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 in plant olive extracts by real time PCR	
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?	Yes	
Plant species tested (if relevant)	Olea europea	
Matrices tested (if relevant)	leaves extracts	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	DNA extraction of plant extracts by LoConsole et al 2015) (procedure B); DNA extraction from bacterial bacterial suspension
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Real-time PCR following Francis et al., 2006 Real-time PCR following Harper et al., 2010 Real-time PCR following Harper et al., 2010 and Li et al. 2006
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?	Real-time PCR (Francis et al., 2006): 10^1 CFU/ml Real-time PCR (Harper et al., 2010): 10^2 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	Real-time PCR (Francis et al., 2006): 90% Real-time PCR (Harper et al., 2010): 100% Real-time PCR following Harper et al., 2010 and Li et al. 2006: 100%	
Specify the standard test	Real-time PCR following Francis et al., 2006 Real-time PCR following Harper et al., 2010 Real-time PCR following Harper et al., 2010 and Li et al. 2006	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested	Agrobacterium tumefaciens bv1 L.C.58 Agrobacterium tumefaciens bv2 AT 20 N5 Agrobacterium tumefaciens bv1 B6 Xanthomonas campestris pv. vesicatoria CREA-DC 1009 Xanthomonas arboricola pv. juglandis NCPPB 411 Xanthomonas arboricola pv. corylina NCPPB 935 Xanthomonas arboricola pv. pelargonii CREA-DC 1033 Xanthomonas arboricola pv. pelargonii CREA-DC 1040 Clavibacter michiganensis pv. michiganensis CREA-DC 1046 Pseudomonas savastanoi CREA-DC 1180 Pseudomonas syringae pv. syringae CREA-DC 1204 Pseudomonas syringae pv. syringae CREA-DC 1205 Pseudomonas marginalis pv. marginalis CREA-DC 1229 Pseudomonas marginalis pv. marginalis CREA-DC 1230 Pantoea agglomerans ISF 438 Xanthomonas campestris pv. citri CREA-DC 1264 Xanthomonas arboricola pv. juglandis CREA-DC 1300 Brenneria rubrifaciens NCPPB 2020^T Brenneria quercina NCPPB 1852^T Brenneria populi NCPPB 4249^T Ralstonia solanacearum NCPPB 325^T Xanthomonas arboricola pv. pruni IVIA-3287.1 Erwinia amylovora CREA-DC 208 Xanthomonas arboricola pv. celebensis NCPPB 1832 Xanthomonas campestris pv. populi NCPPB 2987 Xanthomonas arboricola Uni-Mo 279 Pantoea stewartii subsp. stewartii 2766 Pantoea stewartii subsp. stewartii 1788 Agrobacterium vitis CREA-DC 1822 Agrobacterium vitis CREA-DC 1824	
Number of non-target organisms	35 non target organism tested belonging to the following	

tested	species: Xanthomonas arboricola pv juglandis, Xanthomonas arboricola pv. pruni, Xanthomonas arboricola pv. corylina, Xanthomonas arboricola pv. fragariae, Xanthomonas arboricola pv. celebensis, X. campestris pv. campestris, X. campestris pv. populi, X. axonopodis pv. citri, X. hortorum pv. pelargonii, Pseudomonas savastanoi pv. savastanoi, P. marginalis, P. syringae pv. syringae, Brenneria rubrifaciens, B. quercina, B. salicis, B. populi, Pantoea stewartii, P. agglomerans, Erwinia amylovora, Agrobacterium tumefaciens, Rhizobium vitis
Cross reacts with (specify the species)	Real-time (Francis et al., 2006): X. arboricola pv. celebensis (NCPPB 1832) and Brenneria populi (NCPPB 4299^T) gave an amplification curve corresponding to melt peak values of respectively 87.5°C and 87°C (differently from Xylella fastidiosa that have a melt peak at 84-85°C). Pantoea agglomerans (ISF 438), B. quercina (NCPPB 1852^T), Pseudomonas marginalis (CREA-PAV 1229), X. hortorum pv. pelargonii gave amplification curves with inconsistent melting peak. No amplification were obtained with the tested non-target organisms by real-time PCR of Harper et al., 2010
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Real-time PCR following Francis et al., 2006: 100% Real-time PCR following Harper et al., 2010: 97% Real-time PCR following Harper et al., 2010 and Li et al. 2006:100%
Specify the standard test	Real-time PCR following Francis et al., 2006 Real-time PCR following Harper et al., 2010 Real-time PCR following Harper et al., 2010 and Li et al. 2006
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Concordance) Real-time PCR following Francis et al., 2006: 85 % Real-time PCR following Harper et al., 2010: 99% Real-time PCR following Harper et al., 2010 and Li et al. 2006: 100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Accordance) Real-time PCR following Francis et al., 2006: 91% Real-time PCR following Harper et al., 2010: 99% Real-time PCR following Harper et al., 2010 and Li et al. 2006: 100%
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	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) 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)
<u>Other information</u>	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	Accuracy Real-time PCR following Francis et al., 2006: 92% Real-time PCR following Harper et al., 2010: 100% Real-time PCR following Harper et al., 2010 and Li et al. 2006: 100% Validation was carried out by the Laboratories listed below, under the supervision of the reference laboratory CREA-PAV with the collaboration of CNR-IPSP: • 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