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	Study on the performance of molecular methods for the detection and identification of tomato mild mottle virus (TMMoV, genus Ipomovirus); Test performance study - Report (version V1.0).
Date, reference of the validation report	2026-03-06 - EURL-Virology TPS-2025-01-TMMoV
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	Test performance study (TPS) on the detection and identification of TMMoV.
Description of the test	
Organism(s)	Tomato mild mottle virus / Ipomovirus lycopersici (TOMMOV)
Detection / identification	detection and identification
Matrix(ces) tested	Leaves, Other extracted RNA
Plant species tested	Solanum lycopersicum
Method(s)	Molecular Extraction DNA RNA Molecular real time RT PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
As or adapted from an IPPC diagnostic protocol	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?	no extraction was performed without using 2-mercaptoethanol and the final RNA elution was performed with two consecutive additions of 50 µL of RNase-free water pre-warmed to 65°C (total elution volume 100 µL).
Other information	
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Vučurović et al. 2026 (article in preparation)
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
Other details on the test	Final reaction volume was 10 µL. Final concentration of primers was 0.3 µM and final concentration of probes was 0.25 µM.
Performance Criteria :	
Organism 1.:	**Ipomovirus lycopersici(TOMMOV)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	at least to 1 x 10^7 dilution of isolate TMMoV DSMZ PV-0993 and at least to 1 x 10^9 dilution of gBlocks HQ840786.2 in RNA from healthy tomato leaves (level of agreement between experiments: 100%).
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	79.6%. Calculation was done on the basis of assigned reference statuses. Isolates used: TMMoV DSMZ PV-0993 dilutions 1x 10^4 to 1x 10^10 and TMMoV gBlocks HQ840786.2 diluted in RNA from healthy tomato leaves dilutions 1x 10^4 to 1x 10^11.

Standard test(s)	/
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	three isolates of TMMoV (TMMoV DSMZ PV-0993 and TMMoV DSMZ PV-1015 and TMMoV gBlocks HQ840786.2).
Specificity value	100% evaluated on three isolates of TMMoV.
Analytical specificity - exclusivity	
Number of non-target organisms tested	One healthy tomato sample, four isolates of other ipomovirus species CBSV (DSMZ PV0957), CVYV (DSMZ PV0776), SPMMV (DSMZ PV0900), UCBSV (DSMZ PV0926).
Specificity value	100%
Cross-reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	/
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	93%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	/
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	Preparation for test performance study organized in the framework of the EURL Virology.
Other information	
Any other information considered useful	Test performance study organized in the framework of the EURL Virology involving 13 laboratories from 12 countries. Full validation report is available: https://eurlplanthealth.nl/files/view/1fbc280d-ad99-4c73-9e17-85236bffbd32/eurl_virology_tps-2025-01-tmmov_report_v1.0.pdf.

Creation date: 2026-05-06 14:14:48 - Last update: 2026-05-06 15:17:44