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	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Development and validation of a real-time RT-PCR assay for generic detection of Pospiviroids
Date, reference of the validation report	2012-04-04 - NRC-ref: 2010.molbio.015
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)	Pospiviroid(1POSPG)
Detection / identification	detection
Matrix(ces) tested	Leaves leaves
Plant species tested	Solanum lycopersicum
Method(s)	Molecular Extraction DNA RNA Molecular real time RT PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN

Specify the kit used	RNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	M. Botermans, B.T.L.H. van de Vossenberg, J.Th.J. Verhoeven, J.W. Roenhorst, M. Hooftman, R. Dekter, E.T.M. Meekes (2013) Development and validation of a real-time RT-PCR assay for generic detection of Pospiviroids. Journal of Virological Methods 187 43– 50
Is the test modified compared to the reference test	
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.:	**Pospiviroid(1POSPG)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Undiluted infected leaf sap was considered 100% infected. Starting with undiluted plant sap from infected hosts up to 10^7 times dilutes in sap of healthy tomato leaves, all pospiviroids species were detected up to a relative infection rate of 0.13% (=770 times diluted)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	1
Standard test(s)	PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and TPMVd). For other pospiviroids primers

	Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	28 pospiviroid strains, see table 1 Botermans et al. (2013)
Specificity value	1
Analytical specificity - exclusivity	
Number of non-target organisms tested	8 non -ospiviroid strains and 8 tomato viruses, see table 1 Botermans et al. (2013)
Specificity value	
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
Specify the test(s)	PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% at undiluted, 500 x, and 1000x diluted
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% at undiluted, 500 x, and 1000x diluted
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	Three laboratories participated in the inter-laboratory comparison: Dutch General Inspection Service for Agricultural Seed and Seed Potatoes (NAK, Emmeloord), Naktuinbouw (Roelofarendsveen) and the National Reference Centre of the National Plant Protection Organization (Wageningen). Sixteen samples of tomato leaves infected with PSTVd, TASVd or Tomato chlorotic dwarf viroid (TCDVd) at different relative infection rates were tested at the three laboratories (Table 6, Botermans et al. 2013)
Other information	
Any other information considered useful	For tomato leaves the assay is not influenced by the matrix (selectivity). To determine the robustness of the test an alternative RNA extraction kit was used: the Sbeadex maxi plant kit on a Kingfisher KF96 system, and Real-time RT-PCRs were carried out on different real-time PCR

	machines. The test results were similar for all samples tested.
The following complementary files are available online:	• Development and validation of a real-time RT-PCR assay for generic detection of pospiviroids

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