

**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> by real time PCR in samples of oleander plants using the automatized DNA extraction protocol by Promega (Maxwell® RSC PureFood GMO and Authentication Kit AS1600)
<b>Date, reference of the validation report</b>	2019-01-30 - Implementation and validation of rapid diagnostic procedures for <i>Xylella fastidiosa</i> . In: Book of Abstract, 3RD JOINT ANNUAL MEETING POnTE-XF-AXTORS, AJACCIO (FRANCE), 28-30 OCTOBER 2019.
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	XF-ACTORS
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xylella fastidiosa</i> (XYLEFA)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Loconsole et al., 2019
<b>Other information</b>	
<b>Method: Molecular Extraction DNA RNA</b>	

<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Loconsole et al., 2019
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	PROMEGA
<b>Specify the kit used</b>	Maxwell® RSC PureFood GMO and Authentication Kit
Kit used following the manufacturer's instructions?	no the amount of starting plant material is increased: at least 0.5 gr are homogenized with 5 ml of CTAB buffer (Promega) (1:10 w/v)
<b>Other information</b>	
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 4)
<b>Name of the test</b>	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes BSA was not included in the amplification MIX
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<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>	10 <sup>2</sup> cfu/ml
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the</b>	100% (on 3 replicates for each 10-fold serial dilutions from 10 <sup>7</sup> to 10 CFU/ml of bacterial

<b>standard test, see appendix 2 of PM 7/98</b>	suspension spiked in plant sap from healthy plant)
<b>Standard test(s)</b>	CTAB-based extraction protocol and Modified DNeasy® Mericon™ Food Standard Protocol (Qiagen), followed by real time PCR Harper et al., 2010
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100% (3 replicates of plant sap from healthy plant)
<b>Specify the test(s)</b>	CTAB-based extraction protocol and Modified DNeasy® Mericon™ Food Standard Protocol (Qiagen), followed by real time PCR Harper et al., 2010
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% (on 3 replicates for each 10-fold serial dilutions from 10 <sup>7</sup> to 10 CFU/ml of bacterial suspension spiked in plant sap from healthy plant; and 3 replicates of plant sap from healthy plant)
<b>Test performance study</b>	
<b>Test performance study?</b>	no
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">Poster detection</a></li> </ul>

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