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	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)
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 (prevalidation study) - 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 (prevalidation study) - 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 Roberts et al. 2000) in symptomatic tomato leaves (prevalidation study) - TSWV V1.0 Detection and identification of tomato spotted wilt tospovirus by RT-PCR in symptomatic tomato leaves (prevalidation study) - TSWV V1.0 Detection and identification of tomato spotted wilt tospovirus by RT-PCR 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)
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
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 Extraction was performed as described in Appendix 3 of PM 7/139 (1). Samples were extracted by placing the 0.6 g of freeze dried leaves in Bioreba extraction bag and adding 3 ml of PBS-extraction buffer. An aliquot of 100 µL was send to TPS participants. TPS participants added 450 µL of RLT buffer (without ß-mercaptoethanol) (Qiagen) and continued with the manufacturer's procedure. 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	Mortimer-Jones et al. 2009 (Journal of Virological Methods, 161, 289-296)
Is the test modified compared to the reference test	yes AgPath-IDTM One-step RT-PCR kit was used instead of JumpStart Taq Ready Mix (Sigma). 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 (Ambion - Thermo Scientific) Final reaction volume was 10 µL. Final concentration of primers was 0.3 µM and final concentration of probe was 0.2 µM. Primer concentration and cycling conditions were the same for all used kits. Cycling conditions were: 48°C for 10 min, 95 °C for 10 min, then 45 cycles at 95 °C for 15 s and 60 °C for 1 min. Kit from Ambion (Thermo Scientific) was suggested by TPS organizer and was used by 10 TPS participants. One TPS participant used kit from VWR, Quantabio (Quanta OneStep Toughmix qScript™ XLT One-StepRT-qPCR ToughMix® | VWR). One TPS participant used kit from Invitrigen/Thermo Fisher Scientific (PCR SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase). One TPS participant used kit from Quantabio (Ultraplex 1-step Toughmix (4X)). No impact was observed when using different kits.
Performance Criteria :	
Organism 1.:	**Orthotospovirus tomatomaculae(TSWV00)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	at least to 1,000,000x dilution of isolate TSWV-PV-1175 (level of agreement between experiments: 100%)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	99.50%
Standard test(s)	Calculation was done on the basis of expected results which was for molecular test the same as health status of the samples (1,000,000x and 100,000x diluted isolate TSWV-PV-1175 was expected to be positive). Additionally, in the TPS study, 1,000x diluted TSWV-PV-0182 isolate and 100,000x diluted TSWV-PV-0389 isolate have been used as medium and as low concentrated TSWV positive sample, respectively.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	3 TSWV isolates from DSMZ collection (PV-1175, PV-0182, PV-0389)
Specificity value	/
Analytical specificity - exclusivity	
Number of non-target organisms tested	3 healthy tomato samples, 5 other tospovirus species (ANSV00 isolate PV-1027; CSNV00 isolate PV-0529; GRSV00 isolate PV-0205; INSV00 isolate PV-0281; TCSV00 isolate PV-0390)
Specificity value	/
Diagnostic Specificity	
Proportion of uninfected/uninfested samples	95.20%

(true negatives) testing negative compared to results from a standard test	
Specify the test(s)	Calculation was done based on the results of samples listed in sections inclusivity and exclusivity. One TSWV isolate was tested as several dilutions (see section diagnostic sensitivity) and also these results are included in calculation of diagnostic specificity.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	97.90%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	96%
Test performance study	
Test performance study?	yes
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
Other information	
Any other information considered useful	Test performance study organized in the framework of the VALITEST project involving 15 laboratories from 12 countries. Results of two laboratories have been excluded from calculation of diagnostic parameters due to non-conforming results of controls and/or healthy tomato samples. Full validation report is available on request (send the e-mail to: niblabfito@nib.si and natasa.mehle@nib.si).

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