

**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 real time RT PCR in leaves and 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>	Tomato brown rugose fruit virus(TOBRFV)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Fruits, Leaves
<b>Plant species tested</b>	Capsicum annum, Solanum lycopersicum
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time 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>	no
<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>Reference of the test</b>	
<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 material was grounded on 0.1M phosphate buffer 7.2 pH
<b>Other information</b>	
<b>Other details on the test</b>	
<b>Method: Molecular real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<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>Reference of the test</b>	ISF (2020) Detection of Infectious Tomato brown rugose fruit virus (ToBRFV) in Tomato and Pepper Seed. <a href="https://www.worldseed.org/wp-content/uploads/2020/03/Tomato-ToBRFV_2020.03.pdf">https://www.worldseed.org/wp-content/uploads/2020/03/Tomato-ToBRFV_2020.03.pdf</a> [accessed 15 April 2020].
<b>Is the test modified compared to the reference test</b>	yes It was selected a commercial kit for RNA extraction and a master mix for amplification; and primers and probes concentrations were decreased
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Duplex
<b>Other details on the test</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Tomato brown rugose fruit virus(TOBRFV)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	LOD (evaluated according the PM 7/98) $10^{-7}$ level of ten-fold serial dilution (<10 copies/ $\mu$ l) for tomato and $10^{-5}$ level of ten-fold serial dilution for pepper Probability of detection 4.6 evaluated during the TPS on 5 samples at five leve of dilution from $10^0$ to $10^{-8}$
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	88%
<b>Standard test(s)</b>	Comparison with samples of known status during TPS

<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	PV-1236; PV-1241 (DSMZ collection); Sicily isolates and Piedmont isolates from CREA-DC collection evaluated in preliminary studies
<b>Specificity value</b>	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) evaluated in preliminary studies
<b>Specificity value</b>	100%
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	86%
<b>Specify the test(s)</b>	Comparison with samples of known status during TPS
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	73% (TPS)
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	78% (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
<b>Other information</b>	
<b>Any other information considered useful</b>	Due to the high number of deviations from the proposed protocol robustness was evaluated comparing the accuracy obtained 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:	
	<ul style="list-style-type: none"> <li>• <a href="#">Report TPS ToBREV</a></li> </ul>

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