

**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 by real-time RT-PCR
<b>Date, reference of the validation report</b>	2024-04-30 - Validation report on the testing of Chrysanthemum stem necrosis virus by real-time RT-PCR
<b>Link to other validation data</b>	- Validation report on the testing of Chrysanthemum stem necrosis virus and other American clade 1 tospoviruses by RT-PCR. Validation report on the testing of Chrysanthemum stem necrosis virus and other American clade 1 tospoviruses by 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)
<b>Detection / identification</b>	detection and identification
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time 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 real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/139 Tospoviruses (genus Orthotospovirus) (version 1)
<b>Name of the test</b>	Real-time RT-PCR test for CSNV (Boben et al., 2007)
<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>	ThermoFisher Scientific
<b>Specify the kit used</b>	AgPath-ID™ One-Step RT-PCR
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<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 isolates in water and in RNA of chrysanthemum. LOD 10 <sup>-4</sup> .
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	No of targets tested: 8 (3 isolates + 5 gBlocks).
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	

<b>Number of non-target organisms tested</b>	No of non-targets tested: 30 (29 isolates of other tospoviruses and one CSVd).
<b>Specificity value</b>	100%
<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: 2 (for each isolate different dilutions were evaluated) No. of operators: up to 3 No. of real-time PCR instruments: up to 5 No. of different days: up to 13 Percentage of identical results (positive replicates) is 100% No. of target isolates tested: 2 (for each isolate different dilutions were evaluated) No. of operators: up to 3 No. of real-time PCR instruments: up to 5 No. of different days: up to 13 Percentage of identical results (positive replicates) is 100%.
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	No. of samples tested: 3 (high, medium and low target concentration) No. of replicates tested: 5 Percentage of identical results (positive replicates) is 100%.
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	Additionally, Impact of the matrix was evaluated and No effect of the matrix on the test result was detected. Full validation report is available on the EURL webpage: <a href="https://eurlplanthealth.nl/files/view/b8e2cedf-f922-495a-82b1-b362aba8a4e6/eurl_virology_csnv_real-time-rt-pcr_validation-report_final.pdf">https://eurlplanthealth.nl/files/view/b8e2cedf-f922-495a-82b1-b362aba8a4e6/eurl_virology_csnv_real-time-rt-pcr_validation-report_final.pdf</a>

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