

**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, 49000 Angers, France
<b>Short description of the test</b>	Detection of <i>Xylella fastidiosa</i> and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the CTAB DNA extraction method and the Harper real time PCR (Harper et al., 2010)
<b>Date, reference of the validation report</b>	2024-09-13 - 23-XfDORM
<b>Link to other validation data</b>	<ul style="list-style-type: none"> <li>- 23-XfDORM Detection of <i>Xylella fastidiosa</i> and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the Quickpick SML Plant DNA extraction kit and the tetraplex Dupas real time PCR (Dupas et al., 2019)</li> <li>- 23-XfDORM Detection of <i>Xylella fastidiosa</i> and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the Quickpick SML Plant DNA extraction kit and the Harper real time PCR (Harper et al., 2010)</li> <li>- 23-XfDORM Detection of <i>Xylella fastidiosa</i> and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the CTAB DNA extraction and the Hodgetts simplex real time PCR (Xfp) (Hodgetts et al., 2021)</li> <li>- 23-XfDORM Detection of <i>Xylella fastidiosa</i> and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the CTAB DNA extraction and the Hodgetts simplex real time PCR (Xfm) (Hodgetts et al., 2021)</li> <li>- 23-XfDORM Detection of <i>Xylella fastidiosa</i> and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the DNeasy Plant Mini Kit for DNA extraction and the Harper real time PCR (Harper et al., 2010)</li> <li>- 23-XfDORM Detection of <i>Xylella fastidiosa</i> and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the Quickpick SML plant DNA extraction kit and the Hodgetts simplex real time PCR (Xfp) (Hodgetts et al., 2021)</li> <li>- 23-XfDORM Detection of <i>Xylella fastidiosa</i> and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the Quickpick SML plant DNA extraction kit and the Hodgetts simplex real time</li> </ul>

	PCR (Xff) (Hodgetts et al., 2021) - 23-XfDORM Detection of Xylella fastidiosa and identification of subspecies in naturally infected and not infected dormant plant samples (woody cuttings) using the CTAB DNA extraction and the Hodgetts simplex real time PCR (Xff) (Hodgetts et al., 2021)
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Euphresco
<b>If yes, please specify</b>	Euphresco 2022-A-406
<b>Description of the test</b>	
<b>Organism(s)</b>	Xylella fastidiosa (XYLEFA)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Woody cuttings dormant woody twigs naturally infected with X. fastidiosa and not infected
<b>Plant species tested</b>	Prunus domestica, Prunus dulcis, Vitis vinifera
<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 5)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	CTAB DNA extraction
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic</b>	yes

<b>protocol</b>	
<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 5)
<b>Name of the test</b>	Real-time PCR (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>	no
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Other details on the test</b>	Reagent: TaqMan Fast Universal PCR Master Mix (Applied Biosystems)
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<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>	98.6% (evaluated with 9 samples, 7 positive and 2 negative samples, on 4 replicates, tested by 10 laboratories)
<b>Standard test(s)</b>	samples of known status
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100% (evaluated with 9 samples, 7 positive and 2 negative samples, on 4 replicates, tested by 10 laboratories)
<b>Specify the test(s)</b>	samples of known status
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	97.8% (evaluated with 9 samples, 7 positive and 2 negative samples, on 4 replicates, tested by 10 laboratories on different days and with 5 different PCR equipments)
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	97.1% (evaluated with 9 samples, 7 positive and 2 negative samples, on 4 replicates, tested by 10 laboratories)
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output.It available, link to published article/report</b>	Test Performance Study organized in the framework of the Euphresco project 2022-A-406 involving 14 laboratories from 10 countries to evaluate the performance of several molecular protocols for the detection of Xylella fastidiosa and identification of subspecies in naturally infected

	dormant plant samples.
The following complementary files are available online:	<ul style="list-style-type: none"><li>• <a href="#">TPS report</a></li><li>• <a href="#">Annex I</a></li></ul>



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