

**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 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 ELISA - 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 Real time PCR
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<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>Matrix(ces) tested</b>	Leaves leaf petiols
<b>Plant species tested</b>	<i>Acacia saligna</i> , <i>Citrus</i> sp., <i>Nerium oleander</i> , <i>Olea europaea</i> , <i>Polygala myrtifolia</i> , <i>Prunus avium</i> , <i>Prunus dulcis</i> , <i>Quercus ilex</i> , <i>Vitis</i> sp.
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Conventional PCR
<b>Method: Molecular Extraction DNA RNA</b>	

<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	CTAB-based total nucleic acid extraction 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 Xylella fastidiosa in olive trees by serological and molecular methods. Journal of Plant Pathology, 96, 7-14.
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 1)
<b>Name of the test</b>	Conventional PCR (Minsavage et al., 1994)
<b>Is the test modified compared to the reference test</b>	yes Total nucleic acids were extracted following the protocol reported in: 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 Xylella fastidiosa 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>	Minsavage GV, Thompson CM, Hopkins DL & Leite RMVBC and Stall RE (1994) Development of a polymerase chain reaction protocol for detection of Xylella fastidiosa in plant tissue. Phytopathology 84, 456-461.
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	no
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	up to $10^4$ cfu/ml (corresponding to $0.7 \times 10^3$ cfu/reaction), using dilutions ranging from $10^7$ to $10^8$ 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>	97.92%
<b>Standard test(s)</b>	141 obtained positive samples/144 expected positive samples (distributed as blind 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>	120 obtained negative samples/ 120 expected negative samples (distributed as blind samples)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	98.86%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	Validation of the PCR protocol was carried out by the Laboratories listed below, under the supervision of the reference laboratory CNR-UNIBA. • IPSP-CNR: Istituto per la Protezione Sostenibile delle Piante CNR, UOS Bari (Italy) • UNIBA: Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Bari (Italy); • CRSFA: Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia, Locorotondo (BA), (Italy); • IAMB: Istituto Agronomico Mediterraneo, Valenzano (BA), (Italy). • Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale - Università degli Studi di Foggia, (Italy) A panel of blind samples was distributed.
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 2014</a></li> <li>• <a href="#">Report interlaboratory validation 2015</a></li> </ul>

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