

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 (Harper et al., 2010, erratum 2013) in plant material
Laboratory contact details	National Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 111, 1000 Ljubljana, Slovenia
Date and reference of the validation report	2018-09-17 - 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 (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 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 5 - Real time PCR (Harper et al., 2010; erratum 2013)
Is the test the same as described in the EPPO DP?	Modified For modification see report 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).
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, Helichrysum, Hydrangea, Juglans, Laurus, Lavandula, Lonicera, Morus, Myrtus, Origanum, Nerium, Olea, Polygala, Prunus, Quercus, Rhamnus, Rosa, Rosmarinus, Rubus, Spartium, Vaccinium, 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 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>

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

Analytical sensitivity (= limit of detection)

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 (5×10^3) 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).
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.
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:	<ul style="list-style-type: none"> • qPCR for detection of Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Analytical sensitivity - standard curves (No. D0024/18) • qPCR for detection of Xylella fastidiosa based on

[Harper et al., 2010, erratum 2013: Diagnostic specificity and sensitivity determined in spiked samples \(PKIe\) \(No. D0025/18\)](#)

- [qPCR for detection of Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Literature review and modification of test \(No. D0023/18\)](#)
- [qPCR for detection of Xylella fastidiosa based on Schaad et al. \(2000\), Francis et al. \(2006\), Harper et al., 2010, erratum 2013: Analytical specificity \(No. D0027/18\)](#)