

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)

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.

Target Organism	Tospoviruses Tomato spotted wilt tospovirus Chrysanthemum stem necrosis tospovirus	
Short description	Detection of Tospoviruses by RT-PCR in plant material	
Laboratory contact details	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France	
Date and reference of the validation report	2015 - Leguay A., Gentit P. (2015) Evaluation de méthodes de RT-PCR pour la détection polyvalente des virus du genre Tospovirus - Laboratoire de la santé des végétaux - Angers (France)	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 Chen T.C., Li J.T., Lin Y.P., Yeh Y.C., Kang Y.C., Huang L.H., Yeh S.D. (2012) Genomic characterization of Calla lily chlorotic spot virus and design of broad-spectrum primers for detection of tospoviruses. Plant Pathology 61:183-194.	
Is the test the same as described in the EPPO DP?	No Chen T.C., Li J.T., Lin Y.P., Yeh Y.C., Kang Y.C., Huang L.H., Yeh S.D. (2012) Genomic characterization of Calla lily chlorotic spot virus and design of broad-spectrum primers for detection of tospoviruses. Plant Pathology 61:183-194.	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Solanum lycopersicum, Nicotiana benthamiana, Chrysanthemum sp., Cineraria sp., Allium sp., Capsicum sp.	
Matrices tested (if relevant)	Leaves	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Plant RNeasy minikit from Qiagen
Molecular methods, e.g. hybridization, PCR and real time PCR	X	RT-PCR - Chen et al. (2012)
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting,		

plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	Not concerned because a virus is not quantifiable	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100 %	
Specify the standard test	30 / 30 (3 replicate for each sample)	
<u>Analytical specificity</u>		
Specificity value	100%	
Number of strains/populations of target organisms tested	Target organisms tested 1. ANSV Alstroemeria necrotic streak virus) 2. CaCV Capsicum chlorosis virus 3. CSNV Chrysanthemum stem necrosis virus 4. GRSV Groundnut ringspot virus 5. INSV Impatiens necrotic spot virus 6. IYSV Iris yellow spot virus 7. TCSV Tomato chlorotic spot virus 8. TYRV Tomato yellow (fruit) ring virus 9. TSWV Tomato spotted wilt virus 10. WSMoV Watermelon silver mottle virus	
Number of non-target organisms tested	Non-target organisms tested 1. healthy Allium 2. healthy Chrysanthemum 3. healthy Cineraria 4. healthy Allium 5. healthy Allium 6. healthy Solanum lycopersicum 7. healthy Capsicum 8. Solanum Tomato infected by virus (TICV); Tomato chlorosis virus (ToCV); Tomato yellow leaf curl virus (TYLCV) 9. Solanum Tomato infected by Pepino mosaic virus (PepMV) 10. Solanum Tomato infected by Tomato torrado virus (ToTV).	
Cross reacts with (specify the species)	No cross reaction observed	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing	100%	

negative compared to results from a standard test	
Specify the standard test	30 samples agreement / 30 (3 replicate for each sample)
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not tested
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% - 60 samples agreement /60 (3 replicate for each sample)
<u>Test performance study</u>	
Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
<u>Other information</u>	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	