

**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.

<b>Laboratory contact details</b>	Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands
<b>Short description of the test</b>	Detection of PSTVd ??in potato leaf using Boonham real-time RT -PCR. version 1
<b>Date, reference of the validation report</b>	2015-01-01 -
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Potato spindle tuber viroid / Pospiviroid fusituberis (PSTVD0)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular real time RT PCR
<b>Method: Molecular real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/033 Potato spindle tuber viroid (version 1)
<b>Name of the test</b>	Taqman real time RT PCR
<b>Is the test modified compared to the reference test</b>	no
<b>Other information</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	no
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Pospiviroid fusituberis(PSTVD0)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	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)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	Potato spindle viroid: isolates PD5627996, M16826, N, PD3077695, Howell, W202, W223, W224.
<b>Specificity value</b>	
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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.
<b>Specificity value</b>	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.
<b>Cross reacts with</b>	Tomato chlorotic dwarf viroid Tomato planta macho viroid Mexican papita viroid
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Reproducibility: 100%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Repeatability: 100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	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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