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 Xylella fastidiosa in composite samples of cherry
Date, reference of the validation report	2019-03-13 - 2019-03-13 - 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.
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Method(s)	Extraction Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) Molecular real time PCR
Method: Extraction	
Reference of the test description	
Other information	
Other details on the test	The preparation of the olive samples is different from the description reported in the EPPO DP
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 Xylella fastidiosa (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 MericonTM Food Standard Protocol
Other information	
Other details on the test	Total DNA were extracted from composite samples of cherry, prepared as reported in the attached additional file, by using “Modified DNeasy MericonTM Food Standard Protocol” (Qiagen)
Method: Molecular Extraction DNA RNA (2)	
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)
Is the test modified compared to the reference test	no
Other information	
Other details on the test	Total DNA were extracted from composite samples of cherry, prepared as reported in the attached additional file, by using CTAB-based protocol;
Method: Molecular Extraction DNA RNA (3)	
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 samples of cherry, prepared as reported in the attached additional file, by using “Maxwell® RSC PureFood GMO and Authentication Kit” protocol (Promega)
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO 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?	0.2 gr of xylem tissue scraped from 2 shoots recovered form one infected plant of cherry, in 20 gr of xylem tissue recovered from Xylella-free plant
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 each test reported above to extract the total DNA
Standard test(s)	Standard tests reported in 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 each test reported above to extract the total DNA
Specify the test(s)	Standard tests reported in 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 each test reported above to extract the total DNA
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:	• composite samples of cherry

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