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 perennial host species by Real time PCR (Harper et al. 2010 erratum 2013)	
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	
Matrices tested (if relevant)	leaf petioles	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	"Dneasy mericon food kit" (QIAGEN) for total DNA extraction
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Real time PCR with Taqman probe Harper S.J., Ward L.I., Clover G.R.G., 2010. Development of LAMP and real-time PCR methods for the rapid detection of Xylella fastidiosa for quarantine and field applications. Phytopathology 100: 1282–1288.
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?	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.	
Diagnostic sensitivity		
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	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested		
Number of non-target organisms tested		
Cross reacts with (specify the species)		
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%	
Specify the standard test	10 obtained negative samples/ 10 expected negative samples	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)		
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%	
Test performance study		
Test performance study?	No	

Include brief details of the test performance study and its output.It 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.**	This protocol is designed for the extraction of total DNA from a large-scale sample of raw or processed food material. The protocol can be performed manually or authomaized using a dedicated workstation starting from 0,5-0,8 g of fresh small pieces of midribs and petioles into extraction bags and homogenized adding 5ml of Food Lysis Buffer, using available equipments (Polytron, Homex, etc); 1 ml of sap is incubate for 30 min at 60°C and after on ice for several minutes, then centrifuged for 5 min at 2500 x g. From this step, total nucleic acids are purified following the manufacturer’s instructions (Qiagen) and eluted in a final volume of 100 µl.