

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	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49000 Angers, France
Short description of the test	Detection of PepMV by RT-Q PCR in tomato seed and leaves
Date, reference of the validation report	2012-10-01 - 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 PM7/98?	no
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Pepino mosaic virus / Potexvirus pepini (PEPMV0)
Detection / identification	detection
Method(s)	Extraction Molecular Extraction DNA RNA Molecular real time RT PCR
Method: Extraction	
Reference of the test description	
Other information	
Other details on the test	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
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	RNeasy Plant Mini Kit

Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	RNA was extracted with the RNeasy Plant Mini Kit from Qiagen
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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 comparison :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)
Other information	
Other details on the test	Kit Invitrogen Super Script III One-Step RT-PCR
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Potexvirus pepini(PEPMV0)
Analytical sensitivity	
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
Standard test(s)	DAS ELISA serum PRI & BIOREBA
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	14 (see table as separate file or full validation report for detail)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	19 (see table as separate file or full validation report for detail)
Specificity value	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 test(s)	DAS ELISA serum PRI & BIOREBA
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
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 OPCR avec la méthode de référence ELISA pour la détection du Pepino Mosaic Virus (PepMV)

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