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	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Detection of Tomato brown rugose fruit virus by conventional RT PCR (Loewe kit) in seeds of tomato and pepper
Date, reference of the validation report	2021-12-01 - Euphresco 2019-A-327 project report
Link to other validation data	- 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. - 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 - 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 - 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 - Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by LAMP (Agdia AmplifyRP) in seeds of tomato and pepper - 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
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	Euphresco
If yes, please specify	Euphresco 2019-A-327
Description of the test	
Organism(s)	Tomato brown rugose fruit virus / Tobamovirus fructirugosum (TOBRFV)
Detection / identification	detection
Matrix(ces) tested	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
Plant species tested	Capsicum, Solanum lycopersicum
Method(s)	Extraction Molecular Extraction DNA RNA Molecular Conventional RT PCR
Method: Extraction	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/146 Tomato brown rugose fruit virus (version 1)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Other information	
Other details on the test	GH+ buffer
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/146 Tomato brown rugose fruit virus (version 1)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes Centrifugation at 4°C
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 Centrifugation at 4°C
Other information	
Method: Molecular Conventional RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/146 Tomato brown rugose fruit virus (version 1)
Name of the test	One step conventional RT-PCR Loewe (Rodríguez-Mendoza et al. 2019)

As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	LOEWE
Specify the kit used	Tomato Brown Rugose Fruit Virus RNA PCR Cat No 09175
Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex
Performance Criteria :	
Organism 1.:	**Tobamovirus fructirugosum(TOBRFV)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Preliminary study on tomato seeds spiked with ToBRFV: 10^-2 with GH+ buffer for extraction 10^-2 with phosphate buffer for extraction
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Tomato: 64.2% Pepper: 2.2%
Standard test(s)	Comparison with samples of known status
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Tomato: 100% Pepper: 97.8%
Specify the test(s)	Comparison with samples of known status
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 Euphresco project 2019-A-327 involving 26 laboratories from 16 countries. The performance of this test is based on data from 9 laboratories.
The following complementary files are available online:	• Report_2019-A-327_Euphresco

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