

**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>	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
<b>Short description of the test</b>	Detection of Tomato brown rugose fruit virus by real time RT PCR (Menzel and Winter, 2021) 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 (Abiopep) in seeds of tomato and pepper.</li> <li>- Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by LAMP (Sarkes et al., 2020) 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 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 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 / Tobamovirus fructirugosum (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 real time RT PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	GH+ buffer
<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>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<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
<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 Centrifugation at 4°C
<b>Other information</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>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<b>Name of the test</b>	Real-time RT-PCR Menzel and Winter (2021)
<b>As or adapted from an IPPC diagnostic</b>	no

<b>protocol</b>	
<b>Is the test modified compared to the reference test</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Tobamovirus fructirugosum(TOBRFV)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	Preliminary study on tomato seeds spiked with ToBRFV: 10 <sup>-5</sup> with GH+ buffer for extraction 10 <sup>-4</sup> with phosphate buffer for extraction
<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>	Tomato: 99.1% Pepper: 97.1%
<b>Standard test(s)</b>	Comparison with samples of known status
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Tomato: 98.8% Pepper: 100%
<b>Specify the test(s)</b>	Comparison with samples of known status
<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 14 laboratories.
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">Report_2019-A-327_Euphresco</a></li> </ul>

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