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 LAMP-PCR	
Laboratory contact details	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy	
Date and reference of the validation report	2015-07 - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS	
Validation process according to EPPO Standard PM 7/98:	No	
Reference of the test description	N/R - T. YASEEN, S. DRAGO, F. VALENTINI, T. ELBEAINO, G. STAMPONE, M. DIGIARO and A.M. D'ONGHIA. On-site detection of Xylella fastidiosa in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method, 2015. Phytopathologia Mediterranea 54: 488−496. - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS	
Is the test the same as described in the EPPO DP?	No	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Olea Europaea L., Nerium Oleander	
Matrices tested (if relevant)	leaf petioles	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	- T. YASEEN, S. DRAGO, F. VALENTINI, T. ELBEAINO, G. STAMPONE, M. DIGIARO and A.M. D'ONGHIA. On-site detection of Xylella fastidiosa in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method, 2015. Phytopathologia Mediterranea 54: 488−496. - manufacturer instructions provided by Enbiotech s.r.l. - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS.

Molecular methods, e.g. hybridization, PCR and real time PCR	X	- T. YASEEN, S. DRAGO, F. VALENTINI, T. ELBEAINO, G. STAMPONE, M. DIGIARO and A.M. D'ONGHIA. On-site detection of Xylella fastidiosa in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method, 2015. Phytopathologia Mediterranea 54: 488−496. - manufacturer instructions provided by Enbiotech s.r.l. - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS
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		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	up to 10^2 cfu/ml using dilutions ranging from 10^7 to 10 CFU/ml, prepared by adding to the extraction buffer the proper aliquots of bacterial suspension after the incubation with a piece of healthy olive stem.	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100%	
Specify the standard test	33 obtained positive samples/ 33 expected positive samples	
<u>Analytical specificity</u>		
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	27 obtained negative samples/ 27 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
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 Lamp-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); • Enblotech s.r.l. (PA) which provided the kit and the manufacturer instructions
The following complementary files are available online:	• protocols for diagnosis of Xylella fastidiosa • report interlaboratory validation 2015