

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 Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 111, 1000 Ljubljana, Slovenia
Short description of the test	Detection of <i>Xylella fastidiosa</i> by real-time PCR (Schaad et al., 2002) in plant material
Date, reference of the validation report	2018-06-14 - 172 ; Dreo, Tanja, 2018. qPCR for detection of <i>Xylella fastidiosa</i> based on Schaad et al., <i>Phytopathology</i> , 2002, 92 (7): 721-728: Review of existing validation data, modification of test and in silico analysis. (No. D0008/18). 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., <i>Phytopathology</i> , 2002, 92 (7): 721-728: Diagnostic specificity and sensitivity determined in spiked samples (PKIe) (No. D0009/18). 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., <i>Phytopathology</i> , 2002, 92 (7): 721-728: Analytical sensitivity - standard curves (No. D0010/18). 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.
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?	
If yes, please specify	
Description of the test	
Organism(s)	<i>Xylella fastidiosa</i> (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves, Shoots Plant material (leaf veins and

	petioles, vascular tissue [xylem] from shoots)
Plant species tested	Acacia sp., Acer sp., Asparagus sp., Callistemon sp., Citrus sp., Coffea sp., Cytisus sp., Ficus sp., Ginkgo sp., Grevillea sp., Hebe sp., Hedera sp., Heliotropium sp., Hydrangea sp., Juglans sp., Laurus sp., Lavandula sp., Lonicera sp., Morus sp., Myrtus sp., Nerium sp., Olea sp., Origanum sp., Polygala sp., Prunus sp., Quercus sp., Rhamnus sp., Rosa sp., Rubus sp., Salvia, Spartium sp., Vinca sp., Vitis sp.
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
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Name of the test	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
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	
Other details on the test	
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Schaad, N. W., Opgenorth, D., Gaush, P. 2002. Real-Time Polymerase Chain Reaction for One-Hour On-Site Diagnosis of Pierce's Disease of Grape in Early Season Asymptomatic Vines. Phytopathology 2002 92:7, 721-728.

Is the test modified compared to the reference test	yes
Kit	
Is a kit used	
Other information	
Reaction type	
Other details on the test	
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: In total 500 target copies per mL extracted DNA (log 2,1 cps/mL as determined with digital PCR) were reliably detected (minimum 2/3 parallel reactions) 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. Concentration of 10 ³ cps/mL was reliably detected in all three tested strains. Standard curves in plant material: Concentrations from 5x10 ⁴ to down to 10 ³ to (target cps/mL) can be reliably detected in samples of olives (10 ⁴), oleander (10 ³), rosemary (5x10 ³) and lavender (5x10 ⁴) plants tested for latent infection. Spiked PKle controls: 100 % analytical sensitivity (111 different symptomatic samples of 27 different genera and 66 asymptomatic (latent) samples of 20 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.
Standard test(s)	
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	Xanthomonas campestris pv. citri (even with high concentrations as tested there was only one reaction positive out of two tested (Cq 37.5))
Cross reacts with	Xanthomonas campestris pv. citri

Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	No data available.
Specify the test(s)	
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
Brief details of the test performance study and its output. It available, link to published article/report	
Other information	
Any other information considered useful	
The following complementary files are available online:	<ul style="list-style-type: none"> • D0010_18_qPCR_Xyf_Schaad_2002_AnalyticalSensitivity_SCs • D0009_18_qPCR_Xyf_Schaad_2002_DiagnosticSensitivityPKle • D0008_18_qPCR_Xyf_Schaad_2002_ModificationInSilico • D0027_qPCR_Xyf_HarperSchaadFrancis_AnalyticalSpecificity

Creation date: 2018-10-05 00:00:00 - Last update: 2021-01-22 09:48:02