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	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49000 Angers, France
Short description of the test	detection of Xylella fastidiosa Xylella fastidiosa by Molecular real time PCR in Leaves, Shoots
Date, reference of the validation report	2021-09-16 - Dduplex real-time PCR Ouyang et al., 2013 / Harper et al., 2010 - report version 1
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves, Shoots HealthypPlants collected in uninfected areas in France
Plant species tested	Helichrysum italicum, Lavandula sp., Polygala myrtifolia
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
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 4)
As or adapted from an IPPC diagnostic protocol	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 25 DP 25: Xylella fastidiosa (version 2018)
Name of the test	QuickPick SML Plant DNA kit (Bio-Nobile)

Is the test modified compared to the reference test	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?	yes
Other information	
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 - Master mix - Addition of BSA - Volume per reaction - PCR program - Duplex real-time PCR with Harper et al., 2010 - Cut-off value of 38
Kit	
Is a kit used	no
Other information	
Reaction type	Duplex - Probe
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	With a detection rate of 100% : Polygala myrtifolia : 10^4 cells/mL Helichrysum italicum : 10^3 cells/mL Lavandula sp. : 10^4 cells/mL
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	On artificially contaminated sample at the bacterial concentration of 10^3 cells/mL : Polygala myrtifolia : 94% Helichrysum italicum : 100% Lavandula sp. : 94% On naturally contaminated sample (24 sampes – 14 plant species) : 100%
Standard test(s)	Real-time PCR Harper et al., 2010 (MA039v4)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	15 target strains Cf. attached file "Rapport de validation duplex Ouyang"

Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	43 non target organisms Cf. attached file "Rapport de validation duplex Ouyang"
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 Harper et al., 2010 (MA039v4)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not evaluated
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Matrix Bacterial concentration (cells/mL) Repeatability Polygala myrtifolia 10^5 100% 10^4 100% 10^3 89% Helichrysum italicum 10^5 100% 10^4 100% 10^3 100% Lavandula sp. 10^5 100% 10^4 100% 10^3 89%
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	This test based on Ouyang et al., 2013 is complementary to the real-time PCR Harper et al., 2010 in order to confirm positive results as their genomic targets are different
The following complementary files are available online:	• Rapport de validation duplex Ouyang

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