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 conventional PCR in plant material	
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 Minsavage et al., 1994	
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, Prunus persica, Citrus sinensis, Coffea arabica, C. canephora	
Matrices tested (if relevant)		
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	DNeasy® Plant mini kit (Qiagen)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Conventional PCR
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?	Data from intra-laboratory study performed in 2013(Anses): - Grapevine (Vitis vinifera): ≈ 10^2 bact./mL - Peach tree (Prunus persica): ≈ 10^2 bact./mL - Orange tree (Citrus sinensis): ≈ 10^3 bact./mL - Coffee tree (Coffea arabica): ≈ 10^4 bact./mL (diluted DNA 1/10) - Coffee tree (C. canephora): ≈ 10^4 bact./mL (non-specific bands are present near 750 bp; expected band is 733 bp) 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 (Vitis vinifera): 81% - Peach tree (Prunus persica): 81% - Orange tree (Citrus sinensis): 82% - Coffee tree (Coffea arabica): 81% - Coffee tree (C. canephora): 74%	
Specify the standard test	- Spiked matrices with bacterial concentration from 10^1 to 10^6 bact./mL - Grapevine spiked with X. f. subsp. fastidiosa (CFBP7970) - Peach tree spiked with X. f. subsp. multiplex (CFBP8173 and CFBP8070) - Orange tree spiked with X. f. subsp. pauca (CFBP8072) - Coffee tree (Coffea arabica) spiked with X. f. subsp. pauca (CFBP8072) - Coffee tree (C. canephora) spiked with X. f. subsp. fastidiosa (CFBP8073) 21 samples per matrix 63 DNA extraction per matrix 126 amplifications per matrix (on orange tree 18 samples per matrix 54 DNA extraction per matrix 108 amplifications per matrix)	
Analytical specificity		
Specificity value	100%	
Number of strains/populations of target organisms tested	Inclusivity tested with 10 target strains: 100% - X.f. subsp. fastidiosa (CFBP8069 -LSV0056/ CFBP8071 -LSV4041/ CFBP8083 -LSV4042/ CFBP7970-LSV2434/ CFBP8082 -LSV4040) - X.f. subsp. pauca (CFBP8072 - LSV4103) - X.f. subsp. sandyi (CFBP8077-LSV4236) - X.f. subsp. multiplex (CFBP8173 -LSV4039/ CFBP8068-LSV0054/ CFBP8070-LSV4038)	

Number of non-target organisms tested	Exclusivity tested with 17 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)
Cross reacts with (specify the species)	None
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	- Grapevine (Vitis vinifera): NA - Peach tree (Prunus persica): NA - Orange tree (Citrus sinensis): 100% - Coffee tree (Coffea arabica): 100% - Coffee tree (C. canephora): 100%
Specify the standard test	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	- Grapevine (Vitis vinifera): 80% - Peach tree (Prunus persica): 92% - Orange tree (Citrus sinensis): 98% - Coffee tree (Coffea arabica): 94% - Coffee tree (C. canephora): 89%
Test performance study	
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 on a new set of spiked samples: Performance criteria Analytical sensitivity (with a probability of detection of 100% on coffee and orange only): - Coffee tree: ≈10^4 bact./mL (100%: 5 labs/5) - Olive tree: 10^6bact/mL (3 labs/5) - Grapevine: 10^6bact/mL (2 labs/5) - Orange: ≈10^2 bact./mL (100%: 5 labs/5) - Peach tree: 10^4bact/mL (3 labs/5) Diagnostic sensitivity (based on results on spiked samples to the following concentrations):

	- Coffee tree: 70% (10^2-10^4 bact/mL) - Olive tree: 30% (10^4 – 10^6 bact/mL) - Grapevine: 40% (10^4 – 10^6 bact/mL) - Orange: 80% (10^1-10^3 bact/mL) - Peach tree: 60% (10^2-10^4 bact/mL) Note: these results got by 7 laboratories are different of those got in intra-laboratory, mainly on grapevine (variability linked to a matrix effect?) Diagnostic Specificity: 100% Reproducibility: 84% Repeatability: 95% (from 88% to 100% according to the 7 laboratories) 4 samples per matrix 2 extractions per sample 2 amplifications per DNA extract TPS performed with extraction kit from Qiagen (DNeasy Plant mini kit)
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	