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.

Short description Detection of Xylella fastidiosa subsp. pauca ceppo CoDiRo by LAMP-PCR from plant olive crude extracts 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 - 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 L. List of methods used X Method for extraction / isolation / baiting of target organism from matrix X Method for extraction / isolation / baiting of target organism from matrix X Plant olive crude extract obtained after homogenization, PCR and real time PCR X Serological methods; e.g. hybridization, PCR and real time PCR X Plating methods; selective isolation I Bioassay methods; selective isolation I Bioassay methods: selective isolation I	Target Organism	Xylella fastidiosa subsp. pauca		
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Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.				
enrichment in host plants, baiting, plant test and grafting.	Plating methods: selective isolation			
Pathogonicity test	enrichment in host plants, baiting,			
	Pathogenicity test			
Fingerprint methods: protein	Fingerprint methods: protein			

nucfiling fathers and nucfiling C DNA			
profiling, fatty acid profiling & DNA profiling			
Morphological and morphometrical methods intended for identification			
Biochemical methods: e.g. enzyme electrophoresis, protein profiling			
Other			
Analytical sensitivity (= limit of detec	tion)		
What is smallest amount of target that can be detected reliably?	10^3/ 10^4 CFU/ml		
Diagnostic sensitivity	•		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	70%		
Specify the standard test	LAMP-PCR (Enbiotech s.r.l.) from crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)		
Analytical specificity			
Specificity value			
Number of strains/populations of target organisms tested			
Number of non-target organisms tested			
Cross reacts with (specify the species)			
Diagnostic Specificity			
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	97%		
Specify the standard test	LAMP-PCR (Enbiotech)from crude extract obtained after homogenization of leaf tissues following Loconsole et al (2014)		
<u>Reproducibility</u>			
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Concordance) 63%		
<u>Repeatability</u>	-		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	(Accordance) 68%		
Test performance study			
Test performance study?	Yes		
Include brief details of the test	1. Two sei	ies of olive extracts spiked with ten fold dilution of	

performance study and its output.It available, provide a link to published article/report	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	1
Any other information considered useful e.g. robustness, ease of performing the test, etc.	Relative accuracy: 77%