

**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.

<b>Target Organism</b>	Xylella fastidiosa subsp. pauca	
<b>Short description</b>	Detection of Xylella fastidiosa subsp. pauca ceppo CoDiRo in plant olive extracts by real time PCR	
<b>Laboratory contact details</b>	Council for Agricultural Research and Economics- Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy	
<b>Date and reference of the validation report</b>	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	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	0	
<b>Is the test the same as described in the EPPO DP?</b>	Yes	
<b>Is the lab accredited for this test?</b>	Yes	
<b>Plant species tested (if relevant)</b>	Olea europea	
<b>Matrices tested (if relevant)</b>	leaves extracts	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>	X	DNA extraction of plant extracts by LoConsole et al 2015) (procedure B); DNA extraction from bacterial bacterial suspension
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	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
<b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>		
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b>		
<b>Pathogenicity test</b>		

<b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA profiling</b>		
<b>Morphological and morphometrical methods intended for identification</b>		
<b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>		
<b>Other</b>		
<b>Analytical sensitivity (= limit of detection)</b>		
<b>What is smallest amount of target that can be detected reliably?</b>	Real-time PCR (Francis et al., 2006): 10 <sup>1</sup> CFU/ml Real-time PCR (Harper et al., 2010): 10 <sup>2</sup> CFU/ml	
<b>Diagnostic sensitivity</b>		
<b>Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98</b>	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%	
<b>Specify the standard test</b>	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	
<b>Analytical specificity</b>		
<b>Specificity value</b>		
<b>Number of strains/populations of target organisms tested</b>	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 <sup>T</sup> Brenneria quercina NCPPB 1852 <sup>T</sup> Brenneria populi NCPPB 4249 <sup>T</sup> Ralstonia solanacearum NCPPB 325 <sup>T</sup> 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	
<b>Number of non-target organisms</b>	35 non target organism tested belonging to the following	

<b>tested</b>	species: <i>Xanthomonas arboricola</i> pv. <i>juglandis</i> , <i>Xanthomonas arboricola</i> pv. <i>pruni</i> , <i>Xanthomonas arboricola</i> pv. <i>corylina</i> , <i>Xanthomonas arboricola</i> pv. <i>fragariae</i> , <i>Xanthomonas arboricola</i> pv. <i>celebensis</i> , <i>X. campestris</i> pv. <i>campestris</i> , <i>X. campestris</i> pv. <i>populi</i> , <i>X. axonopodis</i> pv. <i>citri</i> , <i>X. hortorum</i> pv. <i>pelargonii</i> , <i>Pseudomonas savastanoi</i> pv. <i>savastanoi</i> , <i>P. marginalis</i> , <i>P. syringae</i> pv. <i>syringae</i> , <i>Brenneria rubrifaciens</i> , <i>B. quercina</i> , <i>B. salicis</i> , <i>B. populi</i> , <i>Pantoea stewartii</i> , <i>P. agglomerans</i> , <i>Erwinia amylovora</i> , <i>Agrobacterium tumefaciens</i> , <i>Rhizobium vitis</i>
<b>Cross reacts with (specify the species)</b>	Real-time (Francis et al., 2006): <i>X. arboricola</i> pv. <i>celebensis</i> (NCPPB 1832) and <i>Brenneria populi</i> (NCPPB 4299 <sup>T</sup> ) gave an amplification curve corresponding to melt peak values of respectively 87.5°C and 87°C (differently from <i>Xylella fastidiosa</i> that have a melt peak at 84-85°C). <i>Pantoea agglomerans</i> (ISF 438), <i>B. quercina</i> (NCPPB 1852 <sup>T</sup> ), <i>Pseudomonas marginalis</i> (CREA-PAV 1229), <i>X. hortorum</i> pv. <i>pelargonii</i> 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
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	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%
<b>Specify the standard test</b>	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
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	(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%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	(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%
<b>Test performance study</b>	
<b>Test performance study?</b>	Yes
<b>Include brief details of the test performance study and its output. It available, provide a link to published article/report</b>	1. Two series of olive extracts spiked with ten fold dilution of <i>Xylella fastidiosa</i> CODiRo strain suspensions from 10 <sup>7</sup> to 10 <sup>1</sup> 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. 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)

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. 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.**

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