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	Pospiviroid	
Short description	Detection of pospiviroids by realtime RT-PCR on tomato and pepper seeds, i.e. CEVd, CLVd, PCFVd, TASVd, TCDVd, TPMVd	
Laboratory contact details	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands	
Date and reference of the validation report	2015-10-08 - TESTA Deliverable 5.3 Validated methods for viruses and viroids.	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 Naktuinbouw protocols SPN-V043 v2.0: Real-time RT-PCR (RT Taqman PCR) for pospiviroids (CEVd, CLVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd) on seeds of tomato (Solanum lycopersicum) and SPN-V044 v2.0: Real-time RT-PCR (RT Taqman PCR) for pospiviroids (CEVd, CLVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd) on seeds of pepper (Capsicum annuum) (both available on www.naktuinbouw.eu)	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Solanum lycopersicum and Capsicum annuum	
Matrices tested (if relevant)	seeds	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Tomato seeds: crush seeds in buffer using stomacher Pepper seeds: crush seeds using genogrinder and subsequently add buffer
Molecular methods, e.g. hybridization, PCR and real time PCR	X	RNA isolation using Kingfisher with Sbeadex kit followed by Taqman PCR. Descriptions of the tests are available in complementary files available online (see links at the end of the sheet)
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		

<u>Analytical sensitivity (= limit of detection)</u>	
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)
<u>Diagnostic sensitivity</u>	
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.
Specify the standard test	Detection of PSTVd and TCDVd in tomato seeds as described by Bakker et al (2015), EPPO Bulletin.
<u>Analytical specificity</u>	
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.
Number of strains/populations of target organisms tested	All 18 isolates of 7 species detected (TESTA report, Table 6).
Number of non-target organisms tested	No cross reactions with 29 isolates of other viruses and viroids tested (TESTA report, Table 7).
Cross reacts with (specify the species)	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 standard test	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

Repeatability	
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
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	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