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 fastidiosa		
Short description	detection of Xylella fastidiosa in composite olive samples		
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 - 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:	Νο		
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?	Yes		
Plant species tested (if relevant)	Olea L. europaea		
Matrices tested (if relevant)	petioles/midribs of olive leaves		
List of methods used			
Method for extraction / isolation / baiting of target organism from matrix	X	Total DNA were extracted from composite olive samples, prepared as reported in theattached additional file, by using: - CTAB-based protocol; - "Modified DNeasy MericonTM Food Standard Protocol" (Qiagen) - "Maxwell® RSC PureFood GMO and Authentication Kit" protocol	

		(Promega)
Molecular methods, e.g. hybridization, PCR and real time PCR	Х	qPCR following the condition reported in Appendix 5 – Real-time 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	<u>tion)</u>	
What is smallest amount of target that can be detected reliably?	4 infected olive leaves in 20 gr of leaf petioles/midribs recovered from Xylella-free olive 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% using the 3 different DNA extraction procedures	
Specify the standard test	standard t	tests reported by 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% usir	ng the 3 different DNA extraction procedures
Specify the standard test	standard t	tests reported by appendix 3 and 5 of PM 7/24 (3)
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)		
<u>Repeatability</u>		
Provide the calculated % of	100% usir	ng the 3 different DNA extraction procedures

agreement for a given level of the pest (see PM 7/98)		
Test performance study		
Test performance study?	No	
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:	<u>composite olive samples</u>	