

**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 from plant olive extracts by ELISA test
<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>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xylella fastidiosa</i> (XYLEFA) <i>Xylella fastidiosa</i> subsp. <i>pauca</i> (XYLEFP)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Serological DAS-ELISA Serological DAS-ELISA (2)
<b>Method: Extraction</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>	Method for obtaining plant tissue extract by homogenization of leaf tissues following Loconsole et al. (2014)
<b>Other information</b>	
<b>Method: Serological DAS-ELISA</b>	

<b>Reference of the test description</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	LOEWE
<b>Specify the kit used</b>	Xylella fastidiosa grapevine isolate DAS ELISA Cat. No. 07119
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Method: Serological DAS-ELISA (2)</b>	
<b>Reference of the test description</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	AGRITEST
<b>Specify the kit used</b>	DAS ELISA KIT Xylella fastidiosa (K27A/B)
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b>Organism 2.:</b>	<b>Xylella fastidiosa subsp. pauca(XYLEFP)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	ELISA test (Agritest): 10 <sup>4-5</sup> CFU/ml ELISA test (Loewe): 10 <sup>5</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>	ELISA test (Agritest):33% ELISA test (Loewe): 36%
<b>Standard test(s)</b>	ELISA test (Agritest) following manufacturer instructions ELISA test (Loewe) following manufacturer instructions
<b>Analytical specificity - exclusivity</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>Specificity value</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 test(s)</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>Brief details of the test performance study and its output. It available, 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>10^1</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 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: 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)</p>
<b>Other information</b>	
<b>Any other information considered useful</b>	Accuracy: ELISA test (Agritest): 50% ELISA test (Loewe): 52%

