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	National Instituite of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
Short description of the test	Validation report on the testing of tomato brown rugose fruit virus by ABIOPEP real-time RT-PCR.
Date, reference of the validation report	2023-12-14 - Validation report on the testing of tomato brown rugose fruit virus by ABIOPEP real-time RT-PCR
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	EURL
If yes, please specify	EURL-Virology (European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas)
Description of the test	
Organism(s)	Tobamovirus fructirugosum(TOBRFV)
Detection / identification	detection and identification
Method(s)	Molecular Extraction DNA RNA Molecular real time RT PCR Molecular real time RT PCR (2) Molecular real time RT PCR (3)
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 2)
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	QIAGEN
Specify the kit used	RNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	yes For seed extraction the kit was used with the following modifications: the RLT buffer was replaced by GH+ buffer (EPPO PM7/146(2) Appendix 1) and the centrifugation temperature was decreased to 4°C at all steps to optimize RNA extraction from seed.
Other information	
Method: Molecular real time 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 2)
Name of the test	Real-time RT-PCR Bernabé-Orts et al. (2021)
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	ThermoFisher Scientific
Specify the kit used	TaqManR RNA-to-CtTM 1-Step Kit
Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex
Method: Molecular real time RT PCR (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
EPPO Diagnostic Protocol name	PM 7/146 Tomato brown rugose fruit virus (version 2)
Name of the test	Real-time RT-PCR Bernabé-Orts et al. (2021)
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	ThermoFisher Scientific
Specify the kit used	AgPath-ID™ One-Step RT-PCR
Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex
Method: Molecular real time RT PCR (3)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
EPPO Diagnostic Protocol name	PM 7/146 Tomato brown rugose fruit virus (version 2)
Name of the test	Real-time RT-PCR Bernabé-Orts et al. (2021)
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	KAPA BIOSYSTEMS
Specify the kit used	KAPA PROBE FAST Universal One-Step qRT-PCR Kit
Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex
Performance Criteria :	
Organism 1.:	**Tobamovirus fructirugosum(TOBRFV)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Dilutions of ToBRFV infected tomato leaves in sap from healthy leaves. LOD 10^-6.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Number of targets tested: 29 (seed samples) Number of non-targets tested: 11 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 2 Tomato seeds: 95.7% Pepper seeds: 88.9%
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Number of targets tested: 7 (ToBRFV isolates)
Specificity value	100%
Analytical specificity - exclusivity	

Number of non-target organisms tested	Number of non-targets tested: 19 (isolates of other tobamoviruses).
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Number of targets tested: 29 (seed samples) Number of non-targets tested: 11 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 2 Tomato seeds: 100% Pepper seeds: 100%
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Percentage of identical results is 100%. No. of target samples tested: 2 (RNA samples). No. of different days: 5
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not evaluated.
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	Additionally Robustness of the test was evaluated: Percentage of correct results is 100% Number of targets tested: 2 (seed samples) Number of non-targets tested: 5 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 9 Number of different RNA extraction: 3 Number of different reagents for real-time RT-PCR: 5 Number of different instruments: 5 Full validation report is available on the EURL webpage: https://eurlplanthealth.nl/files/view/e7dde713-181c-4363-aabe-031aa39873e7/20231214_tomato_brown_rugose_fruit_virus_validation_real-time_rt-pcr_bernabe-orts-et-al.pdf

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