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	Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	Detection of Xylella fastidiosa subsp. pauca ceppo CoDiRo by LAMP-PCR from plant olive crude extracts
Date, reference of the validation report	2021-05-04 - Loreti S., Pucci N., Loconsole G., Modesti V, Lucchesi S.,Potere O., Saponari M 2017. Protocollo Diagnostico per XYLELLA FASTIDIOSA subsp. PAUCA ceppo CoDiRO. In Protocolli Diagnostici - ASPROPI- ISBN 9788899595722.pp. 241-278
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	

Description of the test		
Organism(s)	Xylella fastidiosa subsp. pauca (XYLEFP)	
Detection / identification	detection	
Method(s)	Extraction Molecular LAMP	
Method: Extraction		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
As or adapted from an IPPC diagnostic protocol	no	
Reference of the test	Plant olive crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)	
Other information		
Other details on the test	Plant olive crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)	

Method: Molecular LAMP	
Reference of the test description	
Kit	
Is a kit used	yes
Manufacturer name	ENBIOTECH
Specify the kit used	ENBIOTECH Xylella fastidiosa LAMP kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	LAMP-PCR (manufacturer instructions provided by Enbiotech s.r.l.)
Are the performance characteristics included in the EPPO diagnostic protocol?	**yes**
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa subsp. pauca(XYLEFP)**
<u>Analytical sensitivity</u>	
What is smallest amount of target that can be detected reliably?	$10^3/ 10^4$ CFU/ml
<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	0,7
Standard test(s)	LAMP-PCR (Enbiotech s.r.l.) from crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	0,97
Specify the test(s)	LAMP-PCR (Enbiotech)from crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Concordance) 63%
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Accordance) 68%
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	1. Two series of olive extracts spiked with ten fold dilution of Xylella fastidiosa CODiRo strain suspensions from 10^7 to 10 cfu/ml plus two healthy samples (16 samples in total) were tested

	in three different laboratories (CREA-PAV; CNR-IPSP; Plant Protection Service Lombardy) (NTC, healthy and infected olive extracts as control) for analytical sensitivity. 2. To check the diagnostic sensitivity and specificity , the accuracy, the repeatability and reproducibility, olive extract samples spiked with Xylella fastidiosa CODiRo strain suspensions at 10^6 cfu /ml (three repetitions), 10^4 cfu /ml (three repetitions), 10^3 cfu /ml (three repetitions), healthy olive extracts (three repetitions) for a total of 12 samples, were tested by the following TPS participants : 1. CREA-DC (N. Pucci; S. Loreti) 2. SELGE/CNR-IPSP/ DiSSPA-Uniba (M. Saponari, G. Loconsole; O. Potere) 3. PPS Piemonte (C. Morone, G. Mason) 4. PPS Friuli Venezia Giulia (G. Bianchi) 5. PPS Lombardia (F. Gaffuri) 6. PPS Emilia Romagna (A. Alessandrini; R. Gozzi) 7. PPS Trentino Alto Adige (V. Gualandri; L. Tessari) 8. PPS Marche (S. Nardi; S. Talevi) 9. PPS Liguria (M. Guelfi) 10. CIHEAM-IAMB (A.M. D'Onghia; M. Digiaro) 11. CRSFA (F. Palmisano) 12. Centro di Sperimentazione Agraria e Forestale, Laimburg (A. Gallmetzer;A. Kraus) 13. Uni-MI (P. Casati) 14. Uni-CT (V. Catara) 15. PPS Toscana (D. Rizzo) 16. PPS Veneto (A. Saccardi; D. Pasqua di Bisceglie)
Other information	
Any other information considered useful	Relative accuracy: 77%

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