

**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>	INRA Angers 42 rue Georges MOREL, 49070 Beaucouzé, France
<b>Short description of the test</b>	detection and identification of Xylella fastidiosa Xylella fastidiosa by Molecular Conventional PCR (nested) in leaf petiole , Shoots, insects
<b>Date, reference of the validation report</b>	2021-12-16 - INRAE_nested-MLST_Xf
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	XF-ACTORS, Projects E-RTA2017-00004-C06-02 , Project 2018 40E111 Spain.
<b>Description of the test</b>	
<b>Organism(s)</b>	Xylella fastidiosa(XYLEFA)
<b>Detection / identification</b>	detection and identification
<b>Matrix(ces) tested</b>	Leaves, Other, Shoots more than 40 plant species and 2 insect species from France and Spain
<b>Plant species tested</b>	Arbutus, Asparagus officinalis, Centranthus, Cistus, Cupressus, Ficus, Genista, Helichrysum petiolare, Juglans, Lavandula, Myrtus, Nerium, Olea, Phagnalon, Platanus, Polygala myrtifolia, Prunus, Quercus, Spartium
<b>Method(s)</b>	Molecular Conventional PCR
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Cesbron, S., Dupas, E., Beaufèvre, Q., Briand, M., Montes-Borrego, M., Velasco-Amo, M. D. P., ... & Jacques, M. A. (2020). Development of a Nested-

	MultiLocus Sequence Typing approach for a highly sensitive and specific identification of <i>Xylella fastidiosa</i> subspecies directly from plant samples. <i>Agronomy</i> , 10(8), 1099.
<b>Is the test modified compared to the reference test</b>	yes
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Nested
<b>Other details on the test</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b><i>Xylella fastidiosa</i>(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	On <i>Xylella fastidiosa</i> DNA dilution series : up to 22 pg mL <sup>-1</sup> (0.8 x 10 <sup>4</sup> copies mL <sup>-1</sup> ) for gltT, holC, petC, leuA, cysG, and up to 2.2 pg mL <sup>-1</sup> (0.8 x 10 <sup>3</sup> copies mL <sup>-1</sup> ) for nuoL and malF
<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>	On low and high infected samples : 206 plant samples and 26 insect samples, also analysed with Harper's qPCR without threshold: cysG : from 11 to 90% gltT: from 9 to 90% holC : 27 to 93% leuA : 27 to 90% malF : 16 to 90% nuoL: 26 to 90% petC : 25 to 90%
<b>Standard test(s)</b>	Harper's qPCR
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	<i>Xylella fastidiosa</i> subsp. <i>fastidiosa</i> (CFBP 7970), <i>Xylella fastidiosa</i> subsp. <i>multiplex</i> (CFBP 8416; CFBP 8070), <i>Xylella fastidiosa</i> subsp. <i>morus</i> (CFBP 8084), <i>Xylella fastidiosa</i> subsp. <i>pauca</i> (CFBP 8402) in vitro, and on 58 <i>Xylella fastidiosa</i> genome sequences in silico (including all subspecies).
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	34 non-target species ( <i>Agrobacterium rubi</i> CFBP 6448, <i>Agrobacterium tumefaciens</i> CFBP 2413, <i>Agrobacterium vitis</i> CFBP 5523, <i>Clavibacter insidiosus</i> CFBP 2404, <i>Clavibacter michiganensis</i> CFBP 4999, <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> CFBP 3414, <i>Dickeya dianthicola</i> CFBP 1200, <i>Ensifer meliloti</i> CFBP 5561, <i>Erwinia amylovora</i> CFBP 1232, <i>Pantoea agglomerans</i> CFBP 3845, <i>Pantoea stewartii</i> pv. <i>stewartii</i> CFBP 3167, <i>Pseudomonas amygdali</i> CFBP 3205, <i>Pseudomonas cerasi</i> CFBP 7436, <i>Pseudomonas congelans</i> CFBP

	7019, Pseudomonas syringae pv. persicae CFBP 1573, Pseudomonas syringae pv. syringae CFBP 1392, Rhizobium nepotum CFBP 7436, Stenotrophomas maltophilia CFBP 13100, Xanthomonas euvesicatoria pv. citrumelonis CFBP 3371, Xanthomonas arboricola pv. juglandis CFBP 2528, Xanthomonas arboricola pv. pruni CFBP 2535, Xanthomonas axonopodis pv. axonopodis CFBP 4924, Xanthomonas campestris pv. campestris CFBP 5241, Xanthomonas citri pv. aurantifolii CFBP 2901, Xanthomonas citri pv. citri CFBP 2525, Xanthomonas citri pv. viticola CFBP 7660, Xanthomonas gardneri CFBP 2625, Xanthomonas hortorum pv. hederiae CFBP 4925, Xanthomonas hortorum pv. pelargonii CFBP 2533, Xanthomonas hyacinthi CFBP 1156, Xanthomonas oryzae pv. oryzae CFBP 2532, Xanthomonas translucens CFBP 2054, Xanthomonas vasicola pv. holcicola CFBP 2543) in vitro, and on 194 380 bacterial whole genome shotgun sequences in silico.
<b>Specificity value</b>	100%
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100%
<b>Specify the test(s)</b>	qPCR from Harper
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	not tested
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	not tested
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. If available, link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful</b>	

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