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 and other American clade 1 tospoviruses by RT-PCR
Date, reference of the validation report	2024-04-30 - Validation report on the testing of Chrysanthemum stem necrosis virus and other American clade 1 tospoviruses by RT-PCR.
Link to other validation data	- 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
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)	Orthotospovirus chrysanthinecrocaulis(CSNV00) Orthotospovirus(1TOSPG)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular Conventional 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 Conventional RT PCR	
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)
Name of the test	Conventional RT-PCR (Hassani-Mehraban et al., 2016)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes Kit OneTaq® One-Step RT-PCR Kit (NEB) was used.
Kit	
Is a kit used	yes
Manufacturer name	New England Biolabs (NEB)
Specify the kit used	OneTaq® One-Step RT-PCR Kit
Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex
Other details on the test	Primers for American clade 1 (AM1-F and AM1-R) were used.
Performance Criteria :	
Organism 1.:	**Orthotospovirus chrysanthinecrocaulis(CSNV00)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	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: 10^0 For the dilutions in water: CSNV: 10^-1 INSV: 10^-3 TSWV: 10^-4
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	No of targets tested: 27 (tospoviruses of American clade 1).

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

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