

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.

Laboratory contact details	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
Short description of the test	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)
Date, reference of the validation report	2024-09-13 - 23-XfDORM
Link to other validation data	<ul style="list-style-type: none"> - 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) - 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) - 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) - 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) - 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) - 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) - 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 (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)
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	Euphresco
If yes, please specify	Euphresco 2022-A-406
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 5)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Other details on the test	CTAB DNA extraction
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no

EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 5)
Name of the test	Real-time PCR (Harper et al., 2010; erratum 2013)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Probe
Other details on the test	Reagent: TaqMan Fast Universal PCR Master Mix (Applied Biosystems)
Performance Criteria :	
Organism 1.:	Xylella fastidiosa(XYLEFA)
<u>Diagnostic sensitivity</u>	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	98.6% (evaluated with 9 samples, 7 positive and 2 negative samples, on 4 replicates, tested by 10 laboratories)
Standard test(s)	samples of known status
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% (evaluated with 9 samples, 7 positive and 2 negative samples, on 4 replicates, tested by 10 laboratories)
Specify the test(s)	samples of known status
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	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)
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	97.1% (evaluated with 9 samples, 7 positive and 2 negative samples, on 4 replicates, tested by 10 laboratories)
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	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">• TPS report• Annex I
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