

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 samples of oleander	
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:	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 oleander 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)	Nerium oleander	
Matrices tested (if relevant)	leaf petioles of oleander	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Total DNA were extracted from composite samples of oleander, prepared as reported in the attached additional file, by using: - CTAB-based protocol; - “Modified DNeasy Mericon™ Food Standard Protocol” (Qiagen); - “Maxwell® RSC PureFood GMO and Authentication Kit” protocol (Promega)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	qPCR following the condition reported in Appendix 5 – Realtime 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?	2 infected leaf petioles in 20 g of leaf petioles processed as single sample (ca. 200 leaf petioles) for each test	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100%, with all the 3 different protocols used to extract the total DNA	
Specify the standard test	standard tests reported in appendix 3 and 5 of PM 7/24 (3)	
<u>Analytical specificity</u>		
Specificity value		
Number of strains/populations of target organisms tested		
Number of non-target organisms tested		
Cross reacts with (specify the species)		
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%	
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)		
<u>Repeatability</u>		
Provide the calculated % of	100% for each protocol used to extract the total DNA	

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. If 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:	<ul style="list-style-type: none"> • composite samples for oleander