

**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, 49000 Angers, France
<b>Short description of the test</b>	Detection of <i>Xylella fastidiosa</i> by real-time PCR in insects
<b>Date, reference of the validation report</b>	2016-05-31 -
<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>	<i>Xylella fastidiosa</i> (XYLEFA)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Specimen insects ( <i>Philaenus spumarius</i> ) heads without eyes;
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	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)
<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>	BIONOBILE
<b>Specify the kit used</b>	QuickPick Plant DNA kit
<b>Kit used following the manufacturer's instructions?</b>	

<b>Other information</b>	
<b>Other details on the test</b>	QuickPick™ Plant DNA kit (Bio-Nobile) Automated protocol with KingFisher™ mL (Thermo Scientific)
<b>Method: Molecular real time 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>	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	Real-time PCR Harper et al., 2010 (erratum 2013)
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	~ 10 <sup>3</sup> bact./head Ct value = 35 And 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>	100%
<b>Standard test(s)</b>	Spiked matrices (heads) with bacterial concentration from 10 <sup>3</sup> to 10 <sup>5</sup> bact./head 72 samples 72 DNA extractions 144 amplifications
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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 <sup>7</sup> bact./mL
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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 <sup>7</sup> bact./mL
<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>	100% of samples did not get positive results but 1/18 got a Ct value of 39/40
<b>Specify the test(s)</b>	No Spiked matrices (heads) 18 samples 18 DNA extractions 36 amplifications
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not evaluated
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	Real-time PCR duplex Harper et al., 2010 (erratum 2013) + internal control 18S (18Suni-F/18S uni-R/18S uni-P) shows similar performances

Creation date: 2016-06-14 00:00:00 - Last update: 2021-05-20 16:46:03