

**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 composite samples of <i>polygala myrtifolia</i>
<b>Date, reference of the validation report</b>	2019-03-13 - Implementation of sampling procedures for testing composite samples for <i>Xylella fastidiosa</i> . POnTE - XF-ACTORS, 2nd Joint Annual Meeting: European Research on Emerging Plant Diseases. Valencia, 23-26 october 2018. Book of abstract: p. 63.
<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>	<i>Xylella fastidiosa</i> (XYLEFA)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) Molecular real time PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	The preparation of the samples of <i>polygala</i> is different from the description reported in the EPPO DP
<b>Method: Molecular Extraction DNA RNA</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 <i>Xylella fastidiosa</i> (version 4)
<b>Is the test modified compared to the</b>	no

<b>reference test</b>	
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite samples of polygala myrtifolia, prepared as reported in the attached additional file, by using CTAB-based protocol;
<b>Method: Molecular Extraction DNA RNA (2)</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 4)
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	DNeasy mericon Food Kit
Kit used following the manufacturer's instructions?	Modified DNeasy Mericon™ Food Standard Protocol
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite samples of polygala myrtifolia, prepared as reported in the attached additional file, by using: - "Modified DNeasy Mericon™ Food Standard Protocol" (Qiagen);
<b>Method: Molecular Extraction DNA RNA (3)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	PROMEGA
<b>Specify the kit used</b>	Maxwell® RSC PureFood GMO and Authentication Kit
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite samples of polygala myrtifolia, prepared as reported in the attached additional file, by "Maxwell® RSC PureFood GMO and Authentication Kit" protocol (Promega)
<b>Method: Molecular real time 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 3)
<b>Name of the test</b>	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
<b>Is the test modified compared to the reference test</b>	yes BSA was not included in the amplification MIX
<b>Other information</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>	2 portion of 2.5-3 cm/shoot excised from 1 plant, in 20 g of healthy shoots (pieces of 2.5-3 cm)
<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>	standard test reported by appendix 3 and 5 of PM 7/24 (3)
<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>	standard test reported by appendix 3 and 5 of PM 7/24 (3)
<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>	These validation data were obtained by IPSP-CNR in collaboration with the Department of Soil, Plant and Food Science of the University of Bari (ITAY). For any additional detail, see the attached file.
<b>The following complementary files are available online:</b>	<ul style="list-style-type: none"> <li>• <a href="#">Composite sample of polygala</a></li> </ul>

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