

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 121, 1000 Ljubljana, Slovenia
Short description of the test	Detection of <i>Xylella fastidiosa</i> by real-time PCR in plant material
Date, reference of the validation report	2016-02-26 - Dreo, Tanja, 2016. Validation data on the modified real-time PCR for detection of <i>Xylella fastidiosa</i> adapted from Francis et al. (2006) (No. D0002/16). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana.
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	<i>Xylella fastidiosa</i> (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves, Shoots Plant material, mainly mixtures of midribs, petioles and vascular tissues.
Plant species tested	<i>Asparagus acutifolius</i> , <i>Coffea</i> , <i>Lavandula</i> , <i>Nerium oleander</i> , <i>Olea europaea</i> , <i>Polygala myrtifolia</i> , <i>Quercus cerris</i> , <i>Salvia rosmarinus</i> , <i>Spartium junceum</i>
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 <i>Xylella fastidiosa</i> (version 2)
As or adapted from an IPPC diagnostic protocol	no
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	DNA extraction from plant material using QuickPick™ SML Plant DNA kit (Bionobile).
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 2)
Name of the test	Taqman real-time PCR tests (based on Francis et al., 2006)
As or adapted from an IPPC diagnostic protocol	no
Other information	
Reaction type	Simplex - Probe
Other details on the test	Modified real-time PCR adapted from Francis, M., Lin, H., Rosa, J.C.-L., Doddapaneni, H., 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. doi:10.1007/s10658-006-9009-4
Are the performance characteristics included in the EPPO diagnostic protocol?	yes
Performance Criteria :	
Organism 1.:	Xylella fastidiosa(XYLEFA)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	On the DNA extracted from pure cultures of X. fastidiosa: 2.6, 3.2 and 3.5 (log (cells/mL) of Xylella fastidiosa subsp. multiplex, Xylella fastidiosa, and Xylella fastidiosa subsp. pauca CoDiRO strain, respectively. On plant material: 94 % (determined on log 5 cells/mL of plant extracts; the lowest concentration 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	4

Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	15
Specificity value	100% No cross reactions were observed.
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)	0,97
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	No data available.
Test performance study	
Test performance study?	no
The following complementary files are available online:	
	<ul style="list-style-type: none"> • Validation data on the modified real-time PCR for detection of Xylella fastidiosa adapted from Francis et al. (2006) (No. D0002/16)

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