

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**  
**ORGANISATION EUROPEENNE ET MEDITERRANEEENNE 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.

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| <b>Laboratory contact details</b>               | National Institute of Biology, Department of Biotechnology and Systems Biology<br>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>            | - Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests<br>- Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests.<br>Report on the results of the test performance study on detection and identification of tomato mottle mosaic virus (ToMMV) using molecular tests |

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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 Conventional RT PCR   |
| <b>Method: Molecular Conventional 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>  | Levitzky et al. (2019)  |
| <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>  | QIAGEN  |
| <b>Specify the kit used</b>   | OneStep RT-PCR kit  |

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| Kit used following the manufacturer's instructions?  | yes  |
| <b>Other information</b>   |  |
| <b>Reaction type</b>   | Simplex  |
| <b>Other details on the test</b>   | Sequencing of the PCR product is required to identify species.   |
| <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 $2.5 \times 10^5$ 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> | 62.5%  |
| <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>   | 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).  |
| <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>   | 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).  |
| <b>Diagnostic Specificity</b>  |  |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | 100%   |
| <b>Specify the test(s)</b>   | /  |

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| <b>Reproducibility</b>  |   |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                          | 94%   |
| <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 8 laboratories from 7 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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