

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

Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/138 Pospiviroids (genus Pospiviroid) (version 1)
As or adapted from an IPPC diagnostic protocol	no
Kit	
Is a kit used	yes
Manufacturer name	LGC
Specify the kit used	sbeadex maxi plant
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	RNA isolation using Kingfisher with Sbeadex kit
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/138 Pospiviroids (genus Pospiviroid) (version 1)
Name of the test	Real-time RT-PCR for seed testing (Naktuinbouw, 2017)
As or adapted from an IPPC diagnostic protocol	no
Other information	
Reaction type	Probe
Other details on the test	Descriptions of the tests are available in complementary files available online (see links at the end of the sheet)
Are the performance characteristics included in the EPPO diagnostic protocol?	yes
Performance Criteria :	
Organism 1.:	Pospiviroid(1POSPG)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	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)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	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.
Standard test(s)	Detection of PSTVd and TCDVd in tomato seeds as described by Bakker et al (2015), EPPO Bulletin.
<u>Analytical specificity - inclusivity</u>	
Number of strains/populations of target organisms tested	All 18 isolates of 7 species detected (TESTA report, Table 6).
Specificity value	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.
<u>Analytical specificity - exclusivity</u>	
Number of non-target organisms tested	No cross reactions with 29 isolates of other viruses and viroids tested (TESTA report, Table 7).
Specificity value	Only cross-reactivity observed within pospiviroids, no cross-reactivity with other viroids or viruses
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	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.
Specify the test(s)	Detection of PSTVd and TCDVd in tomato seeds as described by Bakker et al (2015), EPPO Bulletin.
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for all target species
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for all target species
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	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:	• Test description Pepper seeds • Test description tomato seeds • Validation report Detection of pospiviroids by PCR in tomato seeds

