

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 subsp. pauca	
Short description	Detection of Xylella fastidiosa subsp. pauca ceppo CoDiRo by LAMP-PCR from plant olive crude extracts	
Laboratory contact details	Council for Agricultural Research and Economics- Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy	
Date and reference of the validation report	- 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 PM 7/98:	Yes	
Reference of the test description	0	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Olea europea L.	
Matrices tested (if relevant)	Leaves, petioles	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Plant olive crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	LAMP-PCR (manufacturer instructions provided by Enbiotech s.r.l.)
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?	10 ³ / 10 ⁴ CFU/ml	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	70%	
Specify the standard test	LAMP-PCR (Enbiotech s.r.l.) from crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)	
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	97%	
Specify the standard test	LAMP-PCR (Enbiotech)from crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Concordance) 63%	
Repeatability		
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	
Include brief details of the test	1. Two series of olive extracts spiked with ten fold dilution of	

<p>performance study and its output. It available, provide a link to published article/report</p>	<p>Xylella fastidiosa CODiRo strain suspensions from 10^7 to 10^2 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.</p> <p>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 :</p> <ol style="list-style-type: none"> 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. Digiario) 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)
<p><u>Other information</u></p>	
<p>Any other information considered useful e.g. robustness, ease of performing the test, etc.</p>	<p>Relative accuracy: 77%</p>