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 oleander
Date, reference of the validation report	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.
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?	
If yes, please specify	
Description of the test	
Organism(s)	Xylella fastidiosa(XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves leaf petioles of oleander
Plant species tested	Nerium oleander
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	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	

Is the test modified compared to the reference test	
Other information	
Other details on the test	The preparation of the oleander samples is different from the description reported in the EPPO DP. (see attached document)
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
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	Maxwell® RSC PureFood GMO and Authentication Kit" protocol (Promega)
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Name of the test	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	no
Kit	
Is a kit used	
Other information	
Other details on the test	CTAB-based protocol

Method: Molecular Extraction DNA RNA (3)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Name of the test	
As or adapted from an IPPC diagnostic protocol	
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?	
Other information	
Other details on the test	Modified DNeasy MericonTM Food Standard Protocol (Qiagen):
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
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)
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	no
Kit	
Is a kit used	
Other information	
Reaction type	
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	

Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	2 infected leaf petioles in 20 g of leaf petioles processed as single sample (ca. 200 leaf petioles) for each test
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%, with all the 3 different protocols used to extract the total DNA
Standard test(s)	standard tests reported in appendix 3 and 5 of PM 7/24 (3)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	
Specificity value	
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	standard tests reported in appendix 3 and 5 of PM 7/24 (3)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for each protocol used to extract the total DNA
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	
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	• composite oleander samples

online:	

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