

**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>	National Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
<b>Short description of the test</b>	Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests
<b>Date, reference of the validation report</b>	2024-09-17 - Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.
<b>Link to other validation data</b>	<ul style="list-style-type: none"><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li><li>- 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</li></ul>

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<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Euphresco
<b>If yes, please specify</b>	Euphresco project 2022-A-394 (Validation of molecular diagnostic methods for the detection and identification of tomato mottle mosaic virus (ToMMV-detect))
<b>Description of the test</b>	
<b>Organism(s)</b>	Tobamovirus maculatusellati(TOMMV0)
<b>Detection / identification</b>	detection and identification
<b>Method(s)</b>	Molecular real time RT PCR
<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>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Tiberini et al. 2022
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	ThermoFisher Scientific
<b>Specify the kit used</b>	AgPath-ID™ One-Step RT-PCR

Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Reaction type</b>	Duplex
<b>Other details on the test</b>	Kit One-step real-time RT-PCR TaqMan® RNA-to-Ct™ 1-Step Kit (ThermoFisher Scientific) can be used.
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Tobamovirus maculatussellati(TOMMV0)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	at least to $2.5 \times 10^6$ dilution of isolate ToMMV NIB V 373 (level of agreement between experiments: 100%).
<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>	78.9%
<b>Standard test(s)</b>	Calculation was done on the basis of health status of the samples. Isolates used: ToMMV NIB V 373 dilutions $2.5 \times 10^1$ to $2.5 \times 10^8$ of isolate ToMMV NIB V 414 dilutions 2x and $2x 10^1$ .
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	two ToMMV isolates (NIB V 373 and NIB V 414).
<b>Specificity value</b>	/
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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).
<b>Specificity value</b>	/
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	94.4%
<b>Specify the test(s)</b>	/
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	93%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	/

<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>	Preparation for test performance study organized in the framework of the Euphresco project 2022-A-394.
<b>Other information</b>	
<b>Any other information considered useful</b>	Test performance study organized in the framework of the Euphresco project 2022-A-394 involving 9 laboratories from 8 countries. Full validation report is available: <a href="https://drop.euphresco.net/data/af730655-4022-4e87-a952-b94cfda3a971/">https://drop.euphresco.net/data/af730655-4022-4e87-a952-b94cfda3a971/</a>

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