

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 DNA extracted from olive leaves and petioles	
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	2015-10-28 - 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 and petioles extract	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	DNA extraction by following Loconsole et al. (2014) (procedure B)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	LAMP-PCR (Qualiplante SAS) LAMP-PCR (Enbiotech s.r.l.) Manufacturer instruction provided by the companies
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 ² /10 ³ CFU/ml (Qualiplante SAS by using real-time PCR machine) 10 ³ CFU/ml (Enbiothec s.r.l. by using real-time PCR machine) 10 ¹⁻² CFU/ml (Enbiothec s.r.l. by using ic-gene)	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	90% LAMP-PCR (Enbiotech s.r.l.)	
Specify the standard test	LAMP-PCR (Enbiotech s.r.l.)	
Analytical specificity		
Specificity value	100%	
Number of strains/populations of target organisms tested		
Number of non-target organisms tested	LAMP-PCR (Enbiotech s.r.l. kit) tested on the following bacterial strains: 3 Xanthomonas arboricola pv. pruni, 1 X. arboricola pv. juglandis, 2 X. arboricola pv. fragariae, 1 X. arboricola pv. corylina, 1 X. arboricola pv. celebensis), 1 X. campestris pv. campestris, 1 X. campestris pv. populi, 2 X. hortorum pv. pelargonii), 3 Pseudomonas savastanoi pv. savastanoi, 1 P. marginalis, 4 P. syringae pv. syringae, 4 Brenneria (ssp. rubrifaciens, quercina, salicis, populi), 2 Pantoea stewartii, 1 Pantoea agglomerans, 1 Erwinia amylovora, 3 Agrobacterium tumefaciens, 2 Rhizobium vitis	
Cross reacts with (specify the species)	No cross-reaction with LAMP-PCR (Enbiotech s.r.l. kit)	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% LAMP-PCR (Enbiotech s.r.l.)	
Specify the standard test		
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Concordance) LAMP-PCR (Enbiotech s.r.l.): 85%	
Repeatability		

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Accordance) LAMP-PCR (Enbiotech s.r.l.): 91%
Test performance study	
Test performance study?	Yes
Include brief details of the test performance study and its output. It available, provide a link to published article/report	<p>1. Two series of olive extracts spiked with ten fold dilution of Xylella fastidiosa CODiRo strain suspensions from 10⁷ to 10¹ 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 10⁶ cfu /ml (three repetitions), 10⁴ cfu /ml (three repetitions), 10³ 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)
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	<p>Accuracy:</p> <p>92% (Enbiotech s.r.l.)</p> <p>Validation data were carried out by the Laboratories listed below, under the supervision of the reference laboratory CREA-PAV with the collaboration of CNR-IPSP:</p> <ul style="list-style-type: none"> • CREA-PAV: Centro di Ricerca per la Patologia Vegetale CREA, Rome (Italy) • CNR-IPSP: 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); • Servizio Fitosanitario Regione Lombardia, Laboratorio Fitopatologico c/o Fondazione Minoprio 22070 Vertemate con Minoprio (CO) Italy