

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	Détection de Xylella fastidiosa par PCR en temps réel sur végétal	
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	2019-1 - MA039 version 4	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/024 CTAB-based DNA extraction QuickPick SML Plant DNA kit-based DNA extraction Real-time PCR Harper et al., 2010	
Is the test the same as described in the EPPO DP?	Modified Insertion of a step of sonication of the macerate prior to DNA extraction in order to break the X. fastidiosa biofilms Real-time PCR Harper et al., 2010: DNA extract added to the PCR mix doubled (4 µL instead of 2 µL)	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Olea europaea; Quercus ilex	
Matrices tested (if relevant)	Petioles	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	CTAB-based DNA extraction QuickPick SML Plant DNA kit-based DNA extraction Real-time PCR Harper et al., 2010
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?	Olea europaea: 1.10^5 bact./mL of macerate (detection rate = 100%) Quercus ilex: 1.10^4 bact./mL of macerate (detection rate = 100%)	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98		
Specify the standard test		
Analytical specificity		
Specificity value	100%	
Number of strains/populations of target organisms tested	74 X. fastidiosa strains belonging to X. f. subsp fastidiosa, morus, sandyi, pauca and multiplex	
Number of non-target organisms tested	29 non-target organism: - 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. hederata (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 bactéries saprophytes isolées de Coffea spp. - 4 bactéries saprophytes isolées de Citrus sinensis	
Cross reacts with (specify the species)	No cross reaction	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a		

standard test	
Specify the standard test	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	
The following complementary files are available online:	<ul style="list-style-type: none"> • Rapport de caractérisation et de validation d'une méthode d'analyse MA0039 version 4