

**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.

<b>Laboratory contact details</b>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixm�ras, 49044 Angers, France
<b>Short description of the test</b>	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)
<b>Date, reference of the validation report</b>	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)
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	Xylella fastidiosa(XYLEFA)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	No scientific publication. French official method MA 039 v6
<b>Is the test modified compared to the reference test</b>	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

<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	PROMEGA
<b>Specify the kit used</b>	Maxwell® HT Environmental TNA kit
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 4)
<b>Name of the test</b>	Real-time PCR - duplex (Harper et al., 2010; erratum 2013)
<b>As or adapted from an IPPC diagnostic protocol</b>	yes
<b>IPPC diagnostic Protocol name</b>	ISPM 27 Annex 25 DP 25: Xylella fastidiosa (version 2018)
<b>Name of the test</b>	Real-time PCR Harper et al., 2010
<b>Is the test modified compared to the reference test</b>	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
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	Applied Biosystems
<b>Specify the kit used</b>	TaqMan™ Fast Universal Master Mix (2X), no AmpErase™ UNG
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	With a detection rate of 100%: Olea europaea : $10^4$ cells/mL Quercus ilex : $10^3$ cells/mL
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	Olea europaea : 153% Quercus ilex : 111%
<b>Standard test(s)</b>	CTAB based DNA extraction protocol (PM7/24(4)) Simplex real-time PCR Harper et al., 2010

<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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
<b>Specificity value</b>	100% (15 non-target strains)
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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 translucens</i> pv. graminis LNPV 08.62 <i>Xanthomonas arboricola</i> pv. juglandis LNPV 17.76 <i>Xanthomonas hortorum</i> pv. carotae LNPV 23.03 <i>Xanthomonas hortorum</i> pv. hederæ LSV 25.29 <i>Clavibacter insidiosus</i> 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 Erwinia 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 europaea</i> Oli B Bactérie saprophyte d' <i>Olea europaea</i> Oli C Bactérie saprophyte d' <i>Olea europaea</i> Oli D Bactérie saprophyte d' <i>Olea europaea</i> Oli E Bactérie saprophyte d' <i>Olea europaea</i> 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.
<b>Specificity value</b>	100% (43 non-target strains)
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100%
<b>Specify the test(s)</b>	CTAB based DNA extraction protocol (PM7/24(4)) Simplex real-time PCR Harper et al., 2010
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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
<b>Test performance study</b>	
<b>Test performance study?</b>	no

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