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 real time RT PCR in leaves and fruits Date, reference of the validation report 2020-12-29 - Validation report 2020 - ToBRFV Validation process according to EPPO yes Standard PM7/98? Is the lab accredited for this test? no Was the validated data generated in the framework of a project? If yes, please specify VALITEST Description of the test Organism(s) Tomato brown rugose fruit virus / Tobamovirus fructirugosum (TOBRFV) Detection / Identification Method(s) Molecular Extraction DNA RNA Reference of the test description As or adapted from an EPPO diagnostic protocol As or adapted from an IPPC diagnostic protocol? 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			
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Other information		
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?	yes	
As or adapted from an IPPC diagnostic protocol	no	
Reference of the test	Menzel, W. and Winter, S. (2021). Identification of novel and known tobamoviruses in tomato and other solanaceous crops using a new pair of generic primers and development of a specific RT-qPCR for ToBRFV. Acta Hortic. 1316, 143-148	
Is the test modified compared to the reference test	yes It was selected a commercial kit for RNA extraction and a different master mix for amplification	
Kit		
Is a kit used	no	
Other information		
Reaction type	Simplex	
Performance Criteria :		
Organism 1.:	Tobamovirus fructirugosum(TOBRFV)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	LOD (evaluated according the PM 7/98) 10^-7 level of ten-fold serial dilution (<10 copies/µl) for tomato and 10^-5 level of ten-fold serial dilution for pepper Probability of detection 5 evaluated during the TPS on 5 samples at five leve 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	88%	
Standard test(s)	Comparison with samples of known status during TPS	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	PV-1236; PV-1241 (DSMZ collection); Sicily isolates and Piedmont isolates from CREA-DC collection in preliminary studies	
Specificity value	100%	
openions, same		
Analytical specificity - exclusivity		

Specificity value	100%	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	89%	
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)	76% (TPS)	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	79% (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	
Other information		
Any other information considered useful	Due to the high number of deviations from the proposed protocol robustness was evaluated comparing the accuracy obtaneid from the lab that made deviations and those who followed the protocol, no statistical difference (p>0.05) were found	
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The following complementary files are available online:	Report TPS ToBRFV	

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