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.

Target Organism	Xylella fag	stidiosa	
	Xylella fastidiosa		
Short description	detection of Xylella fastidiosa in composite samples of herbaceous hosts		
Laboratory contact details	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy		
Date and reference of the validation report	2019-03-13 - 2019-03-11 - 2019-03-13 - G. Loconsole, L. Manco, O. Potere, L. Susca, G. Altamura, S. Zicca, D. Boscia, V. N. Savino, M. Saponari, 2018. Implementation of sampling procedures for testing composite samples for Xylella fastidiosa. POnTE - XF-ACTORS, 2nd Joint Annual Meeting: European Research on Emerging Plant Diseases. Valencia, 23-26 october 2018. Book of abstract: p. 63.		
Validation process according to EPPO Standard PM 7/98:	No		
Reference of the test description	0 The test was include in the last revised version of PM 7/24 (4), which is in consultation to the NPPO member countries		
Is the test the same as described in the EPPO DP?	Modified The preparation of the olive samples is different from the description reported in the EPPO DP. Whereas the extraction of total nucleic acid and the qPCR test are reported in the EPPO DP.		
Is the lab accredited for this test?	No		
Plant species tested (if relevant)	periwinkle plant infecetd by Xylella fastidiosa and cabbage plants		
Matrices tested (if relevant)	stem portions		
List of methods used			
Method for extraction / isolation / baiting of target organism from matrix	X	Total DNA were extracted from composite samples of herbaceous plants, prepared as reported in the attached additional file, by using: - CTAB-based protocol; - "Modified DNeasy MericonTM Food Standard Protocol" (Qiagen); - "Maxwell® RSC PureFood GMO and Authentication Kit" protocol (Promega)	

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5 -Realtime

Molecular methods, e.g.

PCR

hybridization, PCR and real time

qPCR following the condition reported in Appendix

PCR (Harper et al., 2010; erratum 2013) in PM 7/24

		(3)		
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay				
Plating methods: selective isolation				
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.				
Pathogenicity test				
Fingerprint methods: protein 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 detection)				
What is smallest amount of target that can be detected reliably?	one stem portion of 1,5-2 cm excised from 1 infected plant of periwinkle, in 40 gr of stem portion of 1,5-2 cm excised from cabbage plants			
Diagnostic sensitivity				
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100% for each test used to extract total DNA			
Specify the standard test	Standard tests reported in appendix 3 and 5 of PM 7/24 (3)			
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	100% for	each test used to extract the total DNA		
Specify the standard test	Standard	tests reported in appendix 3 and 5 of PM 7/24 (3)		
<u>Reproducibility</u>				
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)	100% for each test used to extract the total DNA	
Test performance study		
Test performance study?	Νο	
Include brief details of the test performance study and its output.It available, provide a link to published article/report		
Other information		
Any other information considered useful e.g. robustness, ease of performing the test, etc.	These validation data were obtained by IPSP-CNR in collaboration with the Department of Soil, Plant and Food Science of the University of Bari (ITAY). For any additional detail, see the attached file.	
The following complementary files are available online:	 composite samples of herbaceous plants (cabbage) 	