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 perennial host species by PCR
Date, reference of the validation report	2014-09-01 - 2014-09 and 2015-07 - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2014 and 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS
Link to other validation data	- 2014-09 and 2015-07 - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2014 and 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS Detection of Xylella fastidiosa in perennial host species by ELISA - 2014-09 and 2015-07 - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2014 and 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS Detection of Xylella fastidiosa in perennial host species by Real time PCR
Validation process according to EPPO Standard PM7/98?	yes
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)	Molecular Extraction DNA RNA Molecular Conventional PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Other information	
Other details on the test	CTAB-based total nucleic acid extraction Loconsole,

	G., Potere, O., Boscia, D., Altamura, G., Djelouah, K., Elbeaino, T., Frasheri, D., Lorusso, D., Palmisano, F., Pollastro, P., Silletti, M. R., Trisciuzzi, N., Valentini, F., Savino V. & Saponari, M. (2014a). Detection of Xylella fastidiosa in olive trees by serological and molecular methods. Journal of Plant Pathology, 96, 7-14.
Method: Molecular Conventional 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 1)
Name of the test	Conventional PCR (Minsavage et al., 1994)
Is the test modified compared to the reference test	yes Total nucleic acids were extracted following the protocl reported in: Loconsole, G., Potere, O., Boscia, D., Altamura, G., Djelouah, K., Elbeaino, T., Frasheri, D., Lorusso, D., Palmisano, F., Pollastro, P., Silletti, M. R., Trisciuzzi, N., Valentini, F., Savino V. & Saponari, M. (2014a). Detection of Xylella fastidiosa in olive trees by serological and molecular methods. Journal of Plant Pathology, 96, 7-14.
Other information	
Other details on the test	Minsavage GV, Thompson CM, Hopkins DL & Leite RMVBC and Stall RE (1994) Development of a polymerase chain reaction protocol for detection of Xylella fastidiosa in plant tissue. Phytopathology 84, 456–461.
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?	up to 10^4 cfu/ml (corrisponding to 0.7 x 10^3 cfu/reaction), using dilutions ranging from 10^7 to 10 CFU/ml prepared by spiking the inactivated bacterial culture in total nucleic acids recovered from olive reference sources known to be not infected by Xylella fastidiosa.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	97.92%
Standard test(s)	141 obtained positive samples/144 expected positive samples (distributed as blind samples)
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%

Specify the test(s)	120 obtained negative samples/ 120 expected negative samples (distributed as blind samples)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	98.86%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	Validation of the PCR protocol was carried out by the Laboratories listed below, under the supervision of the reference laboratory CNR-UNIBA. • IPSP-CNR: Istituto per la Protezione Sostenibile delle Piante CNR, UOS Bari (Italy) • UNIBA: Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Bari (Italy); • CRSFA: Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia, Locorotondo (BA), (Italy); • IAMB: Istituto Agronomico Mediterraneo, Valenzano (BA), (Italy). • Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale - Università degli Studi di Foggia, (Italy) A panel of blind samples was distributed.
The following complementary files are available online:	• protocols for diagnosis of Xylella fastidiosa • Report interlaboratory validation 2014 • Report interlaboratory validation 2015

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