

**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>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
<b>Short description of the test</b>	Détection de Xylella fastidiosa par PCR en temps réel sur végétal
<b>Date, reference of the validation report</b>	2019-01-01 - MA039 version 4
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<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>	Xylella fastidiosa(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>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 3)
<b>Is the test modified compared to the reference test</b>	yes Insertion of a step of sonication of the macerate prior to DNA extraction in order to break the X. fastidiosa biofilms
<b>Other information</b>	
<b>Other details on the test</b>	CTAB-based DNA extraction
<b>Method: Molecular Extraction DNA RNA (2)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 3)
<b>Is the test modified compared to the</b>	yes Insertion of a step of sonication of the

<b>reference test</b>	macerate prior to DNA extraction in order to break the <i>X. fastidiosa</i> biofilms
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	BIONOBILE
<b>Specify the kit used</b>	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	
<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>EPPO Diagnostic Protocol name</b>	PM 7/024 <i>Xylella fastidiosa</i> (version 3)
<b>Name of the test</b>	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
<b>Is the test modified compared to the reference test</b>	yes DNA extract added to the PCR mix doubled (4 µL instead of 2 µL)
<b>Other information</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b><i>Xylella fastidiosa</i>(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	<i>Olea europaea</i> : 1.10 <sup>5</sup> bact./mL of macerate (detection rate = 100%) <i>Quercus ilex</i> : 1.10 <sup>4</sup> bact./mL of macerate (detection rate = 100%)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	74 <i>X. fastidiosa</i> strains belonging to <i>X. f. subsp fastidiosa</i> , <i>morus</i> , <i>sandyi</i> , <i>pauca</i> and <i>multiplex</i>
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	29 non-target organism: - 1 <i>Xylophilus ampelinus</i> (CFBP2098) - 2 <i>Xanthomonas arboricola</i> pv. <i>pruni</i> (LSV2574/LSV 2573) - 1 <i>Xanthomonas arboricola</i> pv. <i>juglandis</i> (LSV0862) - 1 <i>Xanthomonas axonopodis</i> pv. <i>citri</i> (LSV2647) - 1 <i>Xanthomonas axonopodis</i> pv. <i>aurantifolia</i> (LSV2680) - 2 <i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i> (LSV1014/LSV3161) - 1 <i>Xanthomonas axonopodis</i> pv. <i>fragariae</i> (LSV3151) - 1 <i>Xanthomonas fragariae</i> (LSV2553) - 1 <i>Xanthomonas hortorum</i> pv. <i>carotae</i> (LSV1776) - 1 <i>Xanthomonas campestris</i> pv. <i>campestris</i> (LSV0455) - 1 <i>Xanthomonas campestris</i> pv. <i>juglandis</i> (LSV1158) - 1 <i>Xanthomonas hortorum</i> pv. <i>hedera</i> (LSV2303) - 1 <i>Xanthomonas translucens</i>

	pv. graminis (LSV0628) - 1 Xanthomonas translucens pv. hordei (LSV0629) - 1 Xanthomonas oryzae pv. oryzae (LSV0865) - 1 Ca. Liberibacter asiaticus - 1 Ca. L. africanus - 6 bactéries saprophytes isolées de Coffea spp. - 4 bactéries saprophytes isolées de Citrus sinensis
<b>Specificity value</b>	No cross reaction
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<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
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">Rapport de caractérisation et de validation d'une méthode d'analyse MA0039 version 4</a></li> </ul>

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