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	Council for Agricultural Research and Economics- Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy	
Short description of the test	Detection of tomato brown rugose fruit virus by molecular conventional RT PCR in leaves and fruits	
Date, reference of the validation report	2020-12-29 - Validation report 2020 - ToBRFV	
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?	Other_project	
If yes, please specify	VALITEST	
Description of the test		
Organism(s)	Tomato brown rugose fruit virus / Tobamovirus fructirugosum (TOBRFV)	
Detection / identification	detection	
Method(s)	Molecular Extraction DNA RNA Molecular Conventional RT PCR	
Method: Molecular Extraction DNA RNA		
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?	yes	
As or adapted from an IPPC diagnostic protocol	no	
Is the test modified compared to the reference test	no	
Kit		
Is a kit used	yes	
Manufacturer name	QIAGEN	
Specify the kit used	RNeasy Plant Mini Kit	

Kit used following the manufacturer's instructions?	no Plant materials was grounded on 0.1M phosphate buffer 7.2 pH	
Other information		
Method: Molecular Conventional 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?	yes	
As or adapted from an IPPC diagnostic protocol	no	
Reference of the test	Rodríguez-Mendoza J., de Jesús García-Ávila C., López-Buenfil J.A., Araujo-Ruiz K., Quezada- Salinas A., Cambrón-Crisantos J.M., Ochoa-Martínez D.L., 2019. Identification of Tomato brown rugose fruit virus by RT-PCR from a coding region of replicase (RdRP). Mexican Journal of Phytopathology (37)2	
Is the test modified compared to the reference test	yes The published primers were included in a kit	
Kit		
Is a kit used	yes	
Manufacturer name	LOEWE	
Specify the kit used	Tomato Brown Rugose Fruit Virus RNA PCR Cat No 09175	
Kit used following the manufacturer's instructions?	yes	
Other information		
Performance Criteria :		
Organism 1.:	Tobamovirus fructirugosum(TOBRFV)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	LOD (evaluated in accordandance of PM 7/98) 10^-5 level of ten-fold serial dilution fot tomato and 10^-3 level of ten-fold serial dilution for pepper probability of detection 3.3 evaluated during the TPS on 5 samples at five level of dilution from 10^0 to 10^-8	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	85%	
Standard test(s)	Comparison with samples of known status during TPS	
Analytical specificity - inclusivity		
Number of strains/populations of target	PV-1236; PV-1241 (DSMZ collection); Sicily isolates	

Specificity value	100%	
Analytical specificity - exclusivity		
Number of non-target organisms tested	ToMV PV-0141; TMV PV-1252; PMMoV PV-0165; BPeMV PV-0170; TMGMV PV-0124 (DSMZ collection) evaluated in preliminary studies	
Specificity value	100%	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	93%	
Specify the test(s)	Comparison with samples of known status during TPS	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	81% (TPS)	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	81% (TPS)	
Test performance study		
Test performance study?	yes	
Brief details of the test performance study and its output.It available, link to published article/report	Test performance study organized in the framework of the VALITEST project involving 34 laboratories from 18 countries	
The following complementary files are available online:	<u>Report TPS ToBRFV</u>	

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