

**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>	National Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
<b>Short description of the test</b>	Detection of <i>Xylella fastidiosa</i> by real-time PCR (Francis et al., 2006) in plant material
<b>Date, reference of the validation report</b>	2018-06-14 - Dreo, Tanja, 2018. qPCR for detection of <i>Xylella fastidiosa</i> based on Francis et al., 2006, EJPP 115, 203-213: Review of existing validation data, modification of test and in silico analysis (No. D0013/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018. qPCR for detection of <i>Xylella fastidiosa</i> based on Francis et al., 2006, EJPP 115, 203-213: Diagnostic specificity and sensitivity determined in spiked samples (PKIe) (No. D0014/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018. qPCR for detection of <i>Xylella fastidiosa</i> based on Francis et al., 2006, EJPP 115, 203-213: Analytical sensitivity – standard curves (No. D0015/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018 qPCR for detection of <i>Xylella fastidiosa</i> based on Schaad et al. (2002), Francis et al. (2006), Harper et al., 2010, erratum 2013: Analytical specificity (No. D0027/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana.
<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
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR

<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 3)
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	BIONOBILE
<b>Specify the kit used</b>	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<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>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 3)
<b>Name of the test</b>	Taqman real-time PCR tests (based on Francis et al., 2006)
<b>Is the test modified compared to the reference test</b>	yes See attached document
<b>Other information</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	no
<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>	DNA: In total 1000 target copies per mL extracted DNA (log 3 cps/mL as determined with digital PCR) were reliably detected in several X. fastidiosa strains, NIB Z 1962 (X. fastidiosa subsp. multiplex, LMG 9063), NIB Z 1963 (X. fastidiosa subsp. fastidiosa from almond, LMG 15099) and CoDiRo strain. Standard curves in plant material: Concentrations from $5 \times 10^4$ to down to $5 \times 10^3$ to (target cps/mL) can be reliably detected in samples of olives ( $10^4$ ), oleander ( $5 \times 10^3$ ), rosemary ( $10^4$ ) and lavender ( $5 \times 10^4$ ) plants tested for latent infection. Spiked PKIe controls: 98 % analytical sensitivity for symptomatic samples (111 different samples of 27 different genera were tested) and 100% analytical sensitivity for asymptomatic samples (66 different samples of 20 different genera were tested).
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples</b>	No data available.

<b>tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	3
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	90
<b>Specificity value</b>	No cross reactivity.
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	No data available.
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Repeatability</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="#">D0013_18_qPCR_Xyf_Francis_2006_ModificationInSilico</a></li> <li>• <a href="#">D0014_18_qPCR_Xyf_Francis_2006_DiagnosticSensitivityPKle</a></li> <li>• <a href="#">D0015_18_qPCR_Xyf_Francis_2006_AnalyticalSensitivity_SCs</a></li> <li>• <a href="#">D0027_qPCR_Xyf_HarperSchaadFrancis_AnalyticalSpecificity</a></li> </ul>

Creation date: 2018-10-05 00:00:00 - Last update: 2021-01-22 10:01:05