

**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>	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
<b>Short description of the test</b>	identification of <i>Xylella fastidiosa</i> subspecies by real time PCR of Hodgetts et al., 2021
<b>Date, reference of the validation report</b>	2023-09-22 - EURL_BAC_TPS_2023_01_Xf
<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>	EURL
<b>If yes, please specify</b>	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)
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xylella fastidiosa</i> (XYLEFA)
<b>Detection / identification</b>	identification
<b>Method(s)</b>	Molecular real time PCR
<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>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/24 <i>Xylella fastidiosa</i> (version 5)
<b>Name of the test</b>	Real-time PCR test (Hodgetts et al., 2021)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes Use total volume of 18 ul, similar as in paper of Hodgetts et al (2021). PM 7/24(5) uses 20 ul.
<b>Kit</b>	
<b>Is a kit used</b>	no

<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	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> )
<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>	Not determined
<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>	For the different primer/probe sets: XFF: 100% XFM: 100% XFP: 100% XFS: 100%
<b>Standard test(s)</b>	Samples were spiked with strains with known subspecies
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	For the different primer/probe sets: XFF: 100% XFM: 100% XFP: 100% XFS: 100%
<b>Specify the test(s)</b>	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>Xanthomanos citri</i> pv <i>citri</i> (NCPBB 409)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Reproducibility was calculated per primer/probe set as concordance (see PM 7/122(2)). XFF: 100% XFM 100% XFP: 100% XFS: 100%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Repeatability was calculated per primer/probe set as accordance (see PM 7/122(2)). XFF: 100% XFM 100% XFP: 100% XFS: 100%
<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 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: <a href="https://eurlplanthealth.nl/">https://eurlplanthealth.nl/</a>

