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% CI [54.07% - 100%] Against Tiberini et al. real-time RT-PCR 94.74%, 95% CI [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% CI [51.42% - 77.76%] Against Tiberini et al. real-time RT-PCR 83.33%, 95% CI [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-a952-b94cfda3a971/
The following complementary files are available online:	• ToMMV EPPO Summary

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