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	Real-time RT-PCR (TaqMan RT-PCR) for pospivirods in leaves of horticultural crops
Date, reference of the validation report	2012-09-28 - V1.2
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?	
Description of the test	
Organism(s)	Pospiviroid (1POSPG)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular real time RT PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
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	
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
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	
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 (Botermans et al., 2013)
As or adapted from an IPPC diagnostic protocol	no
Other information	
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?	Solanum lycopersicon: Botermans et al., 2013. Ornamentals: Relative sensitivity dependent on initial viroid concentration and host plant species. Validated for bulking rates up to 25 for Brugmansia, Calibrachoa, Cestrum, Dahlia (greenhouse)*, Nematanthus, Petunia, Solanum jasminoides and Streptosolen jamesonii, but test is more sensitive. For Calibrachoa, Solanum jasminoides and Streptosolen jamesonii matrix effects have been observed at dilutions over 100x. For some crops like field Dahlia, only the summer period seems suitable for (reliable) testing.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Standard test(s)	PCR sequencing of the complete viroid genome is considered the standard test.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	28 pospiviroid isolates of 10 species (Botermans et al., 2013)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Avsunviroidae: ASBVd, CChMVd, ELVd Pospiviroidae: ASSVd, CbVd-1, HpLVd, HpSVd, DLVd Viruses: AMV, CMV, PepMV, PVY, ToMV, TMV, ToCV, TYLCV
Specificity value	no cross reactions

Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Inter and intralaboratory testing
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Inter and intralaboratory testing
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 interlaboratory 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 TCDVd at different relative infection rates were tested at the three laboratories (Botermans et al. 2013)
Other information	
Any other information considered useful	Choice of PCR mix is important (Botermans et al., 2013)

Creation date: 2014-06-12 00:00:00 - Last update: 2021-05-14 16:51:59