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, 49044 Angers, France
Short description of the test	Detection of Xylella fastidiosa by conventional PCR in plant material
Date, 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 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)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular Conventional PCR

Method: Molecular Extraction DNA RNA	
Reference of the test description	
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	DNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
Other information	

Method: Molecular Conventional 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	Conventional PCR (Minsavage et al., 1994)
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**yes**
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
<u>**Analytical sensitivity**</u>	
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%
<u>**Diagnostic sensitivity**</u>	
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%
Standard test(s)	- 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)
<u>**Analytical specificity - inclusivity**</u>	
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)
Specificity value	100%
<u>**Analytical specificity - exclusivity**</u>	
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)
Specificity value	100%, no cross reaction

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%

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
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 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: 10^6 bact/mL (3 labs/5) - Grapevine: 10^6 bact/mL (2 labs/5) - Orange: ˜10^2 bact./mL (100%: 5 labs/5) - Peach tree: 10^4 bact/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)