

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 Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
Short description of the test	Detection and identification of tomato spotted wilt tospovirus by real-time RT-qPCR (test adapted from Roberts et al. 2000) in symptomatic tomato leaves (prevalidation study)
Date, reference of the validation report	2020-12-14 - TSWV V1.0
Link to other validation data	- TSWV V1.0 Detection and identification of tomato spotted wilt tospovirus by real-time RT-qPCR (test adapted from Mortimer-Jones et al. 2009) in symptomatic tomato leaves (TPS) - TSWV V1.0 Detection and identification of tomato spotted wilt tospovirus by real-time RT-qPCR (test adapted from Roberts et al. 2000) in symptomatic tomato leaves (TPS) - TSWV V1.0 Detection and identification of tomato spotted wilt tospovirus by real-time RT-qPCR (test adapted from Boonham et al. 2002) in symptomatic tomato leaves (TPS) - TSWV V1.0 Detection and identification of tomato spotted wilt tospovirus by RT-PCR in symptomatic tomato leaves (TPS)
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?	Other_project
If yes, please specify	VALITEST
Description of the test	
Organism(s)	Tomato spotted wilt virus / Orthotospovirus tomatomaculae (TSWV00)
Detection / identification	detection and identification
Matrix(ces) tested	Leaves different tospovirus isolates from the collections of DSMZ, NIB, INRA, CREA, UB-FA, WSU and NVWA tested directly or spiked into tomato leaf extract
Plant species tested	Solanum lycopersicum
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 Tosspoviruses (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 Extraction was performed as described in Appendix 3 of PM 7/139 (1): 0.2-0.3 g of freeze dried leaves was ground in 1 mL PBS-extraction buffer. An aliquot of 100 µL was used and 450 µL of RLT buffer (without β-mercaptoethanol) (Qiagen) added. Total RNA was eluted twice with 50 µL (total of 100 µL) of RNase-free water pre-warmed to 65°C. Undiluted RNA was used for testing.
Other information	
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Roberts et al. 2000 (Journal of Virological Methods, 88(1), 1-8)
Is the test modified compared to the reference test	yes AgPath-IDTM One-step RT-PCR kit was used instead of The Gold RT-PCR kit (PE Biosystems). Reaction volume was 10 µL instead of 25µL.
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Other details on the test	Reagent: AgPath-IDTM One-step RT-PCR kit Final reaction volume was 10 µL. Final concentration of primers was 0.2 µM and final concentration of probe was 0.1 µM.
Performance Criteria :	

Organism 1.:	Orthospovirus tomatomaculae(TSWV00)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	at least to 1,000,000x dilution of the isolates TSWV-PV-0182 and TSWV-PV-1175, 1,000,000x dilution of isolate TSWV-PV-0389
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	/
Standard test(s)	/
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	15 TSWV isolates (5 isolates from DSMZ collection and 10 isolates from other collections)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	10 other tospovirus species, represented with 17 isolates (ANSV00 isolate PV-1027; CaCV isolate PV-0864; CSNV00 isolate PV-0529 and NIB V 038; GRSV00 isolate PV-0205; INSV00 isolates PV-0281, PV-0280, PV-0485, PV-1123, and PV-1189; IYSV isolate PV-0528; MSMV isolate VE440; TCSV00 isolates PV-0390 and PV-0391; TYRV00 isolates PV-0526, and PV-0535, WSMoV isolate PV-0283).
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	/
Specify the test(s)	/
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	/
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	/
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	Preparation for test performance study organized in the framework of the VALITEST project

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