

**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.

<b>Laboratory contact details</b>	Council for Agricultural Research and Economics- Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
<b>Short description of the test</b>	Detection of Tomato brown rugose fruit virus by Molecular Conventional RT PCR, Molecular real time RT PCR in leaves, fruits
<b>Date, reference of the validation report</b>	2020-12-29 - Validation report 2020 - ToBRFV
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	VALITEST
<b>Description of the test</b>	
<b>Organism(s)</b>	Tobamovirus fructirugosum(TOBRFV)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Conventional RT PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes Plant materials was grounded on 0.1M phosphate buffer 7.2 pH
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	RNeasy Plant Mini Kit

Kit used following the manufacturer's instructions?	no Plant materials was grounded on 0.1M phosphate buffer 7.2 pH
<b>Other information</b>	
<b>Method: Molecular Conventional RT PCR</b>	
<b>Reference of the test description</b>	
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
<b>Kit</b>	
Is a kit used	no
<b>Other information</b>	
Reaction type	Simplex
Other details on the test	Amplification was performed using the One step RT-PCR kit from Qiagen
<b>Performance Criteria :</b>	
Organism 1.:	<b>Tobamovirus fructirugosum(TOBRFV)</b>
<b>Analytical sensitivity</b>	
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 for 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 level of dilution from $10^0$ to $10^{-8}$
<b>Diagnostic sensitivity</b>	
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
<b>Analytical specificity - inclusivity</b>	
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%
<b>Analytical specificity - exclusivity</b>	

<b>Number of non-target organisms tested</b>	ToMV PV-0141; TMV PV-1252; PMMoV PV-0165; BPeMV PV-0170; TMGMV PV-0124 (DSMZ collection) tested in preliminary studies
<b>Specificity value</b>	100%
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	98%
<b>Specify the test(s)</b>	Comparison with samples of known status during the TPS
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	87% (TPS)
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	88% (TPS)
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Test performance study organized in the framework of the VALITEST project involving 34 laboratories from 18 countries
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">Report TPS ToBRFV</a></li> </ul>

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