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.

Laboratory contact details	Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	Detection of Xylella fastidiosa subsp. pauca ceppo CoDiRo from plant olive extracts by ELISA test
Date, 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 PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
If yes, please specify	
Description of the test	
Organism(s)	Xylella fastidiosa(XYLEFA) Xylella fastidiosa subsp. pauca(XYLEFP)
Detection / identification	detection
Matrix(ces) tested	Leaves leaves and petioles
Plant species tested	Olea europaea
Method(s)	Extraction Serological DAS-ELISA Serological DAS-ELISA (2)
Method: Extraction	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	no

Reference of the test	Method for obtaining plant tissue extract by homogenization of leaf tissues following Loconsole et al. (2014)
Is the test modified compared to the reference test	
Other information	
Other details on the test	
Method: Serological DAS-ELISA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	yes
Manufacturer name	LOEWE
Specify the kit used	Xylella fastidiosa grapevine isolate DAS ELISA Cat. No. 07119
Kit used following the manufacturer's instructions?	
Other information	
Reaction type	
Other details on the test	
Method: Serological DAS-ELISA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	yes
Manufacturer name	AGRITEST
Specify the kit used	DAS ELISA KIT Xylella fastidiosa
Kit used following the manufacturer's instructions?	

Other information	
Reaction type	
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	
Specificity value	
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Organism 2.:	**Xylella fastidiosa subsp. pauca(XYLEFP)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	ELISA test (Agritest): 10^4-5 CFU/ml ELISA test (Loewe): 10^5 CFU/ml
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the	ELISA test (Agritest):33% ELISA test (Loewe): 36%

standard test, see appendix 2 of PM 7/98	
Standard test(s)	ELISA test (Agritest) following manufacturer instructions ELISA test (Loewe) following manufacturer instructions
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	
Specificity value	ELISA test (Agritest: 100% ELISA test (Loewe): 100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	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
Specificity value	no cross-reactions
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	ELISA test (Agritest): 100% ELISA test (Loewe): 100%
Specify the test(s)	ELISA test (Agritest) following manufacturer instructions ELISA test (Loewe) following manufacturer instructions
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Accordance) ELISA test (Agritest): 49% ELISA test (Loewe): 51%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Concordance) ELISA test (Agritest): 49% ELISA test (Loewe): 50%
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, 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)
Other information	
Any other information considered useful	Accuracy: ELISA test (Agritest): 50% ELISA test (Loewe): 52%

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