

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	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	identification of <i>Xylella fastidiosa</i> subspecies by real time PCR of Hodgetts et al., 2021
Date, reference of the validation report	2023-09-22 - EURL_BAC_TPS_2023_01_Xf
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	EURL
If yes, please specify	TPS EURL_BAC_TPS_2023_01_Xf: Molecular detection and subspecies determination of <i>Xylella fastidiosa</i> by real-time PCR (Dupas et al., 2019, Hodgetts et al., 2021)
Description of the test	
Organism(s)	<i>Xylella fastidiosa</i> (XYLEFA)
Detection / identification	identification
Method(s)	Molecular real time PCR
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 <i>Xylella fastidiosa</i> (version 5)
Name of the test	Real-time PCR test (Hodgetts et al., 2021)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes Use total volume of 18 ul, similar as in paper of Hodgetts et al (2021). PM 7/24(5) uses 20 ul.
Kit	
Is a kit used	no

Other information	
Reaction type	Simplex
Other details on the test	The Hodgetts real-time PCR is a set of five simplex real-time PCR reactions, one reaction for each of the <i>X. fastidiosa</i> subspecies (<i>fastidiosa</i> , <i>multiplex</i> , <i>pauca</i> , <i>sandyi</i> and <i>morus</i>)
Performance Criteria :	
Organism 1.:	<i>Xylella fastidiosa</i>(XYLEFA)
<u>Analytical sensitivity</u>	
What is smallest amount of target that can be detected reliably?	Not determined
<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	For the different primer/probe sets: XFF: 100% XFM: 100% XFP: 100% XFS: 100%
Standard test(s)	Samples were spiked with strains with known subspecies
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	For the different primer/probe sets: XFF: 100% XFM: 100% XFP: 100% XFS: 100%
Specify the test(s)	Samples were spiked with known strains, Two strains <i>Xf</i> subsp. <i>fastidiosa</i> (CFBP 8071/LMG 15099 and CFBP 7969/LMG 15553) Two strains <i>Xf</i> subsp. <i>multiplex</i> (LMG 9063 and CFBP 8430) One strain <i>Xf</i> subsp. <i>pauca</i> (NCPBB 4595) One strain <i>Xf</i> subsp. <i>sandyi</i> (NCPBB 460) One strain of <i>X. taiwanensis</i> (NCPBB 4612) One strain of <i>Xanthomonas citri</i> pv <i>citri</i> (NCPBB 409)
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Reproducibility was calculated per primer/probe set as concordance (see PM 7/122(2)). XFF: 100% XFM 100% XFP: 100% XFS: 100%
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability was calculated per primer/probe set as accordance (see PM 7/122(2)). XFF: 100% XFM 100% XFP: 100% XFS: 100%
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 performed by the EURL for pests on plants on bacteria in 2023, evaluating the use of the real-time PCRs of Dupas et al. (2019) and Hodgetts et al (2021) for <i>Xylella fastidiosa</i> subspecies determination. The full report of the Test Performance Study can be found on the website of the EURL: https://eurlplanthealth.nl/

