

**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>	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 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>	Chrysanthemum stem necrosis virus / Orthotospovirus chrysanthinecrocaulis (CSNV00) Orthotospovirus (1TOSPG)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Leaves
<b>Plant species tested</b>	Chrysanthemum x morifolium, Solanum lycopersicum
<b>Method(s)</b>	Molecular Extraction DNA RNA 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
<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 EPPO diagnostic protocol</b>	yes
<b>EPPO 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 the 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 (tosspoviruses of American clade 1).
<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 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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