

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, 49044 Angers, France
Short description of the test	Detection of <i>Xylella fastidiosa</i> by real-time PCR in plant material (Harper et al., 2010, Erratum 2013)
Date, reference of the validation report	2015-09-30 - Rapport de caract�risation et de validation de m�thode d'analyse - D�tection de <i>Xylella fastidiosa</i> par PCR en temps r�el sur plantes h�tes MA039ver01
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?	
If yes, please specify	
Description of the test	
Organism(s)	<i>Xylella fastidiosa</i> (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves Petioles
Plant species tested	Citrus sinensis, Olea europaea, Vitis vinifera
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?	
EPPO Diagnostic Protocol name	PM 7/024 <i>Xylella fastidiosa</i> (version 4)
Name of the test	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	

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	
Other details on the test	QuickPick™ Plant DNA kit (Bio-Nobile) Automated protocol with KingFisher™ mL (Thermo Scientific)
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?	
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 4)
Name of the test	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	no
Kit	
Is a kit used	
Other information	
Reaction type	
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	
Performance Criteria :	
Organism 1.:	Xylella fastidiosa(XYLEFA)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	- Grapevine: ~ 10 ³ bact./mL - Orange tree: ~ 10 ² bact./mL - Olive tree: ~ 10 ⁵ bact./mL With a probability of detection of 100%
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	- Grapevine: 94% - Orange tree: 100% - Olive tree: 67%
Standard test(s)	- Spiked matrices with bacterial concentration from 10 ² to 10 ⁵ bact./mL - Grapevine spiked with X. f. subsp. fastidiosa (CFBP7970) - Orange tree spiked with X. f. subsp. pauca (CFBP8072) - Olive

	tree spiked with <i>X. f. subsp. multiplex</i> (CFBP8173) 15 samples per matrix 30 DNA extraction per matrix 60 amplifications per matrix
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Inclusivity tested with 19 target strains: 100% - <i>X.f. subsp. fastidiosa</i> (CFBP8069 - LSV 0056 / CFBP7970 - LSV 2434 / CFBP8082 - LSV 4040 / CFBP8071 - LSV 4041 / CFBP8083 - LSV 4042 / CFBP8073 - LSV4209 / CFBP8351 - LSV4626) - <i>X.f. subsp. pauca</i> (CFBP8072 - LSV 4103) - <i>X.f. subsp. sandyi</i> (CFBP8077 - LSV 4236 / CFBP 8356 - LSV4627 / LSV4628 / LSV4639 / LSV4659) - <i>X.f. subsp. multiplex</i> (CFBP8068 - LSV 0054 / CFBP8070 - LSV 4038/ CFBP8173 - LSV 4039 / CFBP8075 - LSV 4230/ CFBP8076 - LSV 4231 / CFBP8078 - LSV 4311) Bacterial suspension concentration of about 10 ⁷ bact./mL
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Exclusivity tested with 29 non-target strains: 100% - 1 <i>Xylophilus ampelinus</i> (CFBP2098) - 2 <i>Xanthomonas arboricola</i> pv. <i>pruni</i> (LSV2574/LSV 2573) - 1 <i>Xanthomonas arboricola</i> pv. <i>juglandis</i> (LSV0862) - 1 <i>Xanthomonas axonopodis</i> pv. <i>citri</i> (LSV2647) - 1 <i>Xanthomonas axonopodis</i> pv. <i>aurantifolia</i> (LSV2680) - 2 <i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i> (LSV1014/LSV3161) - 1 <i>Xanthomonas axonopodis</i> pv. <i>fragariae</i> (LSV3151) - 1 <i>Xanthomonas fragariae</i> (LSV2553) - 1 <i>Xanthomonas hortorum</i> pv. <i>carotae</i> (LSV1776) - 1 <i>Xanthomonas campestris</i> pv. <i>campestris</i> (LSV0455) - 1 <i>Xanthomonas campestris</i> pv. <i>juglandis</i> (LSV1158) - 1 <i>Xanthomonas hortorum</i> pv. <i>hedera</i> (LSV2303) - 1 <i>Xanthomonas translucens</i> pv. <i>graminis</i> (LSV0628) - 1 <i>Xanthomonas translucens</i> pv. <i>hordei</i> (LSV0629) - 1 <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> (LSV0865) - 1 <i>Ca. Liberibacter asiaticus</i> - 1 <i>Ca. L. africanus</i> - 6 saprophytic bacteria saprophytes isolated from <i>Coffea</i> spp. - 4 bactéries saprophytes isolées de <i>Citrus sinensis</i> Bacterial suspension concentration of about 10 ⁷ bact./mL
Specificity value	100% no cross reaction
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	- Grapevine: 100% - Orange tree: 100% - Olive tree: 100%
Specify the test(s)	Spiked matrices with bacterial concentration from 10 ³ to 10 ⁵ bact./mL - Grapevine spiked with <i>X. f. subsp. fastidiosa</i> (CFBP7970) - Orange tree spiked with <i>X. f. subsp. pauca</i> (CFBP8072) - Olive tree spiked with <i>X. f. subsp. multiplex</i> (CFBP8173) 15 samples per matrix 30 DNA extraction per matrix 60 amplifications per matrix

Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	98%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	- Grapevine: 96% - Orange tree: 100% - Olive tree: 100%
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output. It available, link to published article/report	A test performance study was performed in 2014 for the Real time PCR Harper et al., 2010 method but with another DNA extraction method (DNeasy® Plant mini kit (Qiagen) Analytical sensitivity (with a probability of detection of 100%): - Orange tree: ~ 10 ² bact./mL - Grapevine: ~ 10 ⁶ bact./mL - Peach tree: ~ 10 ⁴ bact./mL - Olive tree: ~ 10 ⁵ bact./mL - Coffee tree: ~ 10 ⁴ bact./mL Diagnostic sensitivity: 97% Diagnostic specificity: 97% Reproducibility: 84% Repeatability: 91%
Other information	
Any other information considered useful	For information, a proficiency test was performed in 2015 for this method.

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