

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**  
**ORGANISATION EUROPEENNE ET MEDITERRANEEENNE 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 from plant olive extracts by ELISA test	
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	
Matrices tested (if relevant)	leaves and petioles	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Method for obtaining plant tissue extract by homogenization of leaf tissues following Loconsole et al. (2014)
Molecular methods, e.g. hybridization, PCR and real time PCR		
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay	X	ELISA test (Agritest) ELISA test (Loewe)
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		

<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>	ELISA test (Agritest): $10^{4-5}$ CFU/ml ELISA test (Loewe): $10^5$ 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>	ELISA test (Agritest): 33% ELISA test (Loewe): 36%	
<b>Specify the standard test</b>	ELISA test (Agritest) following manufacturer instructions ELISA test (Loewe) following manufacturer instructions	
<b>Analytical specificity</b>		
<b>Specificity value</b>	ELISA test (Agritest): 100% ELISA test (Loewe): 100%	
<b>Number of strains/populations of target organisms tested</b>		
<b>Number of non-target organisms tested</b>	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	
<b>Cross reacts with (specify the species)</b>	no cross-reactions	
<b>Diagnostic Specificity</b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	ELISA test (Agritest): 100% ELISA test (Loewe): 100%	
<b>Specify the standard test</b>	ELISA test (Agritest) following manufacturer instructions ELISA test (Loewe) following manufacturer instructions	
<b>Reproducibility</b>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	(Accordance) ELISA test (Agritest): 49% ELISA test (Loewe): 51%	

<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	(Concordance) ELISA test (Agritest): 49% ELISA test (Loewe): 50%
<b>Test performance study</b>	
<b>Test performance study?</b>	Yes
<b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b>	<p>1. Two series of olive extracts spiked with ten fold dilution of Xylella fastidiosa CODiRo strain suspensions from <math>10^7</math> to <math>10</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.</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 <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>
<b>Other information</b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	Accuracy: ELISA test (Agritest): 50% ELISA test (Loewe): 52%