

**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.

<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>Short description of the test</b>	Detection of <i>Xylella fastidiosa</i> subsp. <i>pauca</i> ceppo CoDiRo in plant olive extracts by real time PCR
<b>Date, 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 PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	ASPROPI (Founded by MIPAAF_Italy)
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xylella fastidiosa</i> subsp. <i>pauca</i> (XYLEFP)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Leaves leaves extracts
<b>Plant species tested</b>	<i>Olea europaea</i>
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular real time PCR Molecular real time PCR (2) Molecular real time PCR (3)
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	DNA extraction of plant extracts by LoConsole et al 2015) (procedure B)

<b>Other information</b>	
<b>Other details on the test</b>	DNA extraction of plant extracts by LoConsole et al 2015) (procedure B)
<b>Method: Molecular Extraction DNA RNA (2)</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	DNA extraction from bacterial bacterial suspension
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	Real-time PCR following Harper et al., 2010 and Li et al. 2006
<b>Method: Molecular real time PCR (2)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>Other information</b>	
<b>Other details on the test</b>	Real-time PCR following Harper et al., 2010
<b>Method: Molecular real time PCR (3)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>Other information</b>	
<b>Other details on the test</b>	Real-time PCR following Francis et al., 2006
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa subsp. pauca(XYLEFP)</b>
<b>Analytical sensitivity</b>	
<b>What is the 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>Standard test(s)</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 - inclusivity</b>	
<b>Number of strains/populations of target</b>	Agrobacterium tumefaciens bv1 L.C.58

<b>organisms tested</b>	<p>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</p>
<b>Specificity value</b>	
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	<p>35 non target organism tested belonging to the following 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</p>
<b>Specificity value</b>	<p>Real-time (Francis et al., 2006): X. arboricola pv. celebensis (NCPBP 1832) and Brenneria populi (NCPBP 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 (NCPBP 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</p>
<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 test(s)</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>Brief details of the test performance study and its output. If available, link to published article/report</b>	<p>1. Two series of olive extracts spiked with ten fold dilution of <i>Xylella fastidiosa</i> CODiRo strain suspensions from <math>10^7</math> to <math>10^0</math> 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 <i>Xylella fastidiosa</i> CODiRo strain suspensions at <math>10^6</math> cfu /ml (three repetitions), <math>10^4</math> cfu /ml (three repetitions), <math>10^3</math> 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"> <li>1. CREA-DC (N. Pucci; S. Loreti)</li> <li>2. SELGE/CNR-IPSP/ DiSSPA-Uniba (M. Saponari, G. Loconsole; O. Potere)</li> <li>3. PPS Piemonte (C. Morone, G. Mason)</li> <li>4. PPS Friuli Venezia Giulia (G. Bianchi)</li> <li>5. PPS Lombardia (F. Gaffuri)</li> <li>6. PPS Emilia Romagna (A. Alessandrini; R. Gozzi)</li> <li>7. PPS Trentino Alto Adige (V. Gualandri; L. Tessari)</li> <li>8. PPS Marche (S. Nardi; S. Talevi)</li> <li>9. PPS Liguria (M. Guelfi)</li> <li>10. CIHEAM-IAMB (A.M. D'Onghia; M. Digiario)</li> <li>11. CRSFA (F. Palmisano)</li> <li>12. Centro di Sperimentazione Agraria e Forestale, Laimburg (A. Gallmetzer; A. Kraus)</li> <li>13. Uni-MI (P. Casati)</li> <li>14. Uni-CT (V. Catara)</li> <li>15. PPS Toscana (D. Rizzo)</li> <li>16. PPS Veneto (A. Saccardi; D. Pasqua di Bisceglie)</li> </ol> <p>Olive extract samples spiked with <i>Xylella fastidiosa</i> CODiRo strain suspensions at <math>10^6</math> cfu /ml (three repetitions), <math>10^4</math> cfu /ml (three repetitions), <math>10^3</math> 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"> <li>1. CREA-DC (N. Pucci; S. Loreti)</li> <li>2.</li> </ol>

	<p>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>
<b>Other information</b>	
<p><b>Any other information considered useful</b></p>	<p>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</p>
<p>The following complementary files are available online:</p>	<ul style="list-style-type: none"> <li>• <a href="#">Xylella_protocol_validation</a></li> </ul>

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