EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
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.

Laboratory contact details	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy
Short description of the test	Detection of Xylella fastidiosa in perennial host species by Real time PCR (Harper et al. 2010 erratum 2013)
Date, reference of the validation report	2015-10-22 - 0
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	
If yes, please specify	
Description of the test	
Organism(s)	Xylella fastidiosa(XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves leaf petioles
Plant species tested	Nerium oleander, Olea europaea, Prunus avium, Prunus dulcis
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN

Specify the kit used	DNeasy mericon Food Kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	"Dneasy mericon food kit" (QIAGEN) for total DNA extraction
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	Probe
Other details on the test	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.
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
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%
Standard test(s)	26 obtained positive samples/ 26 expected positive samples
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	

Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	
Specificity value	
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	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
Brief details of the test performance study and its output.It available, link to published article/report	
Other information	
Any other information considered useful	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.

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