

**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.

<b>Laboratory contact details</b>	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy
<b>Short description of the test</b>	Detection of <i>Xylella fastidiosa</i> in perennial host species by Real time PCR Francis et al., 2006 modified using SYBR green
<b>Date, reference of the validation report</b>	2015-10-22 - internal report
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xylella fastidiosa</i> (XYLEFA)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	DNeasy mericon Food Kit
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Method: Molecular Extraction DNA RNA (2)</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	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.
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPD diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Real-time PCR following Francis et al., 2006 modified using SYBR green.
<b>Other information</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	up to 10 <sup>2</sup> cfu/ml (corresponding to 7 cfu/reaction) using dilutions ranging from 10 <sup>7</sup> 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
<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>Standard test(s)</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>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 test(s)</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>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no