

**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 herbaceous hosts
<b>Date, reference of the validation report</b>	2019-03-13 - 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>	no
<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>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 reference test</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite samples of herbaceous plants, prepared as reported in the attached additional file
<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 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>	no
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite samples of herbaceous plants, 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>	no
<b>As or adapted from an IPPC 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
<b>Kit used following the manufacturer's instructions?</b>	yes proceed as indicated in the manufacture's instruction
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite samples of herbaceous plants, prepared as reported in the attached additional file, by using: - "Maxwell® RSC PureFood GMO and Authentication Kit" protocol (Promega)
<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>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 4)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<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?	
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite samples of herbaceous plants, prepared as reported in the attached additional file, by using: - "Modified DNeasy Mericon™ Food Standard Protocol" (Qiagen);
<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>	no
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Other details on the test</b>	qPCR following the condition reported in Appendix 5 -Realtime PCR (Harper et al.,2010; erratum 2013) in PM 7/24 (3)
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	one stem portion of 1,5-2 cm excised from 1 infected plant of periwinkle, in 40 gr of stem portion of 1,5-2 cm excised from cabbage plants
<b><u>Diagnostic sensitivity</u></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% for each test used to extract total DNA
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100% for each test used to extract the total DNA
<b>Specify the test(s)</b>	Standard tests reported in appendix 3 and 5 of PM 7/24 (3)
<b><u>Repeatability</u></b>	

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% for each test used to extract the total DNA
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	<p>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. The test was include in the last revised version of PM 7/24 (4), which is in consultation to the NPPO member countries Reference: 2019-03-13 - 2019-03-11 - 2019-03-13 - G. Loconsole, L. Manco, O. Potere, L. Susca, G. Altamura, S. Zicca, D. Boscia, V. N. Savino, M. Saponari, 2018. Implementation of sampling procedures for testing composite samples for Xylella fastidiosa. POnTE - XF-ACTORS, 2nd Joint Annual Meeting: European Research on Emerging Plant Diseases. Valencia, 23-26 october 2018. Book of abstract: p. 63.</p>
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">composite samples of herbaceous plants (cabbage)</a></li> </ul>

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