## 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	Fera Sand Hutton, YO41 1LZ York, United Kingdom	
Short description of the test	Detection and identification of tomato mottle mosaic virus (Tobamovirus maculatessellati) by Molecular real-time RT-PCR in Seeds	
Date, reference of the validation report	2023-06-05 - VAL/074 Method validation for development of a tomato mottle mosaic virus real- time RT-PCR assay	
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?	no	
Description of the test		
Organism(s)	Tomato mottle mosaic virus / Tobamovirus maculatessellati (TOMMV0)	
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	no	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no	
As or adapted from an IPPC diagnostic protocol	no	
Reference of the test	Fox, A., Fowkes, A.R., Skelton, A., Harju, V., Buxton- Kirk, A., Kelly, M., Forde, S.M.D., Pufal, H., Conyers, C., Ward, R., Weekes, R., Boonham, N. and Adams, I.P. (2019), Using high-throughput sequencing in support of a plant health outbreak reveals novel viruses in Ullucus tuberosus (Basellaceae). Plant Pathol, 68: 576-587.	
Is the test modified compared to the reference test	no	

Kit		
Is a kit used	yes	
Manufacturer name	Invitek	
Specify the kit used	Invimag Virus DNA/RNA mini-kit	
Kit used following the manufacturer's instructions?		
Other information		
Method: Molecular real time RT PCR		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes	
As or adapted from an IPPC diagnostic protocol	no	
Reference of the test	Fowkes, A.R., Botermans, M., Frew, L., de Koning, P.P.M., Buxton-Kirk, A., Westenberg, M., et al. (2022) First report of Tomato mottle mosaic virus in Solanum lycopersicum seeds in The Netherlands and intercepted in seed imported from Asia. New Disease Reports, 45, e12067.	
Is the test modified compared to the reference test	no	
Kit		
Is a kit used	yes	
Manufacturer name	Bio-Rad	
Specify the kit used	iTaq Universal Probes One-Step Kit	
Kit used following the manufacturer's instructions?	yes	
Other information		
Reaction type	Simplex	
Performance Criteria :		
Organism 1.:	Tobamovirus maculatessellati(TOMMV0)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	10-5 dilution in water, 10-4 dilution in seed	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Against Levitzky et al. RT-PCR 100%, 95% Cl [54.07% - 100%] Against Tiberini et al. real-time RT- PCR 94.74%, 95% Cl [73.97% - 99.87%]	
Standard test(s)	Levitzky, N., Smith, E., Lachman, O., Luria, N., Mizrahi, Y., Bakelman, H., Sela, N., Laskar, O., Milrot, E., & Dombrovsky, A. (2019). The bumblebee Bombus terrestris carries a primary inoculum of Tomato brown rugose fruit virus	

	contributing to disease spread in tomatoes. PloS one, 14(1), e0210871. https://doi.org/10.1371/journal.pone.0210871 Tiberini, A., Manglli, A., Taglienti, A., Vučurović, A., Brodarič, J., Ferretti, L., Luigi, M., Gentili, A., & Mehle, N. (2022). Development and Validation of a One-Step Reverse Transcription Real-Time PCR Assay for Simultaneous Detection and Identification	
	of Tomato Mottle Mosaic Virus and Tomato Brown Rugose Fruit Virus. Plants (Basel, Switzerland), 11(4), 489. https://doi.org/10.3390/plants11040489	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	31 samples (including sub-samples) of tomato mottle mosaic virus	
Specificity value	100%	
Analytical specificity - exclusivity		
Number of non-target organisms tested	139 non-target samples (including sub-samples) tested. Tobamoviruses: ToMV, PMMoV, PSTVd, TMV, ToBRFV and ulluco tobamovirus. Other viruses and viroids included in the evaluation of the exclusivity: CEVd, CLVd, PCFVd, PepMV EU, PepMV Ch1, PepMV Ch2, PVX, PVY, STV, TASVd, TPMVd, TSWV, TYLCV.	
Specificity value		
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Against Levitzky et al. RT-PCR 65.45%, 95% Cl [51.42% - 77.76%] Against Tiberini et al. real-time RT-PCR 83.33%, 95% Cl [68.64% - 93.03%]	
Specify the test(s)	Levitzky, N., Smith, E., Lachman, O., Luria, N., Mizrahi, Y., Bakelman, H., Sela, N., Laskar, O., Milrot, E., & Dombrovsky, A. (2019). The bumblebee Bombus terrestris carries a primary inoculum of Tomato brown rugose fruit virus contributing to disease spread in tomatoes. PloS one, 14(1), e0210871. https://doi.org/10.1371/journal.pone.0210871 Tiberini, A., Manglli, A., Taglienti, A., Vučurović, A., Brodarič, J., Ferretti, L., Luigi, M., Gentili, A., & Mehle, N. (2022). Development and Validation of a One-Step Reverse Transcription Real-Time PCR Assay for Simultaneous Detection and Identification of Tomato Mottle Mosaic Virus and Tomato Brown Rugose Fruit Virus. Plants (Basel, Switzerland), 11(4), 489. https://doi.org/10.3390/plants11040489	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% in both 10-5 dilution in water, 10-4 dilution in seed	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% in both 10-5 dilution in water, 10-4 dilution in seed	
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 Euphresco project 2022-A-394.	
Other information		
Any other information considered useful	Test performance study organized in the framework of the Euphresco project 2022-A-394 involving 7 laboratories from 6 countries. The results of one laboratory were excluded from calculation of diagnostic parameters because of deviation from the protocol seems to have an impact on the sensitivity and specificity of the test. Full validation report is available: http s://drop.euphresco.net/data/af730655-4022-4e87-a 952-b94cfda3a971/	
The following complementary files are available online:	<u>ToMMV EPPO Summary</u>	

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