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 (Francis et al., 2006) 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 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 Xylella fastidiosa 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 Xylella fastidiosa 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 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	0 EPPO PM 7/24 (3) - Appendix 6 - Real time test (based on Francis et al., 2006) Taqman version
Is the test the same as described in the EPPO DP?	Yes
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 Francis, M., Lin, H., Rosa, J.C.-L., Doddapaneni, H., and Civerolo, E.L. (2006). Genome-based PCR Primers for Specific and Sensitive Detection and Quantification of Xylella fastidiosa. European Journal of Plant Pathology 115, 203–213.
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 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 5x10^4 to down to 5x10^3 to (target cps/mL) can be reliably detected in samples of olives (10^4), oleander (5x10^3), rosemary (10^4) and lavender (5x10^4) 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).	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared	No data available.	

to results from the standard test , see appendix 2 of PM 7/98	
Specify the standard test	
Analytical specificity	
Specificity value	100%
Number of strains/populations of target organisms tested	3
Number of non-target organisms tested	90
Cross reacts with (specify the species)	No cross reactivity.
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 Francis et al., 2006, EJPP 115, 203–213: Analytical sensitivity – standard curves (No. D0015/18) • qPCR for detection of Xylella fastidiosa based on Francis et al., 2006, EJPP 115, 203–213: Diagnostic specificity and sensitivity determined in spiked samples (PKIe) (No. D0014/18) • qPCR for detection of Xylella fastidiosa 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) • 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)