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 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 real time 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
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 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%
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) in preliminary studies

Specificity value	100%
Diagnostic Specificity	
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
The following complementary files are available online:	• Report TPS ToBRFV

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