

**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>                                      | National Institute of Biology, Department of Biotechnology and Systems Biology<br>Vecna pot 121, 1000 Ljubljana, Slovenia  |
| <b>Short description of the test</b>                                   | Validation report on the testing of Chrysanthemum stem necrosis virus and other American clade 1 tospoviruses by RT-PCR  |
| <b>Date, reference of the validation report</b>                        | 2024-04-30 - Validation report on the testing of Chrysanthemum stem necrosis virus and other American clade 1 tospoviruses by RT-PCR.  |
| <b>Link to other validation data</b>                                   | - Validation report on the testing of Chrysanthemum stem necrosis virus by real-time RT-PCR<br>Validation report on the testing of Chrysanthemum stem necrosis virus by real-time RT-PCR |
| <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> | EURL   |
| <b>If yes, please specify</b>  | EURL-Virology (European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas)   |
| <b>Description of the test</b>   |  |
| <b>Organism(s)</b>   | Orthotospovirus chrysanthinecrocaulis(CSNV00)<br>Orthotospovirus(1TOSPG)   |
| <b>Detection / identification</b>                                      | detection  |
| <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 protocol</b>                  | yes  |
| <b>EPPO Diagnostic Protocol name</b>                                   | PM 7/139 Tospoviruses (genus Orthotospovirus) (version 1)  |
| <b>As or adapted from an IPPC diagnostic protocol</b>                  | no   |

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| <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>   | RNeasy Plant Mini Kit   |
| Kit used following the manufacturer's instructions?                     | no Total RNA was eluted twice with 50 µL (total of 100 µL) of RNase-free water pre-warmed to 65 °C.   |
| <b>Other information</b>  |   |
| <b>Method: Molecular Conventional RT PCR</b>                            |   |
| <b>Reference of the test description</b>                                |   |
| <b>As or adapted from an EPO diagnostic protocol</b>                    | yes   |
| <b>EPO Diagnostic Protocol name</b>                                     | PM 7/139 Tospoviruses (genus Orthotospovirus) (version 1)   |
| <b>Name of the test</b>   | Conventional RT-PCR (Hassani-Mehraban et al., 2016)   |
| <b>As or adapted from an IPPC diagnostic protocol</b>                   | no  |
| <b>Is the test modified compared to the reference test</b>              | yes Kit OneTaq® One-Step RT-PCR Kit (NEB) was used.   |
| <b>Kit</b>  |   |
| <b>Is a kit used</b>  | yes   |
| <b>Manufacturer name</b>  | New England Biolabs (NEB)   |
| <b>Specify the kit used</b>   | OneTaq® One-Step RT-PCR Kit   |
| Kit used following the manufacturer's instructions?                     | yes   |
| <b>Other information</b>  |   |
| <b>Reaction type</b>  | Simplex   |
| <b>Other details on the test</b>  | Primers for American clade 1 (AM1-F and AM1-R) were used.   |
| <b>Performance Criteria :</b>   |   |
| <b>Organism 1.:</b>   | <b>Orthotospovirus chrysanthinecrocaulis(CSNV00)</b>  |
| <b>Analytical sensitivity</b>   |   |
| <b>What is smallest amount of target that can be detected reliably?</b> | Dilutions of CSNV RNA in RNA of chrysanthemum. Dilutions of CSNV, INSV and TSWV RNAs in water. LOD: For the dilutions in RNA of chrysanthemum: CSNV: 10 <sup>0</sup> For the dilutions in water: CSNV: 10 <sup>-1</sup> INSV: 10 <sup>-3</sup> TSWV: 10 <sup>-4</sup> |
| <b>Analytical specificity - inclusivity</b>                             |   |
| <b>Number of strains/populations of target organisms tested</b>         | No of targets tested: 27 (tospoviruses of American clade 1).  |

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| <b>Specificity value</b>   | 100%   |
| <b>Analytical specificity - exclusivity</b>  |  |
| <b>Number of non-target organisms tested</b>   | No of non-targets tested: 6 (5 isolates of other tospoviruses and one CSVd).   |
| <b>Specificity value</b>   | 83% (one false positive result; sequencing of amplicon confirmed that this was nonspecific amplification of the host (N. benthamiana) tissue)  |
| <b>Reproducibility</b>   |  |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b> | No. of target isolates tested: 4 (for two isolates, two dilutions were evaluated) No. of different days: 3<br>Percentage of identical results (positive replicates): 100%  |
| <b>Organism 2.:</b>  | <b>Orthotospovirus(1TOSPG)</b>   |
| <b>Test performance study</b>  |  |
| <b>Test performance study?</b>   | no   |
| <b>Other information</b>   |  |
| <b>Any other information considered useful</b>   | Full validation report is available on the EURL webpage: <a href="https://eurlplanthealth.nl/files/view/b5e4d1fe-fba0-4bb6-9cee-0a6d600d9f88/20240430_eurl_virology_tospovirus-am-c1_rt-pcr_validation-report_final.pdf">https://eurlplanthealth.nl/files/view/b5e4d1fe-fba0-4bb6-9cee-0a6d600d9f88/20240430_eurl_virology_tospovirus-am-c1_rt-pcr_validation-report_final.pdf</a> |

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