

**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>	Detection and identification of <i>Xylella fastidiosa</i> by real time PCR in extract of the midvein (midrib) and leaf stalk
<b>Date, reference of the validation report</b>	2019-09-02 - 2018molbio006
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xylella fastidiosa</i> (XYLEFA)
<b>Detection / identification</b>	detection and 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>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 <i>Xylella fastidiosa</i> (version 4)
<b>Name of the test</b>	Real-time PCR (adapted from Ouyang et al., 2013)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes Duration of initial denaturing step shortened to 2 minutes
<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>	Reaction mixtures used: (1) Premix Ex Taq (perfect real-time) (TaKaRa) (2) PerfeCTa qPCR ToughMix (Quanta Biosciences)
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	<60 copies of the genome per real-time PCR reaction
<b><u>Diagnostic sensitivity</u></b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	100%
<b>Standard test(s)</b>	real-time PCR of Harper et al. 2010
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	100%
<b>Specificity value</b>	40 target isolates: Xylella fastidiosa pathovars fastidiosa, multiplex, pauca, sandyi and unknown
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	94 non-target isolates, amongst them Pseudomonas syringae pathovars aesculi, coronofaciens, theae, ciccaronei, syringae, savastanoi, primulae, morsprunorum, persicae, ribicola, ulmi, viburni, and unknown; Xanthomonas axonopodis pathovars dieffenbachiae, begoniae, citri, corylina, maculifoliigardeniae, juglandis, phaseoli, pruni, and unknown; Xanthomonas campestris pathovars vesicatoria, carotae, campestris, hyacinti, hederiae, poinsetticola, raphani, lobeliae, gramini, pelargonii, bilvae, armoraciae and unknown; Xanthomonas citri subsp. citri and aurantifolii; two unknown endophytes of Coffea arabica; Xanthomonas cucurbitae; Xanthomonas sp. pv. euphorbiae; Xanthomonas sp. pv. fici; Xanthomonas fragariae; Erwinia pyrifoliae; Xanthomonas hortorum; Xanthomonas populi; Burkholderia gladioli; Brenneria quercina; Xanthomonas perforans; Xylophilus ampelinus; Sphingomonas melonis; Xylella taiwanensis
<b>Specificity value</b>	100%
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100%
<b>Specify the test(s)</b>	real-time PCR of Harper et al. 2010
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a</b>	100%

<b>given level of the pest (see PM 7/98)</b>	
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">Validation report real-time PCR Xylella fastidiosa (Dutch)</a></li> </ul>

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