

**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.

<b>Target Organism</b>	Xylella fastidiosa	
<b>Short description</b>	detection of Xylella fastidiosa in composite samples of herbaceous hosts	
<b>Laboratory contact details</b>	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy	
<b>Date and reference of the validation report</b>	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.	
<b>Validation process according to EPPO Standard PM 7/98:</b>	No	
<b>Reference of the test description</b>	0 The test was include in the last revised version of PM 7/24 (4), which is in consultation to the NPPO member countries	
<b>Is the test the same as described in the EPPO DP?</b>	Modified The preparation of the herbaceous 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.	
<b>Is the lab accredited for this test?</b>	Yes	
<b>Plant species tested (if relevant)</b>	Periwinkle infected by Xylella fastidiosa and tomato plants	
<b>Matrices tested (if relevant)</b>	stems	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>	X	Total DNA were extracted from composite herbaceous samples, 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)
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	X	qPCR following the condition reported in Appendix 5 - Realtime PCR (Harper et al., 2010; erratum 2013) in PM 7/24

		(3)
<b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>		
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b>		
<b>Pathogenicity test</b>		
<b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA profiling</b>		
<b>Morphological and morphometrical methods intended for identification</b>		
<b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>		
<b>Other</b>		
<b><u>Analytical sensitivity (= limit of detection)</u></b>		
<b>What is smallest amount of target that can be detected reliably?</b>	One stem portion of 1,5-2 cm excised from an infected plant of periwinkle, in 40 gr of 1,5-2 cm stem portions of tomato plants	
<b><u>Diagnostic sensitivity</u></b>		
<b>Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98</b>	100% using all the 3 tests reported for the DNA extraction.	
<b>Specify the standard test</b>	Standard tests reported in appendix 3 and 5 of PM 7/24 (3)	
<b><u>Analytical specificity</u></b>		
<b>Specificity value</b>		
<b>Number of strains/populations of target organisms tested</b>		
<b>Number of non-target organisms tested</b>		
<b>Cross reacts with (specify the species)</b>		
<b><u>Diagnostic Specificity</u></b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100% using all the 3 tests reported for the DNA extraction.	
<b>Specify the standard test</b>	Standard tests reported in appendix 3 and 5 of PM 7/24 (3)	
<b><u>Reproducibility</u></b>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>		

<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% using all the 3 tests reported for the DNA extraction.
<b>Test performance study</b>	
<b>Test performance study?</b>	No
<b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	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"> <li>• <a href="#">composite samples of herbaceous plants (tomato)</a></li> </ul>