

**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 olive samples
<b>Date, reference of the validation report</b>	2021-09-26 - Diagnostic Procedures to Detect <i>Xylella fastidiosa</i> in Nursery Stocks and Consignments of Plants for Planting. Agriculture 2021, 11, 922. <a href="https://doi.org/10.3390/agriculture11100922">https://doi.org/10.3390/agriculture11100922</a>
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	XF-ACTORS
<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 petioles/midribs of olive leaves
<b>Plant species tested</b>	<i>Olea europaea</i>
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) 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>	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>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?	yes "Modified DNeasy Mericon™ Food Standard Protocol" (Qiagen)
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite olive samples, prepared as reported in the attached additional file, by using "Modified DNeasy Mericon™ Food Standard Protocol" (Qiagen)
<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>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 olive samples, 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>	
<b>Specify the kit used</b>	
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA were extracted from composite olive samples, prepared as reported in the attached additional file, by using CTAB-based extraction

	protocol, Modified DNeasy® Mericon™ Food Standard Protocol and the Maxwell® RSC PureFood GMO and Authentication Kit” (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 the smallest amount of target that can be detected reliably?</b>	4 infected olive leaves in 20 gr of leaf petioles/midribs recovered from Xylella-free olive plants
<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% using the 3 different DNA extraction procedures
<b>Standard test(s)</b>	standard tests 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% using the 3 different DNA extraction procedures
<b>Specify the test(s)</b>	standard tests 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% using the 3 different DNA extraction procedures
<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>	• <a href="#">paper 2021</a>

