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	Xylella fastidiosa detection by duplex real-time PCR Harper et al., 2010 / loos et al., 2009 on Olea europaea and Quercus spp. after DNA extraction with Maxwell® HT Environmental TNA kit (Promega) and KingFisher™ Flex automate (French official method MA 039 v6)
Date, reference of the validation report	2023-02-01 - Xylella fastidiosa detcection by duplex real-time PCR – DNA extraction on Olea europaea and Quercus ilex with Maxwell® HT Environmental TNA kit (Promega)
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
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	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	No scientific publication. French official method MA 039 v6
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	yes
Manufacturer name	PROMEGA
Specify the kit used	Maxwell® HT Environmental TNA kit
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 - duplex (Harper et al., 2010; erratum 2013)
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	Real-time PCR Harper et al., 2010
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	yes
Manufacturer name	Applied Biosystems
Specify the kit used	TaqMan™ Fast Universal Master Mix (2X), no AmpErase™ UNG
Kit used following the manufacturer's instructions?	yes
Other information	
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%: Olea europaea : 10^4 cells/mL Quercus ilex : 10^3 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	Olea europaea : 153% Quercus ilex : 111%
Standard test(s)	CTAB based DNA extraction protocol (PM7/24(4)) Simplex real-time PCR Harper et al., 2010

Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	LNPV 00.54 Xylella fastidiosa subsp. multiplex ST41 LNPV 00.56 Xylella fastidiosa subsp. fastidiosa ST1 LNPV 24.34 Xylella fastidiosa subsp. fastidiosa ST2 LSV 40.38 Xylella fastidiosa subsp. multiplex ST10 LSV 42.09 Xylella fastidiosa subsp. proche fastidiosa ST75 LSV 42.10 Xylella fastidiosa subsp. proche pauca ST74 LSV 42.30 Xylella fastidiosa subsp. multiplex ST27 LSV 42.31 Xylella fastidiosa subsp. multiplex ST9 LSV 42.36 Xylella fastidiosa subsp. sandyi ST5 LSV 46.27 Xylella fastidiosa subsp. sandyi ST72 LSV 46.63 Xylella fastidiosa subsp. morus ST29 LSV 46.77 Xylella fastidiosa subsp. multiplex ST7 LSV 46.79 Xylella fastidiosa subsp. multiplex ST6 LSV 47.09 Xylella fastidiosa subsp. pauca ST53 LSV 47.29 Xylella fastidiosa subsp. pauca ST53
Specificity value	100% (15 non-target strains)
Analytical specificity - exclusivity	
Number of non-target organisms tested	LNPV 0.78 Pseudomonas syringae pv. morsprunorum LNPV 0.79 Pseudomonas syringae pv. persicae LNPV 04.55 Xanthomonas campestris pv. campestris LNPV 06.28 Xanthomonas translucens pv. graminis LNPV 08.62 Xanthomonas arboricola pv. juglandis LNPV 17.76 Xanthomonas hortorum pv. carotae LNPV 23.03 Xanthomonas hortorum pv. hederae LSV 25.29 Clavibacter insidiosus LSV 25.53 Xanthomonas fragariae LSV 25.73 Xanthomonas arboricola pv. pruni LSV 26.80 Xanthomonas axonopodis pv. aurantifolia LSV 28.76 Pseudomonas fluorescens LSV 29.24 Pseudomonas syringae pv. mori LSV 32.54 Agrobacterium tumefaciens LSV 34.17 Pseudomonas savastanoi pv. savastanoi LSV 36.75 Xanthomonas hortorum pv. pelargonii LSV 42.32 Pseudomonas syringae pv. aesculi LSV 46.74 Xanthomonas axonopodis pv. viticola LNPV 01.01 Pseudomonas syringae pv. syringae LNPV 08.19 Pseudomonas viridiflava CFBP 1232 Erwinia amylovora CFBP 2098 Xylophilus ampelinus BN 16/306.1 BN (bois noir)* FD 16/309.1 FD (flavescence dorée)* Poly A Bactérie saprophyte de Polygala myrtifolia Poly B Bactérie saprophyte de Polygala myrtifolia Poly C Bactérie saprophyte de Polygala myrtifolia Poly D Bactérie saprophyte de Polygala myrtifolia Oli A Bactérie saprophyte d’Olea europaea Oli B Bactérie saprophyte d’Olea europaea Oli C Bactérie saprophyte d’Olea europaea Oli D Bactérie saprophyte d’Olea europaea Oli E Bactérie saprophyte d’Olea europaea Immo A Bactérie saprophyte d’Helichrysum italicum Immo B Bactérie saprophyte d’Helichrysum italicum Immo C Bactérie saprophyte d’Helichrysum italicum Immo D Bactérie saprophyte d’Helichrysum italicum Immo E Bactérie saprophyte d’Helichrysum italicum Lav A Bactérie saprophyte de Lavandula sp. Lav B

	Bactérie saprophyte de Lavandula sp. Lav C Bactérie saprophyte de Lavandula sp. Lav D Bactérie saprophyte de Lavandula sp. Lav E Bactérie saprophyte de Lavandula sp.
Specificity value	100% (43 non-target strains)
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	CTAB based DNA extraction protocol (PM7/24(4)) Simplex real-time PCR Harper et al., 2010
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Samples with bacterial concentrations from 10^2 cells/ mL to 10^5 cells/mL - DNA extraction: 3 rep. - Amplificatio: 3 rep. on 3 different days
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Samples with bacterial concentrations from 10^2 cells/ mL to 10^5 cells/mL - DNA extraction: 3 rep. - Amplificatio: 3 rep. on 3 different days
Test performance study	
Test performance study?	no

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