

**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 Dixm�ras, 49044 Angers, France
Short description of the test	Detection of <i>Xylella fastidiosa</i> by real-time PCR in insects
Date, reference of the validation report	2016-05-31 -
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?	
If yes, please specify	
Description of the test	
Organism(s)	<i>Xylella fastidiosa</i> (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Specimen insects (<i>Philaenus spumarius</i>) heads without eyes;
Plant species tested	
Method(s)	Extraction Molecular Extraction DNA RNA Molecular real time PCR
Method: Extraction	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Other information	
Other details on the test	Grinding, one head with 200 �l steril demineralized water in 2 mL microtubes with metallic beads (3

	mm diameter) and high frequency agitator (RETSCH MM400 during 2 min / 30 Hertz)
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	yes
Manufacturer name	BIONOBILE
Specify the kit used	QuickPick Plant DNA kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	QuickPick™ Plant DNA kit (Bio-Nobile) Automated protocol with KingFisher™ mL (Thermo Scientific)
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 4)
Name of the test	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	
Other details on the test	Real-time PCR Harper et al., 2010 (erratum 2013)
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	

Organism 1.:	Xylella fastidiosa(XYLEFA)
Analytical sensitivity	
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%
Standard test(s)	Spiked matrices (heads) with bacterial concentration from 10 ³ to 10 ⁵ bact./head 72 samples 72 DNA extractions 144 amplifications
Analytical specificity - inclusivity	
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
Specificity value	100%
Analytical specificity - exclusivity	
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. hedera (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 saprophytic bacteria saprophytes isolated from Coffea spp. 4 saprophytic bacteria isolated from Citrus sinensis Bacterial suspension concentration of about 10 ⁷ bact./mL
Specificity value	100% - No cross reaction
Cross reacts with	

Diagnostic Specificity	
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 test(s)	No Spiked matrices (heads) 18 samples 18 DNA extractions 36 amplifications
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not evaluated
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
Brief details of the test performance study and its output. If available, link to published article/report	
Other information	
Any other information considered useful	Real-time PCR duplex Harper et al., 2010 (erratum 2013) + internal control 18S (18Suni-F/18S uni-R/18S uni-P) shows similar performances

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