EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

Target Organism	Xylella fastidiosa
Short description	Detection of Xylella fastidiosa by real-time PCR (Schaad et al., 2002) in plant material
Laboratory contact details	National Institiute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
Date and reference of the validation report	2018-06-14 - Dreo, Tanja, 2018. qPCR for detection of Xylella fastidiosa based on Schaad et al., Phytopathology, 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 Xylella fastidiosa based on Schaad et al., Phytopathology, 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 Xylella fastidiosa based on Schaad et al., Phytopathology, 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 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 PM 7/98:	Yes
Reference of the test description	N/R 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 the same as described in the EPPO DP?	No
Is the lab accredited for this test?	Yes
Plant species tested (if relevant)	Genera: Acacia, Acer, Asparagus, Callistemon, Citrus, Coffea, Cytisus, Ficus, Ginko, Grevillea, Hebe, Hedera, Heliotropium, Hydrangea, Juglans, Laurus, Lavandula, Lonicera, Morus, Myrtus, Origanum, Nerium, Olea, Polygala, Prunus, Quercus, Rhamnus, Rosa, Rosmarinus, Rubus, Spartium, Vinca, and Vitis

Matrices tested (if relevant)	Plant material (leaf veins and petioles, vascular tissue [xylem] from shoots)	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	DNA extraction from plant material using QuickPick™ SML Plant DNA kit (Bionobile). Modified real-time PCR adapted from 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.
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
<u>Analytical sensitivity (= limit of detection)</u>		
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 103 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 PKIe controls: 100 % analytical sensitivity (111 different symptomatic samples of 27 different genera and 66 asymptomatic (latent) samples of 20 different genera were tested).	
<u>Diagnostic sensitivity</u>		

Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	No data available.
Specify the standard test	
Analytical specificity	
Specificity value	99%
Number of strains/populations of target organisms tested	3
Number of non-target organisms tested	90
Cross reacts with (specify the species)	Xanthomonas campestris pv. citri (even with high concentrations as tested there was only one reaction positive out of two tested (Cq 37.5))
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	No data available.
Specify the standard test	
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
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	
The following complementary files are available online:	• qPCR for detection of Xylella fastidiosa based on Schaad et al. (2000), Francis et al. (2006), Harper et al., 2010, erratum 2013: Analytical specificity (No. D0027/18) • qPCR for detection of Xylella fastidiosa based on Schaad et al., Phytopathology, 2002, 92 (7): 721-728: Analytical sensitivity – standard curves (No. D0010/18)

	• qPCR for detection of Xylella fastidiosa based on Schaad et al., Phytopathology, 2002, 92 (7): 721-728: Diagnostic specificity and sensitivity determined in spiked samples (PKIe) (No. D0009/18) • qPCR for detection of Xylella fastidiosa based on Schaad et al., Phytopathology, 2002, 92 (7): 721-728: Review of existing validation data, modification of test and in silico analysis (No. D0008/18)