

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	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 caractérisation et de validation de méthode d'analyse - Détection de <i>Xylella fastidiosa</i> par PCR en temps réel sur plantes hôtes 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?	
Description of the test	
Organism(s)	<i>Xylella fastidiosa</i> (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	yes
EPPO Diagnostic Protocol name	PM 7/024 <i>Xylella fastidiosa</i> (version 4)
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
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)
Is the test modified compared to the reference test	no
Other information	
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 X. f. subsp. multiplex (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% - X.f. subsp. fastidiosa (CFBP8069 - LSV 0056 / CFBP7970 - LSV 2434 / CFBP8082 - LSV 4040 / CFBP8071 - LSV 4041 / CFBP8083 - LSV 4042 / CFBP8073 - LSV 4209 / CFBP8351 - LSV 4626) - X.f. subsp. pauca (CFBP8072 - LSV 4103) - X.f. subsp. sandyi (CFBP8077 - LSV 4236 / CFBP 8356 - LSV 4627 / LSV 4628 / LSV 4639 / LSV 4659) - X.f. subsp. multiplex (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 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 saprophytic bacteria saprophytes isolated from Coffea spp. - 4 bactéries saprophytes isolées de Citrus sinensis Bacterial suspension concentration of about 10 ⁷ bact./mL
Specificity value	100% no cross reaction
<u>Diagnostic Specificity</u>	
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 X. f. subsp. fastidiosa (CFBP7970) - Orange tree spiked with X. f. subsp. pauca (CFBP8072) - Olive tree spiked with X. f. subsp. multiplex (CFBP8173) 15 samples per matrix 30 DNA extraction per matrix 60 amplifications per matrix
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	98%
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	- Grapevine: 96% - Orange tree: 100% - Olive tree: 100%
<u>Test performance study</u>	
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%
<u>Other information</u>	
Any other information considered useful	For information, a proficiency test was performed in 2015 for this method.