

**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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>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>	Tomato mottle mosaic virus / 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 maculatusellati(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 $2 \times 10^1$ .
<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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