

**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>	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)
<b>Date, reference of the validation report</b>	2020-12-14 - TSWV V1.0
<b>Link to other validation data</b>	<ul style="list-style-type: none"> <li>- 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)</li> <li>- 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)</li> <li>- 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)</li> <li>- TSWV V1.0 Detection and identification of tomato spotted wilt tospovirus by RT-PCR in symptomatic tomato leaves (TPS)</li> </ul>
<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>	Other_project
<b>If yes, please specify</b>	VALITEST
<b>Description of the test</b>	
<b>Organism(s)</b>	Tomato spotted wilt virus / Orthotospovirus tomatomaculae (TSWV00)
<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 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.
<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>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Mortimer-Jones et al. 2009 (Journal of Virological Methods, 161, 289-296)
<b>Is the test modified compared to the reference test</b>	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.
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	Reagent: AgPath-IDTM One-step RT-PCR kit Final reaction volume was 10 µL. Final concentration of primers was 0.3 µM and final concentration of probe was 0.2 µM.
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Orthotospovirus tomatomaculae(TSWV00)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be</b>	at least to 100,000x dilution of the isolate TSWV-

<b>detected reliably?</b>	PV-0182, 1,000,000x dilution of the isolate TSWV-PV-0389; at least to 1,000,000x dilution of isolate TSWV-PV-1175
<b><u>Diagnostic sensitivity</u></b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	/
<b>Standard test(s)</b>	/
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	15 TSWV isolates (5 isolates from DSMZ collection and 10 isolates from other collections)
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	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).
<b>Specificity value</b>	100%
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	/
<b>Specify the test(s)</b>	/
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	/
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	/
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Preparation for test performance study organized in the framework of the VALITEST project.

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