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	Pepino mosaic virus	
Short description	Detection of PepMV by RT-Q PCR in tomato seed and leaves	
Laboratory contact details	Anses, Laboratoire de la Santé des Végétaux - Unité de bactériologie, virologie OGM 7 rue Jean Dixméras, 49044 Angers, France	
Date and reference of the validation report	2012-10 - Saison & Gentit & Tassus (2012) Comparaison des méthodes RT PCR et RT QPCR avec la méthode de référence ELISA pour la détection du Pepino Mosaic Virus (PepMV)	
Validation process according to EPPO Standard PM 7/98:	No	
Reference of the test description	0 Ling, K.S. et al., (2008) Genetic composition of Pepino mosaic virus population in North American greenhouse tomatoes. Plant Dis. 92, 1683–1688. Standard method used for the comparaison :Méthode officielle : VHs/04/06 version a : détection du virus de la mosaïque du Pepino (PepMV) sur semences par technique sérologique ELISA. The test has been undertaken using PRI serum. Modification of Ling et al.(2008) assay according to Pepeira project (van der Vlugt et al., 2000)	
Is the test the same as described in the EPPO DP?	No No DP available	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Solanum lycopersicum	
Matrices tested (if relevant)	Seed and leaves	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Each sample was tested twice, one sample grinded in the extraction buffer recommended by the supplier of the antiserum and another sample in the buffer recommended by the Pepeira project.
Molecular methods, e.g. hybridization, PCR and real time PCR	X	For RT-PCR, RNA was extracted with the RNeasy Plant Mini Kit from Qiagen and the Kit Invitrogen Super Script III One-Step RT-PCR is designed.
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		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	Not concerned because the concentration of viruses is never known	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	14/14	
Specify the standard test	DAS ELISA serum PRI & BIOREBA	
Analytical specificity		
Specificity value	100%	
Number of strains/populations of target organisms tested	14 (see table as separate file or full validation report for detail)	
Number of non-target organisms tested	19 (see table as separate file or full validation report for detai	
Cross reacts with (specify the species)	No cross reaction observed with RT QPCR	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	19/19	
Specify the standard test	DAS ELISA serum PRI & BIOREBA	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)		
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	1/4000 with PBS T PVP buffer; 1/120000 with phosphate buffer	
Test performance study		

Test performance study?	No
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	
The following complementary files are available online:	• List of target strains and non-target organisms • Saison & Tassus (2012) Comparaison des méthodes RT PCR et RT QPCR avec la méthode de référence ELISA pour la détection du Pepino Mosaic Virus (PepMV)