

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	D�tection de Xylella fastidiosa par PCR en temps r�el sur v�g�tal
Date, reference of the validation report	2019-01-01 - MA039 version 4
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Is the test modified compared to the reference test	yes Insertion of a step of sonication of the macerate prior to DNA extraction in order to break the X. fastidiosa biofilms
Other information	
Other details on the test	CTAB-based DNA extraction
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 3)
Is the test modified compared to the	yes Insertion of a step of sonication of the

reference test	macerate prior to DNA extraction in order to break the <i>X. fastidiosa</i> biofilms
Kit	
Is a kit used	yes
Manufacturer name	BIONOBILE
Specify the kit used	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	
Other information	
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/024 <i>Xylella fastidiosa</i> (version 3)
Name of the test	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
Is the test modified compared to the reference test	yes DNA extract added to the PCR mix doubled (4 µL instead of 2 µL)
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	<i>Xylella fastidiosa</i>(XYLEFA)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	<i>Olea europaea</i> : 1.10 ⁵ bact./mL of macerate (detection rate = 100%) <i>Quercus ilex</i> : 1.10 ⁴ bact./mL of macerate (detection rate = 100%)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	74 <i>X. fastidiosa</i> strains belonging to <i>X. f. subsp fastidiosa</i> , <i>morus</i> , <i>sandyi</i> , <i>pauca</i> and <i>multiplex</i>
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	29 non-target organism: - 1 <i>Xylophilus ampelinus</i> (CFBP2098) - 2 <i>Xanthomonas arboricola</i> pv. <i>pruni</i> (LSV2574/LSV 2573) - 1 <i>Xanthomonas arboricola</i> pv. <i>juglandis</i> (LSV0862) - 1 <i>Xanthomonas axonopodis</i> pv. <i>citri</i> (LSV2647) - 1 <i>Xanthomonas axonopodis</i> pv. <i>aurantifolia</i> (LSV2680) - 2 <i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i> (LSV1014/LSV3161) - 1 <i>Xanthomonas axonopodis</i> pv. <i>fragariae</i> (LSV3151) - 1 <i>Xanthomonas fragariae</i> (LSV2553) - 1 <i>Xanthomonas hortorum</i> pv. <i>carotae</i> (LSV1776) - 1 <i>Xanthomonas campestris</i> pv. <i>campestris</i> (LSV0455) - 1 <i>Xanthomonas campestris</i> pv. <i>juglandis</i> (LSV1158) - 1 <i>Xanthomonas hortorum</i> pv. <i>hedera</i> (LSV2303) - 1 <i>Xanthomonas translucens</i>

	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
Specificity value	No cross reaction
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
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

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