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.

Laboratory contact details	National Institiute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
Short description of the test	Detection of Xylella fastidiosa by real-time PCR (Harper et al., 2010, erratum 2013) in plant material
Date, reference of the validation report	2018-09-17 - 174 ; Dreo, Tanja, 2018. qPCR for detection of Xylella fastidiosa 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 Xylella fastidiosa 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 Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Diagnostic specificity and sensitivity determined in spiked samples (PKIe) (No. D0025/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018 qPCR for detection of Xylella fastidiosa 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.
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR

Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Kit	
Is a kit used	yes
Manufacturer name	BIONOBILE
Specify the kit used	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	
Other information	
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Name of the test	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
Is the test modified compared to the reference test	yes
Other information	
Other details on the test	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. https://doi.org/10.1094/PHYTO-06-10-0168
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	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 (5x10^3) 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).
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	No data available.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	3
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	90
Specificity value	No cross reactivity.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	No data available.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	no
The following complementary files are available online:	• D0024_18_Xyf_Harper_Analytical sensitivity standard curves • D0025_18 _qPCR_Xyf_Harper_PKIe • D0023_18_qPCR_Xyf_Harper_LitRev • D0027_qPCR_Xyf_HarperSchaadFrancis_AnalyticalSpecificity

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