

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.

Laboratory contact details	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy
Short description of the test	detection of <i>Xylella fastidiosa</i> in composite olive samples
Date, reference of the validation report	2021-09-26 - Diagnostic Procedures to Detect <i>Xylella fastidiosa</i> in Nursery Stocks and Consignments of Plants for Planting. Agriculture 2021, 11, 922. https://doi.org/10.3390/agriculture11100922
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	Other_project
If yes, please specify	XF-ACTORS
Description of the test	
Organism(s)	<i>Xylella fastidiosa</i> (XYLEFA)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/024 <i>Xylella fastidiosa</i> (version 4)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	DNeasy mericon Food Kit

Kit used following the manufacturer's instructions?	yes "Modified DNeasy Mericon™ Food Standard Protocol" (Qiagen)
Other information	
Other details on the test	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)
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
Kit	
Is a kit used	yes
Manufacturer name	PROMEGA
Specify the kit used	Maxwell® RSC PureFood GMO and Authentication Kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	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)
Method: Molecular Extraction DNA RNA (3)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 4)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	
Specify the kit used	
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	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)

Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPP0 diagnostic protocol	yes
EPP0 Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Name of the test	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
Is the test modified compared to the reference test	yes BSA was not included in the amplification MIX
Other information	
Performance Criteria :	
Organism 1.:	Xylella fastidiosa(XYLEFA)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	4 infected olive leaves in 20 gr of leaf petioles/midribs recovered from Xylella-free olive plants
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% using the 3 different DNA extraction procedures
Standard test(s)	standard tests reported by appendix 3 and 5 of PM 7/24 (3)
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% using the 3 different DNA extraction procedures
Specify the test(s)	standard tests reported by appendix 3 and 5 of PM 7/24 (3)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% using the 3 different DNA extraction procedures
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	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 following complementary files are available online:	
	• paper 2021