

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEEENNE 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 perennial host species by Real time PCR Francis et al., 2006 modified using SYBR green	
Laboratory contact details	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy	
Date and reference of the validation report	2015-10-22 - 0	
Validation process according to EPPO Standard PM 7/98:	No	
Reference of the test description	0	
Is the test the same as described in the EPPO DP?	No These tests are not reported in the EPPO standard	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Olea Europaea L., Prunus avium, Prunus dulcis, Nerium Oleander, Polygala myrtifolia, Acacia saligna, Quercus ilex, Citrus spp, Vitis spp	
Matrices tested (if relevant)	leaf petioles	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	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., Frasheri, 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)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Real-time PCR following Francis et al., 2006 modified using SYBR green.
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		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	up to 10 ² cfu/ml (corrisponding 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 Xylella fastidiosa	
<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%	
Specify the standard test	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	
<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	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	
<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 agreement for a given level of the pest (see PM 7/98)	100%
<u>Test performance study</u>	
Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
<u>Other information</u>	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	