

**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>	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy
<b>Short description of the test</b>	Detection of <i>Xylella fastidiosa</i> in perennial host species by Real time PCR
<b>Date, reference of the validation report</b>	2014-09-01 - 2014-09 and 2015-07 - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2014 and 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS
<b>Link to other validation data</b>	- 2014-09 and 2015-07 - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2014 and 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS Detection of <i>Xylella fastidiosa</i> in perennial host species by PCR
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xylella fastidiosa</i> (XYLEFA)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</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>	Loconsole, G., Potere, O., Boscia, D., Altamura, G., Djelouah, K., Elbeaino, T., Frasheri, D., Lorusso, D., Palmisano, F., Pollastro, P., Silletti, M. R., Trisciuzzi, N., Valentini, F., Savino V. & Saponari, M. (2014a). Detection of <i>Xylella fastidiosa</i> in olive trees by

	serological and molecular methods. Journal of Plant Pathology, 96, 7-14.
<b>Other information</b>	
<b>Other details on the test</b>	CTAB-based protocol for total DNA extraction
<b>Method: Molecular real time 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>	Harper S.J., Ward L.I., Clover G.R.G., 2010. Development of LAMP and real-time PCR methods for the rapid detection of Xylella fastidiosa for quarantine and field applications. Phytopathology 100: 1282-1288.
<b>Other information</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>yes</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	up to 10 <sup>2</sup> cfu/ml (corresponding to 7 cfu/reaction) using dilutions ranging from 10 <sup>7</sup> to 10 CFU/ml, prepared by spiking the inactivated bacterial culture in total nucleic acids recovered from olive reference sources known to be not infected by Xylella fastidiosa.
<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>	100%
<b>Standard test(s)</b>	108 obtained positive samples/ 108 expected positive samples
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100%
<b>Specify the test(s)</b>	90 obtained negative samples/ 90 expected negative samples
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%

Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	<p>Validation of the Real time PCR assay was carried out by the Laboratories listed below, under the supervision of the reference laboratory CNR-UNIBA.</p> <ul style="list-style-type: none"> <li>• IPSP-CNR: Istituto per la Protezione Sostenibile delle Piante CNR, UOS Bari, (Italy);</li> <li>• DiSSPA-UNIBA: Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Bari (Italy);</li> <li>• CRSFA: Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia, Locorotondo (BA) (Italy);</li> <li>• IAMB: Istituto Agronomico Mediterraneo, Valenzano (BA) (Italy);</li> <li>• Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale - Università degli Studi di Foggia, (Italy). A panel of blind samples was distributed.</li> </ul>
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">protocols for diagnosis of Xylella fastidiosa</a></li> <li>• <a href="#">Report interlaboratory validation 2015</a></li> <li>• <a href="#">Report interlaboratory validation 2014</a></li> </ul>

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