

**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 (Harper et al., 2010, erratum 2013) in plant material
<b>Date, reference of the validation report</b>	2018-09-17 - 174 ; Dreo, Tanja, 2018. qPCR for detection of <i>Xylella fastidiosa</i> based on Harper et al., 2010, erratum 2013: Literature review and modification of test (No. D0023/18). 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 Harper et al., 2010, erratum 2013: Analytical sensitivity - standard curves (No. D0024/18). 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 Harper et al., 2010, erratum 2013: Diagnostic specificity and sensitivity determined in spiked samples (PKle) (No. D0025/18). 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>	
<i>Reference of the test description</i>	
<b>As or adapted from an EPP0 diagnostic protocol</b>	yes
<b>EPP0 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>	
<i>Reference of the test description</i>	
<b>As or adapted from an EPP0 diagnostic protocol</b>	yes
<b>EPP0 Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 3)
<b>Name of the test</b>	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
<b>Is the test modified compared to the reference test</b>	yes
<b>Other information</b>	
<b>Other details on the test</b>	Modified real-time PCR adapted from Harper, S.J., Ward, L.I., Clover, G.R.G., 2010. Development of LAMP and real-time PCR methods for the rapid detection of Xylella fastidiosa for quarantine and field applications. Phytopathology 100, 1282-1288. <a href="https://doi.org/10.1094/PHYTO-06-10-0168">https://doi.org/10.1094/PHYTO-06-10-0168</a>
<b>Are the performance characteristics included in the EPP0 diagnostic protocol?</b>	<b>no</b>
<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: 500 target copies per mL extracted DNA (log 2,7 cps/mL as determined with digital PCR) were reliably (min. 2/3 parallel reactions positive) with real-time PCR when testing NIB Z 1963 (X. fastidiosa subsp. fastidiosa from almond, LMG 15099), 1000 target copies per mL extracted DNA (log 3,0 cps/mL as determined with digital PCR) were reliably (min. 2/3 parallel reactions positive) detected with real time PCR in when testing CoDiRo strain and 5000 target copies per mL extracted DNA (log 3,7 cps/mL as determined with digital PCR) were reliably (min. 2/3 parallel reactions positive) detected with real time PCR when testing NIB Z 1962 (X. fastidiosa subsp. multiplex)

	Standard curves in plant material: Concentrations from $10^4$ to down to $10^3$ to (target cps/mL) can be reliably detected in samples of olives ( $10^4$ ), oleander ( $10^3$ ), rosemary ( $10^3$ ) and lavender ( $5 \times 10^3$ ) plants tested for latent infection. Spiked PKIe controls: 100 % analytical sensitivity (139 different symptomatic samples of 27 different genera and 77 asymptomatic (latent) samples of 22 different genera were tested).
<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>	No data available.
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	3
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	90
<b>Specificity value</b>	No cross reactivity.
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	No data available.
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<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><u>Test performance study</u></b>	
<b>Test performance study?</b>	no
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">D0024_18_Xyf_Harper_Analytical_sensitivity_standard_curves</a></li> <li>• <a href="#">D0025_18_qPCR_Xyf_Harper_PKIe</a></li> <li>• <a href="#">D0023_18_qPCR_Xyf_Harper_LitRev</a></li> <li>• <a href="#">D0027_qPCR_Xyf_HarperSchaadFrancis_AnalyticalSpecificity</a></li> </ul>

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