

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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| Laboratory contact details | National Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia |
| Short description of the test | Validation report on the testing of tomato brown rugose fruit virus by ABIOPEP real-time RT-PCR. |
| Date, reference of the validation report | 2023-12-14 - Validation report on the testing of tomato brown rugose fruit virus by ABIOPEP real-time RT-PCR |
| 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? | EURL |
| If yes, please specify | EURL-Virology (European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas) |
| Description of the test | |
| Organism(s) | Tomato brown rugose fruit virus / Tobamovirus fructirugosum (TOBRFV) |
| Detection / identification | detection and identification |
| Method(s) | Molecular Extraction DNA RNA Molecular real time RT PCR Molecular real time RT PCR (2) Molecular real time RT PCR (3) |
| Method: Molecular Extraction DNA RNA | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | yes |
| EPPO Diagnostic Protocol name | PM 7/146 Tomato brown rugose fruit virus (version 2) |
| As or adapted from an IPPC diagnostic protocol | no |
| Is the test modified compared to the reference test | no |
| Kit | |

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| Is a kit used | yes |
| Manufacturer name | QIAGEN |
| Specify the kit used | RNeasy Plant Mini Kit |
| Kit used following the manufacturer's instructions? | yes For seed extraction the kit was used with the following modifications: the RLT buffer was replaced by GH+ buffer (EPPO PM7/146(2) Appendix 1) and the centrifugation temperature was decreased to 4°C at all steps to optimize RNA extraction from seed. |
| Other information | |
| Method: Molecular real time RT PCR | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | yes |
| EPPO Diagnostic Protocol name | PM 7/146 Tomato brown rugose fruit virus (version 2) |
| Name of the test | Real-time RT-PCR Bernabé-Orts et al. (2021) |
| As or adapted from an IPPC diagnostic protocol | no |
| Is the test modified compared to the reference test | no |
| Kit | |
| Is a kit used | yes |
| Manufacturer name | ThermoFisher Scientific |
| Specify the kit used | TaqManR RNA-to-CtTM 1-Step Kit |
| Kit used following the manufacturer's instructions? | yes |
| Other information | |
| Reaction type | Simplex |
| Method: Molecular real time RT PCR (2) | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | yes |
| New test being considered for inclusion in the next version of the EPPO diagnostic protocol? | no |
| EPPO Diagnostic Protocol name | PM 7/146 Tomato brown rugose fruit virus (version 2) |
| Name of the test | Real-time RT-PCR Bernabé-Orts et al. (2021) |
| As or adapted from an IPPC diagnostic protocol | no |
| Is the test modified compared to the reference test | no |
| Kit | |

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|---|---|
| Is a kit used | yes |
| Manufacturer name | ThermoFisher Scientific |
| Specify the kit used | AgPath-ID™ One-Step RT-PCR |
| Kit used following the manufacturer's instructions? | yes |
| Other information | |
| Reaction type | Simplex |
| Method: Molecular real time RT PCR (3) | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | yes |
| New test being considered for inclusion in the next version of the EPPO diagnostic protocol? | no |
| EPPO Diagnostic Protocol name | PM 7/146 Tomato brown rugose fruit virus (version 2) |
| Name of the test | Real-time RT-PCR Bernabé-Orts et al. (2021) |
| As or adapted from an IPPC diagnostic protocol | no |
| Is the test modified compared to the reference test | no |
| Kit | |
| Is a kit used | yes |
| Manufacturer name | KAPA BIOSYSTEMS |
| Specify the kit used | KAPA PROBE FAST Universal One-Step qRT-PCR Kit |
| Kit used following the manufacturer's instructions? | yes |
| Other information | |
| Reaction type | Simplex |
| Performance Criteria : | |
| Organism 1.: | Tobamovirus fructirugosum(TOBRFV) |
| Analytical sensitivity | |
| What is smallest amount of target that can be detected reliably? | Dilutions of ToBRFV infected tomato leaves in sap from healthy leaves. LOD 10 ⁻⁶ . |
| Diagnostic sensitivity | |
| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | Number of targets tested: 29 (seed samples) Number of non-targets tested: 11 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 2 Tomato seeds: 95.7% Pepper seeds: 88.9% |
| Analytical specificity - inclusivity | |
| Number of strains/populations of target organisms tested | Number of targets tested: 7 (ToBRFV isolates) |
| Specificity value | 100% |

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|---|---|
| Analytical specificity - exclusivity | |
| Number of non-target organisms tested | Number of non-targets tested: 19 (isolates of other tobamoviruses). |
| Specificity value | 100% |
| Diagnostic Specificity | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | Number of targets tested: 29 (seed samples) Number of non-targets tested: 11 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 2 Tomato seeds: 100% Pepper seeds: 100% |
| Reproducibility | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | Percentage of identical results is 100%. No. of target samples tested: 2 (RNA samples). No. of different days: 5 |
| Repeatability | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | Not evaluated. |
| Test performance study | |
| Test performance study? | no |
| Other information | |
| Any other information considered useful | Additionally Robustness of the test was evaluated: Percentage of correct results is 100% Number of targets tested: 2 (seed samples) Number of non-targets tested: 5 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 9 Number of different RNA extraction: 3 Number of different reagents for real-time RT-PCR: 5 Number of different instruments: 5 Full validation report is available on the EURL webpage: https://eurlplanthealth.nl/files/view/e7dde713-181c-4363-aabe-031aa39873e7/20231214_tomato_brown_rugose_fruit_virus_validation_real-time_rt-pcr_bernabe-orts-et-al.pdf |

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