

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 Dixm�ras, 49000 Angers, France
Short description of the test	Xylella fastidiosa detection by duplex real-time PCR Harper et al., 2010 / Iosifidis 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 detection 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 <i>Xylella fastidiosa</i> subsp. multiplex ST41 LNPV 00.56 <i>Xylella fastidiosa</i> subsp. fastidiosa ST1 LNPV 24.34 <i>Xylella fastidiosa</i> subsp. fastidiosa ST2 LSV 40.38 <i>Xylella fastidiosa</i> subsp. multiplex ST10 LSV 42.09 <i>Xylella fastidiosa</i> subsp. proche fastidiosa ST75 LSV 42.10 <i>Xylella fastidiosa</i> subsp. proche pauca ST74 LSV 42.30 <i>Xylella fastidiosa</i> subsp. multiplex ST27 LSV 42.31 <i>Xylella fastidiosa</i> subsp. multiplex ST9 LSV 42.36 <i>Xylella fastidiosa</i> subsp. sandyi ST5 LSV 46.27 <i>Xylella fastidiosa</i> subsp. sandyi ST72 LSV 46.63 <i>Xylella fastidiosa</i> subsp. morus ST29 LSV 46.77 <i>Xylella fastidiosa</i> subsp. multiplex ST7 LSV 46.79 <i>Xylella fastidiosa</i> subsp. multiplex ST6 LSV 47.09 <i>Xylella fastidiosa</i> subsp. pauca ST53 LSV 47.29 <i>Xylella fastidiosa</i> subsp. pauca ST53
Specificity value	100% (15 non-target strains)
Analytical specificity - exclusivity	
Number of non-target organisms tested	LNPV 0.78 <i>Pseudomonas syringae</i> pv. morsprunorum LNPV 0.79 <i>Pseudomonas syringae</i> pv. persicae LNPV 04.55 <i>Xanthomonas campestris</i> pv. campestris LNPV 06.28 <i>Xanthomonas</i> translucens pv. graminis LNPV 08.62 <i>Xanthomonas</i> arboricola pv. juglandis LNPV 17.76 <i>Xanthomonas</i> hortorum pv. carotae LNPV 23.03 <i>Xanthomonas</i> hortorum pv. hederæ LSV 25.29 <i>Clavibacter</i> insidiosus LSV 25.53 <i>Xanthomonas fragariae</i> LSV 25.73 <i>Xanthomonas arboricola</i> pv. pruni LSV 26.80 <i>Xanthomonas axonopodis</i> pv. aurantifolia LSV 28.76 <i>Pseudomonas fluorescens</i> LSV 29.24 <i>Pseudomonas syringae</i> pv. mori LSV 32.54 <i>Agrobacterium tumefaciens</i> LSV 34.17 <i>Pseudomonas savastanoi</i> pv. savastanoi LSV 36.75 <i>Xanthomonas hortorum</i> pv. pelargonii LSV 42.32 <i>Pseudomonas syringae</i> pv. aesculi LSV 46.74 <i>Xanthomonas axonopodis</i> pv. viticola LNPV 01.01 <i>Pseudomonas syringae</i> pv. syringae LNPV 08.19 <i>Pseudomonas viridiflava</i> CFBP 1232 <i>Erwinia</i> amylovora CFBP 2098 <i>Xylophilus ampelinus</i> BN 16/306.1 BN (bois noir)* FD 16/309.1 FD (flavescence dorée)* Poly A Bactérie saprophyte de <i>Polygala myrtifolia</i> Poly B Bactérie saprophyte de <i>Polygala myrtifolia</i> Poly C Bactérie saprophyte de <i>Polygala myrtifolia</i> Poly D Bactérie saprophyte de <i>Polygala myrtifolia</i> Oli A Bactérie saprophyte d' <i>Olea</i> europaea Oli B Bactérie saprophyte d' <i>Olea</i> europaea Oli C Bactérie saprophyte d' <i>Olea</i> europaea Oli D Bactérie saprophyte d' <i>Olea</i> europaea Oli E Bactérie saprophyte d' <i>Olea</i> europaea Immo A Bactérie saprophyte d' <i>Helichrysum italicum</i> Immo B Bactérie saprophyte d' <i>Helichrysum italicum</i> Immo C Bactérie saprophyte d' <i>Helichrysum italicum</i> Immo D Bactérie saprophyte d' <i>Helichrysum italicum</i> Immo E Bactérie saprophyte d' <i>Helichrysum italicum</i> Lav A Bactérie saprophyte de <i>Lavandula</i> 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)
<u>Diagnostic Specificity</u>	
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
<u>Reproducibility</u>	
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
<u>Repeatability</u>	
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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