

**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.

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| <b>Laboratory contact details</b>   | Council for Agricultural Research and Economics-<br>Research Centre for Plant Protection and<br>Certification<br>Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy |
| <b>Short description of the test</b>  | Detection of Tomato brown rugose fruit virus by<br>Molecular Conventional RT PCR, Molecular real time<br>RT PCR in leaves, fruits                                  |
| <b>Date, reference of the validation report</b>   | 2020-12-29 - Validation report 2020 - ToBRFV   |
| <b>Validation process according to EPPO<br/>Standard PM7/98?</b>  | yes  |
| <b>Is the lab accredited for this test?</b>   | no   |
| <b>Was the validated data generated in the<br/>framework of a project?</b>                              | Other_project  |
| <b>If yes, please specify</b>   | VALITEST   |
| <b>Description of the test</b>  |  |
| <b>Organism(s)</b>  | Tomato brown rugose fruit virus(TOBRFV)  |
| <b>Detection / identification</b>   | detection  |
| <b>Matrix(ces) tested</b>   | Fruits, Leaves symptomatic and asymptomatic  |
| <b>Plant species tested</b>   | Capsicum annum, Solanum lycopersicum   |
| <b>Method(s)</b>  | Molecular Extraction DNA RNA<br>Molecular Conventional RT PCR  |
| <b>Method: Molecular Extraction DNA RNA</b>   |  |
| <b>Reference of the test description</b>  |  |
| <b>As or adapted from an EPPO diagnostic<br/>protocol</b>   | yes  |
| <b>New test being considered for inclusion in the<br/>next version of the EPPO diagnostic protocol?</b> | yes  |
| <b>EPPO Diagnostic Protocol name</b>  | (version )   |
| <b>Name of the test</b>   |  |
| <b>As or adapted from an IPPC diagnostic<br/>protocol</b>   | no   |
| <b>Is the test modified compared to the<br/>reference test</b>  | yes Plant materials was grounded on 0.1M<br>phosphate buffer 7.2 pH  |

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| <b>Kit</b>   |   |
| <b>Is a kit used</b>   | yes   |
| <b>Manufacturer name</b>   | QIAGEN  |
| <b>Specify the kit used</b>  | RNeasy Plant Mini Kit   |
| Kit used following the manufacturer's instructions?  | no Plant materials was grounded on 0.1M phosphate buffer 7.2 pH   |
| <b>Other information</b>   |   |
| <b>Other details on the test</b>   |   |
| <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>   | Alkowni R., Alabdallah O, Fadda Z., 2019. Molecular identification of tomato brown rugose fruit virus in tomato in Palestine. Journal of Plant Pathology 101, pages 719-723.  |
| <b>Is the test modified compared to the reference test</b>   | yes It was selected a commercial kit for RNA extraction and a master mix for amplification  |
| <b>Kit</b>   |   |
| <b>Is a kit used</b>   | no  |
| <b>Other information</b>   |   |
| <b>Reaction type</b>   | Simplex   |
| <b>Other details on the test</b>   | Amplification was performed using the One step RT-PCR kit from Qiagen   |
| <b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>   |   |
| <b>Performance Criteria :</b>  |   |
| <b>Organism 1.:</b>  | <b>Tomato brown rugose fruit virus(TOBRFV)</b>  |
| <b>Analytical sensitivity</b>  |   |
| <b>What is smallest amount of target that can be detected reliably?</b>  | LOD (in agreement of PM7/98) evaluated in preliminary studies $10^{-3}$ level of ten-fold serial dilution for tomato ; $10^{-1}$ level of ten-fold serial dilution for pepper Probability of detection evaluated during TPS at 3.4 on 5 samples at five levels of dilution from $10^0$ to $10^{-8}$ |
| <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> | 81%   |

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| <b>Standard test(s)</b>   | Comparison with samples of known status during the TPS  |
| <b>Analytical specificity - inclusivity</b>   |   |
| <b>Number of strains/populations of target organisms tested</b>   | PV-1236; PV-1241 (DSMZ collection); Sicily isolates and Piedmont isolates from CREA-DC collection (tested in the preliminary studies) |
| <b>Specificity value</b>  | 100%  |
| <b>Analytical specificity - exclusivity</b>   |   |
| <b>Number of non-target organisms tested</b>  | ToMV PV-0141; TMV PV-1252; PMMoV PV-0165; BPeMV PV-0170; TMGMV PV-0124 (DSMZ collection) tested in preliminary studies                |
| <b>Specificity value</b>  | 100%  |
| <b>Cross reacts with</b>  |   |
| <b>Diagnostic Specificity</b>   |   |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b> | 98%   |
| <b>Specify the test(s)</b>  | Comparison with samples of known status during the TPS  |
| <b>Reproducibility</b>  |   |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                      | 87% (TPS)   |
| <b>Repeatability</b>  |   |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                      | 88% (TPS)   |
| <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>             | Test performance study organized in the framework of the VALITEST project involving 34 laboratories from 18 countries                 |
| <b>Other information</b>  |   |
| <b>Any other information considered useful</b>  |   |
|   |   |
| The following complementary files are available online:   | <ul style="list-style-type: none"> <li>• <a href="#">Report TPS ToBRFV</a></li> </ul>   |

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