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, 49044 Angers, France
Short description of the test	identification of Xylella fastidiosa subspeicies on plant extracts by real time PCR Dupas et al., 2019 (Set N° 5)
Date, reference of the validation report	2021-12-27 - Dupas et al., 2019 report version 01
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	identification
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	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Dupas E., Briand M., Jacques M-A. and Cesbron S. Novel tetraplex qPCR assays for simultaneous detection and identification of Xylella fastidiosa subspecies in plant tissues, Frontiers in Plant Science, 2019 ; Volume 10.
Is the test modified compared to the reference test	yes Volume per reaction (20 µL instsead of 10 µL)
Kit	
Is a kit used	no
Other information	
Reaction type	Multiplex (>3) - Probe
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	With a detection rate of 100% XF Harper with set N°5 (on Polygala myrtifolia, Lavandula sp. and Helichrysum italicum) = 10^5 cells/mL XFM with set N°5 = Not tested XFP with set N°5 = Not tested XFFSL with set N°5 (on Polygala myrtifolia, Lavandula sp. and Helichrysum italicum) = 10^5 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	XF Harper with set N°5 = 92% (on pure DNA extract) – 100% (on 10 fold diluted DNA for 1 sample. /12) XFM with set N°5 = 90% XFP with set N°5 = 100% XFFSL with set N°5 = 100%
Standard test(s)	MLST based on the conventional PCR Yuan et al., 2010
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Strain list is available in the joined report
Specificity value	Set N° 5 Primers/probes: • XF-ED : NA • XFSSL :

	100% • XFM : 100% • XFP : 100% • XFF : NA • XFMO : NA • XF Harper : Not evaluated • 18S Uni : NA
Analytical specificity - exclusivity	
Number of non-target organisms tested	Strain list is available in the joined report
Specificity value	Set N° 5 Primers/probes: • XF-ED : NA • XFSSL : 100% • XFM : 100% • XFP : 100% • XFF : NA • XFMO : NA • XF Harper : Not evaluated • 18S Uni : NA
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	XF Harper with set N°5 = 100% XFM with set N°5 = 100% XFP with set N°5 = 100% XFFSL with set N°5 = 100%
Specify the test(s)	MLST based on the conventional PCR Yuan et al., 2010
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	On samples spiked with a bacterial concentration ≥ to the limit of detection XF Harper with set N°5 = 100% XFM with set N°5 = 100% XFP with set N°5 = 100% XFFSL with set N°5 =100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	On samples spiked with a bacterial concentration ≥ to the limit of detection XF Harper with set N°5 = 100% XFM with set N°5 = 100% XFP with set N°5 = 100% XFFSL with set N°5 = 100%
Test performance study	
Test performance study?	no
The following complementary files are available online:	• Validation report Dupas v1

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