

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

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.

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	on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests - Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests. Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests - Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests. Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests
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?	Euphresco
If yes, please specify	Euphresco project 2022-A-394 (Validation of molecular diagnostic methods for the detection and identification of tomato mottle mosaic virus (ToMMV-detect))
Description of the test	
Organism(s)	Tomato mottle mosaic virus / Tobamovirus maculatusellati (TOMMV0)
Detection / identification	detection and identification
Method(s)	Molecular Conventional RT PCR
Method: Molecular Conventional 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	Levitzky et al. (2019)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN

Specify the kit used	OneStep RT-PCR kit
Kit used following the manufacturer's instructions?	yes
<i>Other information</i>	
Reaction type	Simplex
Other details on the test	Sequencing of the PCR product is required to identify species.
Performance Criteria :	
Organism 1.:	Tobamovirus maculatussellati(TOMMV0)
<u>Analytical sensitivity</u>	
What is smallest amount of target that can be detected reliably?	at least to 2.5×10^5 dilution of isolate ToMMV NIB V 373 (level of agreement between experiments: 100%).
<u>Diagnostic sensitivity</u>	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	62.5%
Standard test(s)	Calculation was done on the basis of health status of the samples. Isolates used: ToMMV NIB V 373 dilutions 2.5×10^1 to 2.5×10^8 of isolate ToMMV NIB V 414 dilutions 2x and 2×10^1 .
<u>Analytical specificity - inclusivity</u>	
Number of strains/populations of target organisms tested	two ToMMV isolates (NIB V 373 and NIB V 414).
Specificity value	from 50% (5 laboratories false negative result for ToMMV from seeds) to 100% (3 laboratories) evaluated on two isolates of ToMMV (one from seeds and one from leaf material; the estimated concentration of ToMMV in seeds was lower than the concentration of ToMMV in leaf material).
<u>Analytical specificity - exclusivity</u>	
Number of non-target organisms tested	3 healthy tomato samples (two seeds, one leaves), 11 isolates of 9 other tobamovirus species (CGMMV isolate NIB V 403, ObPV isolate NIB V 364, ORSV isolate NIB V 365, PaMMV isolate NIB V 366, PMMoV isolate NIB V 408, TMGMV isolate NIB V 404, TMV isolates: NIB V 405 and 413, ToBRFV isolate NIB V 331, ToMV isolate NIB V 410, ToMV isolates NIB V 406).
Specificity value	100% evaluated on 3 healthy tomato samples (two seeds, one leaves) and 11 isolates of 9 other tobamovirus species (1 isolate of CGMMV, ObPV, ORSV, PaMMV, PMMoV, TMGMV and ToBRFV, and 2 isolates of ToMV and TMV).
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%

Specify the test(s)	/
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	94%
<u>Repeatability</u>	
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 Euphresco project 2022-A-394.
Other information	
Any other information considered useful	Test performance study organized in the framework of the Euphresco project 2022-A-394 involving 8 laboratories from 7 countries. Full validation report is available: https://drop.euphresco.net/data/af730655-4022-4e87-a952-b94cfda3a971/

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