

**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 Dupas et al, 2019
<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>	Tetraplex real-time PCR (Dupas et al., 2019)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes Used Cy5.5 as phluorescent probe on XFM-P instead of ROX
<b>Kit</b>	
<b>Is a kit used</b>	no

<b>Other information</b>	
<b>Reaction type</b>	Multiplex (>3)
<b>Other details on the test</b>	A combination of a multiplex with XF, XFSL, XFM and XFP, separate PCRs for XFMO and XFF
<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>	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: XF: 97% XFSL: 99% XFF: 100% XFM: 97% XFP: 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: XF: 100% XFSL: 96% XFF: 100% XFM: 99% XFMO:99% XFP: 98%
<b>Specify the test(s)</b>	Samples were spiked with known strains, Two strains Xf subsp. fastidiosa (CFBP 8071/LMG 15099 and CFBP 7969/LMG 15553) Two strains Xf subsp. multiplex (LMG 9063 and CFBP 8430) One strain Xf. subsp. pauca (NCPBB 4595) One strain Xf. subsp. sandyi (NCPBB 460) One strain of X. taiwanensis (NCPBB 4612) One strain of Xanthomonas citri pv citri (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)) XF: 88% XFSL:90% XFF: 95% XFM: 90% XFP: 90%
<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)) XF: 92% XFSL:93% XFF: 96% XFM: 91% XFP: 90%
<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 Xylella fastidiosa 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>