

**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>	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
<b>Short description of the test</b>	Detection of pospiviroids by realtime RT-PCR on tomato and pepper seeds, i.e. CEVd, CLVd, PCFVd, TASVd, TCDVd, TPMVd
<b>Date, reference of the validation report</b>	2015-10-08 - TESTA Deliverable 5.3 Validated methods for viruses and viroids
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	TESTA
<b>Description of the test</b>	
<b>Organism(s)</b>	Pospiviroid(1POSPG)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Seeds seeds
<b>Plant species tested</b>	Capsicum annuum, Solanum lycopersicum
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular real time RT PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	
<b>EPPO Diagnostic Protocol name</b>	PM 7/138 Pospiviroids (genus Pospiviroid) (version 1)
<b>Name of the test</b>	Seeds - Homogenization in GH+ buffer
<b>As or adapted from an IPPC diagnostic protocol</b>	no

<b>Is the test modified compared to the reference test</b>	
<b>Other information</b>	
<b>Other details on the test</b>	Tomato seeds: crush seeds in buffer using stomacher Pepper seeds: crush seeds using genogrinder and subsequently add buffer
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	
<b>EPPO Diagnostic Protocol name</b>	PM 7/138 Pospiviroids (genus Pospiviroid) (version 1)
<b>Name of the test</b>	
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	LGC
<b>Specify the kit used</b>	sbeadex maxi plant
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	RNA isolation using Kingfisher with Sbeadex kit
<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>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	
<b>EPPO Diagnostic Protocol name</b>	PM 7/138 Pospiviroids (genus Pospiviroid) (version 1)
<b>Name of the test</b>	Real-time RT-PCR for seed testing (Naktuinbouw, 2017)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	
<b>Kit</b>	
<b>Is a kit used</b>	

<b>Other information</b>	
<b>Reaction type</b>	Probe
<b>Other details on the test</b>	Descriptions of the tests are available in complementary files available online (see links at the end of the sheet)
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>yes</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Pospiviroid(1POSPG)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	For all seven viroids at least the 1000x dilution was detected and therefore the requirement detection of at least the 100x dilution was met. (Only for TPMVd not all 1000x dilutions were detected below the threshold of 32)
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	100% for reaction mix A (PSTVd, PCFVd, TCDVd), note that primers and probes are similar to those used in the standard test. No data for reaction mixes B, C and D. No data for pepper seeds.
<b>Standard test(s)</b>	Detection of PSTVd and TCDVd in tomato seeds as described by Bakker et al (2015), EPPO Bulletin.
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	All 18 isolates of 7 species detected (TESTA report, Table 6).
<b>Specificity value</b>	The analytical specificity was good since no false-negatives were observed for all primer sets and none of the non-target viroids and viruses reacted with the PCRs. Some acceptable cross-reactivity of TASVd isolates with the CEVd/CLVd primer mix (B) was observed. Objective of the seed assay is detect all relevant pospiviroids and identification of the pospiviroid is relatively less important.
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	No cross reactions with 29 isolates of other viruses and viroids tested (TESTA report, Table 7).
<b>Specificity value</b>	Only cross-reactivity observed within pospiviroids, no cross-reactivity with other viroids or viruses
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100% for reaction mix A (PSTVd, PCFVd, TCDVd), note that primers and probes are similar to those used in the standard test. No data for reaction mixes B, C and D. No data for pepper seeds.
<b>Specify the test(s)</b>	Detection of PSTVd and TCDVd in tomato seeds as described by Bakker et al (2015), EPPO Bulletin.

<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% for all target species
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% for all target species
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. If available, link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful</b>	A proficiency test with four laboratories with naturally PSTVd-contaminated tomato seeds (1 PSTVd genotype) showed that the SPN-V043 2.0 method at Naktuinbouw did perform well. Multiple samples with only 10 PSTVd contaminated seeds amongst 990 healthy tomato seeds were detected.
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Test description Pepper seeds</a></li> <li>• <a href="#">Test description tomato seeds</a></li> <li>• <a href="#">Validation report Detection of pospiviroids by PCR in tomato seeds</a></li> </ul>

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