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	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Detection and identification of Xylella fastidiosa by real time PCR in extract of the midvein (midrib) and leaf stalk
Date, reference of the validation report	2019-09-02 - 2018molbio006
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?	no
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection and identification
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
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?	yes
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 4)
Name of the test	Real-time PCR (adapted from Ouyang et al., 2013)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes Duration of initial denaturing step shortened to 2 minutes
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Probe

Other details on the test	Reaction mixtures used: (1) Premix Ex Taq (perfect real-time) (TaKaRa) (2) PerfeCTa qPCR ToughMix (Quanta Biosciences)
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	<60 copies of the genome per real-time PCR reaction
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Standard test(s)	real-time PCR of Harper et al. 2010
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	100%
Specificity value	40 target isolates: Xylella fastidiosa pathovars fastidiosa, multiplex, pauca, sandyi and unknown
Analytical specificity - exclusivity	
Number of non-target organisms tested	94 non-target isolates, amongst them Pseudomonas syringae pathovars aesculi, coronofaciens, theae, ciccaronei, syringae, savastanoi, primulae, morsprunorum, persicae, ribicola, ulmi, viburni, and unknown; Xanthomonas axonopodis pathovars dieffenbachiae, begoniae, citri, corylina, maculifoliigardeniae, juglandis, phaseoli, pruni, and unknown; Xanthomonas campestris pathovars vesicatoria, carotae, campestris, hyacinti, hederae, poinsetticola, raphani, lobeliae, gramini, pelargonii, bilvae, armoraciae and unknown; Xanthomonas citri subsp. citri and aurantifolii; two unknown endophytes of Coffea arabica; Xanthomonas cucurbitae; Xanthomonas sp. pv. euphorbiae; Xanthomonas sp. pv. fici; Xanthomonas fragariae; Erwinia pyrifoliae; Xanthomonas hortorum; Xanthomonas populi; Burkholderia gladioli; Brenneria quercina; Xanthomonas perforans; Xylophilus ampelinus; Sphingomonas melonis; Xylella taiwanensis
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	real-time PCR of Harper et al. 2010
Reproducibility	
Provide the calculated % of agreement for a	100%

given level of the pest (see PM 7/98)	
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
The following complementary files are available online:	• Validation report real-time PCR Xylella fastidiosa (Dutch)

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