

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
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.

Laboratory contact details	Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands
Short description of the test	Detection of PSTVd ??in potato leaf using Boonham real-time RT -PCR. version 1
Date, reference of the validation report	2015-01-01 -
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
If yes, please specify	
Description of the test	
Organism(s)	Potato spindle tuber viroid(PSTVD0)
Detection / identification	detection
Matrix(ces) tested	Leaves leaves
Plant species tested	Solanum tuberosum
Method(s)	Molecular real time RT PCR
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/033 Potato spindle tuber viroid (version 1)
Name of the test	Taqman real time RT PCR
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	no
Kit	
Is a kit used	

Other information	
Reaction type	
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Potato spindle tuber viroid(PSTVD0)
Analytical sensitivity	
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	
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Potato spindle viroid: isolates PD5627996, M16826, N, PD3077695, Howell, W202, W223, W224.
Specificity value	
Analytical specificity - exclusivity	
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.
Specificity value	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.
Cross reacts with	Tomato chlorotic dwarf viroid Tomato planta macho viroid Mexican papita viroid
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	

Specify the test(s)	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Reproducibility: 100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability: 100%
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output. If available, link to published article/report	
Other information	
Any other information considered useful	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).

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