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, Molecular real time RT PCR in leaves, 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	yes
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	yes Plant materials was grounded on 0.1M phosphate buffer 7.2 pH
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	Alkowni R., Alabdallah O, Fadda Z., 2019. Molecular identification of tomato brown rugose fruit virus in tomato in Palestine. Journal of Plant Pathology 101, pages 719–723.
Is the test modified compared to the reference test	yes It was selected a commercial kit for RNA extraction and a master mix for amplification
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Other details on the test	Amplification was performed using the One step RT-PCR kit from Qiagen
Performance Criteria :	
Organism 1.:	**Tobamovirus fructirugosum(TOBRFV)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	LOD (in agreement of PM7/98) evaluated in preliminary studies 10^-3 level of ten-fold serial dilution fot tomato ;10^-1 level of ten-fold serial dilution for pepper Probability of detection evaluated during TPS at 3.4 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	81%
Standard test(s)	Comparison with samples of known status during the 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 (tested in the 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) tested in preliminary studies
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	98%
Specify the test(s)	Comparison with samples of known status during the TPS
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	87% (TPS)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	88% (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:	• Report TPS ToBRFV

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