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 herbaceous hosts
Date, reference of the validation report	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.
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	no
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	
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 herbaceous plants, prepared as reported in the attached additional file
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	no
Other information	
Other details on the test	Total DNA were extracted from composite samples of herbaceous plants, prepared as reported in the attached additional file, by using: - CTAB-based protocol;
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC 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?	yes proceed as indicated in the manufacture’s instruction
Other information	
Other details on the test	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)
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	QIAGEN

Specify the kit used	DNeasy mericon Food Kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	Total DNA were extracted from composite samples of herbaceous plants, prepared as reported in the attached additional file, by using: - “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
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	no
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Probe
Other details on the test	qPCR following the condition reported in Appendix 5 –Realtime PCR (Harper et al.,2010; erratum 2013) in PM 7/24 (3)
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?	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
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% for each test used to extract total DNA
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% for each test used 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% for each test used 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 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.
The following complementary files are available online:	• composite samples of herbaceous plants (cabbage)

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