

**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 Dixm�ras, 49044 Angers, France
<b>Short description of the test</b>	detection of Xylella fastidiosa Xylella fastidiosa by Molecular real time PCR in Leaves, Shoots
<b>Date, reference of the validation report</b>	2021-09-16 - Dduplex real-time PCR Ouyang et al., 2013 / Harper et al., 2010 - report version 1
<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>	no
<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 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>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>As or adapted from an IPPC diagnostic protocol</b>	yes
<b>IPPC diagnostic Protocol name</b>	ISPM 27 Annex 25 DP 25: Xylella fastidiosa (version 2018)
<b>Name of the test</b>	QuickPick SML Plant DNA kit (Bio-Nobile)
<b>Is the test modified compared to the reference test</b>	no
<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?	yes
<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>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 4)
<b>Name of the test</b>	Real-time PCR (adapted from Ouyang et al., 2013)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes - Master mix - Addition of BSA - Volume per reaction - PCR program - Duplex real-time PCR with Harper et al., 2010 - Cut-off value of 38
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Duplex - Probe
<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>	With a detection rate of 100% : Polygala myrtifolia : 10 <sup>4</sup> cells/mL Helichrysum italicum : 10 <sup>3</sup> cells/mL Lavandula sp. : 10 <sup>4</sup> cells/mL
<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>	On artificially contaminated sample at the bacterial concentration of 10 <sup>3</sup> cells/mL : Polygala myrtifolia : 94% Helichrysum italicum : 100% Lavandula sp. : 94% On naturally contaminated sample (24 samples - 14 plant species) : 100%
<b>Standard test(s)</b>	Real-time PCR Harper et al., 2010 (MA039v4)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	15 target strains Cf. attached file "Rapport de validation duplex Ouyang"
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	43 non target organisms Cf. attached file "Rapport de validation duplex Ouyang"

<b>Specificity value</b>	100%
<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>	Real-time PCR Harper et al., 2010 (MA039v4)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not evaluated
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Matrix Bacterial concentration (cells/mL) Repeatability Polygala myrtifolia 10 <sup>5</sup> 100% 10 <sup>4</sup> 100% 10 <sup>3</sup> 89% Helichrysum italicum 10 <sup>5</sup> 100% 10 <sup>4</sup> 100% 10 <sup>3</sup> 100% Lavandula sp. 10 <sup>5</sup> 100% 10 <sup>4</sup> 100% 10 <sup>3</sup> 89%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	This test based on Ouyang et al., 2013 is complementary to the real-time PCR Harper et al., 2010 in order to confirm positive results as their genomic targets are different
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">Rapport de validation duplex Ouyang</a></li> </ul>

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