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	Détection de Xylella fastidiosa par PCR en temps réel sur végétal
Date, reference of the validation report	2019-01-01 - MA039 version 4
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?	
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves Petioles
Plant species tested	Olea europaea, Quercus ilex
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) 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 Xylella fastidiosa (version 3)
Is the test modified compared to the reference test	yes Insertion of a step of sonication of the macerate prior to DNA extraction in order to break the X. fastidiosa biofilms
Other information	
Other details on the test	CTAB-based DNA extraction
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes

EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Is the test modified compared to the reference test	yes Insertion of a step of sonication of the macerate prior to DNA extraction in order to break the X. fastidiosa biofilms
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	
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 3)
Name of the test	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
Is the test modified compared to the reference test	yes DNA extract added to the PCR mix doubled (4 µL instead of 2 µL)
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Olea europaea: 1.10^5 bact./mL of macerate (detection rate = 100%) Quercus ilex: 1.10^4 bact./mL of macerate (detection rate = 100%)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	74 X. fastidiosa strains belonging to X. f. subsp fastidosa, morus, sandyi, pauca and multiplex
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	29 non-target organism: - 1 Xylophilus ampelinus (CFBP2098) - 2 Xanthomonas arboricola pv. pruni (LSV2574/LSV 2573) - 1 Xanthomonas arboricola pv. juglandis (LSV0862) - 1 Xanthomonas axonopodis pv. citri (LSV2647) - 1 Xanthomonas axonopodis pv. aurantifolia (LSV2680) - 2 Xanthomonas axonopodis pv. phaseoli (LSV1014/LSV3161) - 1 Xanthomonas axonopodis pv. fragariae (LSV3151) - 1 Xanthomonas fragariae (LSV2553) - 1 Xanthomonas hortorum pv. carotae (LSV1776) - 1 Xanthomonas campestris pv.

	campestris (LSV0455) - 1 Xanthomonas campestris pv. juglandis (LSV1158) - 1 Xanthomonas hortorum pv. hedera (LSV2303) - 1 Xanthomonas translucens pv. graminis (LSV0628) - 1 Xanthomonas translucens pv. hordei (LSV0629) - 1 Xanthomonas oryzae pv. oryzae (LSV0865) - 1 Ca. Liberibacter asiaticus - 1 Ca. L. africanus - 6 bactéries saprophytes isolées de Coffea spp. - 4 bactéries saprophytes isolées de Citrus sinensis
Specificity value	No cross reaction
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
The following complementary files are available online:	• Rapport de caractérisation et de validation d'une méthode d'analyse MA0039 version 4

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