

**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>	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands
<b>Short description of the test</b>	Detection of Tomato brown rugose fruit virus by LAMP (Sarkes et al., 2020) in seeds of tomato and pepper
<b>Date, reference of the validation report</b>	2021-12-01 - Euphresco 2019-A-327 project report
<b>Link to other validation data</b>	<ul style="list-style-type: none"> <li>- Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by real time RT PCR (Menzel and Winter, 2021) in seeds of tomato and pepper</li> <li>- Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by real time RT PCR (Abiopep) in seeds of tomato and pepper.</li> <li>- Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by conventional RT PCR (Loewe kit) in seeds of tomato and pepper</li> <li>- Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by conventional RT PCR (Alkowni et al., 2019) in seeds of tomato and pepper</li> <li>- Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by LAMP (Agdia AmplifyRP) in seeds of tomato and pepper</li> <li>- Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by real time RT PCR (ISHI-Veg test) in seeds of tomato and pepper</li> </ul>
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	Euphresco
<b>If yes, please specify</b>	Euphresco 2019-A-327
<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>	Seeds Seed samples consisted of tomato (sample set 1) and pepper (sample set 2). Sample types consisting of samples with different levels of ToBRFV (high, medium) and ToBRFV-negative

	samples were prepared
<b>Plant species tested</b>	Capsicum, Solanum lycopersicum
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular LAMP
<b>Method: Extraction</b>	
<i>Reference of the test description</i>	
<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>	
<b>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<b>Name of the test</b>	
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<i>Other information</i>	
<b>Other details on the test</b>	GH+ buffer
<b>Method: Molecular Extraction DNA RNA</b>	
<i>Reference of the test description</i>	
<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>	
<b>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<b>Name of the test</b>	
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes Centrifugation at 4°C
<i>Kit</i>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	RNeasy Plant Mini Kit
<b>Kit used following the manufacturer's instructions?</b>	no Centrifugation at 4°C
<i>Other information</i>	
<b>Other details on the test</b>	
<b>Method: Molecular LAMP</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>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Sarkes et al., 2020
<b>Is the test modified compared to the reference test</b>	
<b>Kit</b>	
<b>Is a kit used</b>	
<b>Other information</b>	
<b>Reaction type</b>	
<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>	Preliminary study on tomato seeds spiked with ToBRFV: 10 <sup>-3</sup> with GH+ buffer for extraction and 1uL RNA 10 <sup>-3</sup> with phosphate buffer for extraction and 1 uL RNA Did not work with 10uL of raw extract.
<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>	Based on the results of 3 laboratories Tomato: 78.9% Pepper: 6.7%
<b>Standard test(s)</b>	Comparison with samples of known status
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	
<b>Specificity value</b>	
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	
<b>Specificity value</b>	
<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>	Based on the results of 3 laboratories Tomato: 100% Pepper: 100%

<b>Specify the test(s)</b>	Comparison with samples of known status
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<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 Euphresco project 2019-A-327 involving 26 laboratories from 16 countries. The performance of this test is based on data from 3 laboratories.
<b>Other information</b>	
<b>Any other information considered useful</b>	

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