EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

Target Organism	Xylella fastidiosa	
Short description	Detection of Xylella fastidiosa by real-time PCR in plant material (Harper et al., 2010, Erratum 2013)	
Laboratory contact details	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49000 Angers, France	
Date and reference of the validation report	2015-09-30 - Rapport de caractérisation et de validation de méthode d'analyse - Détection de Xylella fastidiosa par PCR en temps réel sur plantes hôtes MA039	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 - Harper et al., 2010, Erratum 2013 - MA 039 version 1 French reference method (www.anses.fr)	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Vitis vinifera, Citrus sinensis, Olea europaea	
Matrices tested (if relevant)	Petioles	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	QuickPick™ Plant DNA kit (Bio-Nobile) Automated protocol with KingFisher™ mL (Themo Scientific)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Real-time PCR Harper et al., 2010 (erratum 2013)
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein		

profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
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%	
Specify the standard test	- 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		
Specificity value	100%	
Number of strains/populations of target organisms tested	Inclusivity tested with19 target strains: 100% - X.f. subsp. fastidiosa (CFBP8069 - LSV 0056 / CFBP7970 - LSV 2434 / CFBP8082 - LSV 4040 / CFBP8071 - LSV 4041 / CFBP8083 - LSV 4042 / CFBP8073-LSV4209 / CFBP8351 - LSV4626) - X.f. subsp. pauca (CFBP8072 - LSV 4103) - X.f. subsp. sandyi (CFBP8077 - LSV 4236 / CFBP 8356 - LSV4627 / LSV4628 / LSV4639 / LSV4659) - 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	
Number of non-target organisms tested	Exclusity 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
Cross reacts with (specify the species)	None
<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 standard test	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
Include brief details of the test performance study and its output.It available, provide a 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 **e.g. robustness, ease of performing the test, etc.**	For information, a proficiency test was performed in 2015 for this method.