

**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.

<b>Laboratory contact details</b>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixm�ras, 49044 Angers, France
<b>Short description of the test</b>	Detection of Xylella fastidiosa by conventional PCR in plant material
<b>Date, reference of the validation report</b>	2015-09-30 - Rapport de caract�risation et de validation de m�thode d'analyse - D�tection de Xylella fastidiosa par PCR en temps r�el sur plantes h�tes MA039
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Xylella fastidiosa(XYLEFA)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Conventional PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	DNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 4)

<b>Name of the test</b>	Conventional PCR (Minsavage et al., 1994)
<b>Other information</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>yes</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Data from intra-laboratory study performed in 2013(Anses): - Grapevine (Vitis vinifera): ~ 10 <sup>2</sup> bact./mL - Peach tree (Prunus persica): ~ 10 <sup>2</sup> bact./mL - Orange tree (Citrus sinensis): ~ 10 <sup>3</sup> bact./mL - Coffee tree (Coffea arabica): ~ 10 <sup>4</sup> bact./mL (diluted DNA 1/10) - Coffee tree (C. canephora): ~ 10 <sup>4</sup> bact./mL (non-specific bands are present near 750 bp; expected band is 733 bp) With a probability of detection of 100%
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	- Grapevine (Vitis vinifera): 81% - Peach tree (Prunus persica): 81% - Orange tree (Citrus sinensis): 82% - Coffee tree (Coffea arabica): 81% - Coffee tree (C. canephora): 74%
<b>Standard test(s)</b>	- Spiked matrices with bacterial concentration from 10 <sup>1</sup> to 10 <sup>6</sup> 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)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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)
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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)
<b>Specificity value</b>	100%, no cross reaction
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	- Grapevine (Vitis vinifera): NA - Peach tree (Prunus persica): NA - Orange tree (Citrus sinensis): 100% - Coffee tree (Coffea arabica): 100% - Coffee tree (C. canephora): 100%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	- Grapevine (Vitis vinifera): 80% - Peach tree (Prunus persica): 92% - Orange tree (Citrus sinensis): 98% - Coffee tree (Coffea arabica): 94% - Coffee tree (C. canephora): 89%
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	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: $\sim 10^4$ bact./mL (100%: 5 labs/5) - Olive tree: $10^6$ bact./mL (3 labs/5) - Grapevine: $10^6$ bact./mL (2 labs/5) - Orange: $\sim 10^2$ 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)

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