

**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 by real-time PCR in plant material	
Laboratory contact details	National Institiute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia	
Date and reference of the validation report	2016-02_26 - Dreo, Tanja, 2016. Validation data on the modified real-time PCR for detection of Xylella fastidiosa adapted from Francis et al. (2006) (No. D0002/16). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana.	
Validation process according to EPPO Standard PM 7/98:	No	
Reference of the test description	N/R Supporting data for the new draft of the EPPO diagnostic protocol on Xylella fastidiosa.	
Is the test the same as described in the EPPO DP?		
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Asparagus acutifolius, Coffea, Lavandula, Nerium oleander, Olea europea, Polygala myrtifolia, Quercus cerris, Rosamrinum officinalis, Spartium junceum.	
Matrices tested (if relevant)	Plant material, mainly mixtures of midribs, petioles and vascular tissues.	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	DNA extraction from plant material using QuickPick™ SML Plant DNA kit (Bionobile). Modified real-time PCR adapted from Francis, M., Lin, H., Rosa, J.C.-L., Doddapaneni, H., Civerolo, E.L., 2006. Genome-based PCR Primers for Specific and Sensitive Detection and Quantification of Xylella fastidiosa. European Journal of Plant Pathology 115, 203-213. doi:10.1007/s10658-006-9009-4
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		

<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>	On the DNA extracted from pure cultures of <i>X. fastidiosa</i> : 2.6, 3.2 and 3.5 (log (cells/mL) of <i>Xylella fastidiosa</i> subsp. multiplex, <i>Xylella fastidiosa</i> , and <i>Xylella fastidiosa</i> subsp. pauca CoDiRO strain, respectively. On plant material: 94 % (determined on log 5 cells/mL of plant extracts; the lowest concentration tested)	
<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>	No data available.	
<b>Specify the standard test</b>		
<b><u>Analytical specificity</u></b>		
<b>Specificity value</b>	100 %	
<b>Number of strains/populations of target organisms tested</b>	4	
<b>Number of non-target organisms tested</b>	15	
<b>Cross reacts with (specify the species)</b>	No cross reactions were observed.	
<b><u>Diagnostic Specificity</u></b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	No data available.	
<b>Specify the standard test</b>		
<b><u>Reproducibility</u></b>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	97 %	
<b><u>Repeatability</u></b>		

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	No data available.
<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>	
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Validation data on the modified real-time PCR for detection of Xylella fastidiosa adapted from Francis et al. (2006) (No. D0002/16)</a></li> </ul>