EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

Target Organism	Xylella fastidiosa	
Short description	Detection of Xylella fastidiosa in perennial host species by PCR	
Laboratory contact details	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy	
Date and reference of the validation report	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	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/024(1) 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.	
Is the test the same as described in the EPPO DP?	Modified 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.	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Olea Europaea L., Prunus avium, Prunus dulcis, Nerium Oleander, Polygala myrtifolia, Acacia saligna, Quercus ilex, Citrus spp, Vitis spp	
Matrices tested (if relevant)	leaf petiols	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	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.
Molecular methods, e.g.	X	PCR by primers RST 31/33 ((Minsavage et al., 1994)

hybridization, PCR and real time PCR		
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
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%	
Specify the standard test	141 obtained positive samples/144 expected positive samples (distributed as blind samples)	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested		
Number of non-target organisms tested		
Cross reacts with (specify the species)		
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%	
Specify the standard test	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
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	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