

**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.

<b>Laboratory contact details</b>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixm�ras, 49044 Angers, France
<b>Short description of the test</b>	Detection of PepMV by RT-Q PCR in tomato seed and leaves
<b>Date, reference of the validation report</b>	2012-10-01 - Saison & Gentit & Tassus (2012) Comparaison des m�thodes RT PCR et RT QPCR avec la m�thode de r�f�rence ELISA pour la d�tection du Pepino Mosaic Virus (PepMV)
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Potexvirus pepini(PEPMV0)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular real time RT PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	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
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	RNeasy Plant Mini Kit

Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	RNA was extracted with the RNeasy Plant Mini Kit from Qiagen
<b>Method: Molecular real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	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)
<b>Other information</b>	
<b>Other details on the test</b>	Kit Invitrogen Super Script III One-Step RT-PCR
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Potexvirus pepini(PEPMV0)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Not concerned because the concentration of viruses is never known
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	14/14
<b>Standard test(s)</b>	DAS ELISA serum PRI & BIOREBA
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	14 (see table as separate file or full validation report for detail)
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	19 (see table as separate file or full validation report for detail)
<b>Specificity value</b>	No cross reaction observed with RT QPCR
<b>Diagnostic Specificity</b>	

<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	19/19
<b>Specify the test(s)</b>	DAS ELISA serum PRI & BIOREBA
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	1/4000 with PBS T PVP buffer; 1/120000 with phosphate buffer
<b>Test performance study</b>	
<b>Test performance study?</b>	no
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">List of target strains and non-target organisms</a></li> <li>• <a href="#">Saison &amp; Tassus (2012) Comparaison des méthodes RT PCR et RT OPCR avec la méthode de référence ELISA pour la détection du Pepino Mosaic Virus (PepMV)</a></li> </ul>

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