

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 real-time PCR in insects	
Laboratory contact details	Anses, Laboratoire de la Santé des Végétaux - Unité de bactériologie, virologie OGM 7 rue Jean Dixméras, 49044 Angers, France	
Date and reference of the validation report	2016-05-31 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/024 Harper et al., 2010, Erratum 2013 MA 039 version 1 French reference method (www.anses.fr)	
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)		
Matrices tested (if relevant)	insects (<i>Philaenus spumarius</i>) heads without eyes; Grinding, one head with 200 µl steril demineralized water in 2 mL microtubes with metallic beads (3 mm diameter) and high frequency agitator (RETSCH MM400 during 2 min / 30 Hertz)	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	QuickPick™ Plant DNA kit (Bio-Nobile) Automated protocol with KingFisher™ mL (Thermo Scientific)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Real-time PCR Harper et al., 2010 (erratum 2013)
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?	≈ 10 ³ bact./head Ct value ≤ 35 And 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	100%	
Specify the standard test	Spiked matrices (heads) with bacterial concentration from 10 ³ to 10 ⁵ bact./head 72 samples 72 DNA extractions 144 amplifications	
Analytical specificity		
Specificity value	100%	
Number of strains/populations of target organisms tested	Inclusivity tested with 19 target strains: 100% - X.f. subsp. fastidiosa (CFBP8069 - LSV 0056 / CFBP7970 - LSV 2434 / CFBP8082 - LSV 4040 / CFBP8071 - LSV 4041 / CFBP8083 - LSV 4042 / CFBP8073-LSV4209 / CFBP8351 - LSV4626) - X.f. subsp. pauca (CFBP8072 - LSV 4103) - X.f. subsp. sandyi (CFBP8077 - LSV 4236 / CFBP 8356 - LSV4627 / LSV4628 / LSV4639 / LSV4659) - X.f. subsp. multiplex (CFBP8068 - LSV 0054 / CFBP8070 - LSV 4038/ CFBP8173 - LSV 4039 / CFBP8075 - LSV 4230/ CFBP8076 - LSV 4231 / CFBP8078 - LSV 4311) Bacterial suspension concentration of about 10 ⁷ bact./mL	
Number of non-target organisms tested	Exclusivity tested with 29 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. hederata (LSV2303)	

	<ul style="list-style-type: none"> - 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 saprophytic bacteria saprophytes isolated from Coffea spp. 4 saprophytic bacteria isolated from Citrus sinensis Bacterial suspension concentration of about 10^7 bact./mL
Cross reacts with (specify the species)	None
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% of samples did not get positive results but 1/18 got a Ct value of 39/40
Specify the standard test	No Spiked matrices (heads) 18 samples 18 DNA extractions 36 amplifications
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not evaluated
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
<u>Test performance study</u>	
Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
<u>Other information</u>	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	Real-time PCR duplex Harper et al., 2010 (erratum 2013) + internal control 18S (18Suni-F/18S uni-R/18S uni-P) shows similar performances