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.

Laboratory contact details	National Instituite 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 Chrysanthemum stem necrosis virus by real-time RT-PCR
Date, reference of the validation report	2024-04-30 - Validation report on the testing of Chrysanthemum stem necrosis virus by real-time RT-PCR
Link to other validation data	- 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
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)	Chrysanthemum stem necrosis virus / Orthotospovirus chrysanthinecrocaulis (CSNV00)
Detection / identification	detection and identification
Method(s)	Molecular Extraction DNA RNA Molecular real time RT PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/139 Tospoviruses (genus Orthotospovirus) (version 1)
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	QIAGEN
Specify the kit used	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.
Other information	
Method: Molecular real time RT PCR	
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 7/139 Tospoviruses (genus Orthotospovirus) (version 1)
Name of the test	Real-time RT-PCR test for CSNV (Boben et al., 2007)
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	AgPath-ID™ One-Step RT-PCR
Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex
Performance Criteria :	
Organism 1.:	**Orthotospovirus chrysanthinecrocaulis(CSNV00)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Dilutions of CSNV isolates in water and in RNA of chrysanthemum. LOD 10^-4.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	No of targets tested: 8 (3 isolates + 5 gBlocks).
Specificity value	100%
Analytical specificity - exclusivity	

Number of non-target organisms tested	No of non-targets tested: 30 (29 isolates of other tospoviruses and one CSVd).
Specificity value	100%
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	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%.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	No. of samples tested: 3 (high, medium and low target concentration) No. of replicates tested: 5 Percentage of identical results (positive replicates) is 100%.
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	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: https://eurlplanthealth.nl/files/view/b8e2cedf-f922-495a-82b1-b362aba8a4e6/eurl_virology_csnv_real-time-rt-pcr_validation-report_final.pdf

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