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 Francis et al., 2006 modified using SYBR green
Date, reference of the validation report	2015-10-22 - internal report
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?	
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves leaf petioles
Plant species tested	Acacia saligna, Citrus sp., Nerium oleander, Olea europaea, Polygala myrtifolia, Prunus avium, Prunus dulcis, Quercus ilex, Vitis sp.
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
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	
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
Other information	

Other details on the test	For total DNA extraction weer 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.
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Real-time PCR following Francis et al., 2006 modified using SYBR green.
Other information	
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is the 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 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
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	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
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

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