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 Real time 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 PCR
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 petiols
Plant species tested	Acacia saligna, Citrus sp., Nerium oleander, Olea europaea, Polygala myrtifolia, Prunus avium, Prunus dulcis, Quercus ilex, Vitis sp.
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
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	no
Reference of the test	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.
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Other details on the test	CTAB-based protocol for total DNA extraction
Method: Molecular real time PCR	
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	no
Reference of the test	Harper S.J., Ward L.I., Clover G.R.G., 2010. Development of LAMP and real-time PCR methods for the rapid detection of Xylella fastidiosa for quarantine and field applications. Phytopathology 100: 1282–1288.
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	**yes**
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	up to 10^2 cfu/ml (corrisponding to 7 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	100%
Standard test(s)	108 obtained positive samples/ 108 expected positive samples
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)	90 obtained negative samples/ 90 expected negative samples
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
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
Brief details of the test performance study and its output.It available, link to published article/report	
Other information	
Any other information considered useful	Validation of the Real time PCR assay 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); • DiSSPA-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 2015 • Report interlaboratory validation 2014

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