspot virus, Potato mop top virus, Tobacco rattle virus, Citrus exocortis viroid, Columnea latent viroid, Chrysanthemum stunt viroid, Mexican pepita viroid, Pepper chat fruit viroid, Tomato apical stunt viroid, Tomato chlorotic dwarf viroid, Tomato plant macho viroid, Iresine viroid.	
Cross reacts with (specify the species)	Mexican pepita viroid, Tomato chlorotic dwarf viroid, Tomato plant macho viroid After performing an in silico analysis on the primers used in this real-time PCR to detect PSTVd the cross reactions could be explained.	
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)	Reproducibility: 100%	
Repeatability		
Provide the calculated % of	Repeatability: 100%	

agreement for a given level of the pest (see PM 7/98)	
Test performance study	
Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	Robustness: Freezing of samples don't influence the test results. The analytical sensitivity of the test is not influenced by using nad5 as internal control (duplex format).