

**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 perennial host species by Real time PCR Francis et al., 2006 modified using SYBR green	
<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>	2015-10-22 - 0	
<b>Validation process according to EPPO Standard PM 7/98:</b>	No	
<b>Reference of the test description</b>	0	
<b>Is the test the same as described in the EPPO DP?</b>	No These tests are not reported in the EPPO standard	
<b>Is the lab accredited for this test?</b>	Yes	
<b>Plant species tested (if relevant)</b>	Olea Europaea L., Prunus avium, Prunus dulcis, Nerium Oleander, Polygala myrtifolia, Acacia saligna, Quercus ilex, Citrus spp, Vitis spp	
<b>Matrices tested (if relevant)</b>	leaf petioles	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>	X	For total DNA extraction we used the following protocols: 1) CTAB-based protocol reported in Loconsole, G., Potere, O., Boscia, D., Altamura, G., Djelouah, K., Elbeaino, T., Frasher, D., Lorusso, D., Palmisano, F., Pollastro, P., Silletti, M. R., Trisciuzzi, N., Valentini, F., Savino V. & Saponari, M. (2014a). Detection of Xylella fastidiosa in olive trees by serological and molecular methods.  2) "Dneasy mericon food kit" (QIAGEN)
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	X	Real-time PCR following Francis et al., 2006 modified using SYBR green.
<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>Analytical sensitivity (= limit of detection)</b>		
<b>What is smallest amount of target that can be detected reliably?</b>	up to $10^2$ cfu/ml (corresponding to $7$ cfu/reaction) using dilutions ranging from $10^7$ to $10$ CFU/ml, prepared by spiking the inactivated bacterial culture in total nucleic acids recovered from olive reference sources known to be not infected by <i>Xylella fastidiosa</i>	
<b>Diagnostic sensitivity</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%	
<b>Specify the standard test</b>	26 obtained positive samples/ 26 expected positive samples using for total nucleic acid extraction both CTAB based protocol and "DNeasy mericon food kit"-Qiagen, followed by the modified real time protocol Francis et al., 2006	
<b>Analytical specificity</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>Diagnostic Specificity</b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100%	
<b>Specify the standard test</b>	9 obtained negative samples/ 9 expected negative samples using for total nucleic acid extraction both CTAB based protocol and "DNeasy mericon food kit"-Qiagen, followed by the modified real time protocol Francis et al., 2006	
<b>Reproducibility</b>		

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	No
<b>Include brief details of the test performance study and its output. It available, provide a link to published article/report</b>	
<b><u>Other information</u></b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	