

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 Xylella fastidiosa Xylella fastidiosa by Molecular real time PCR in Leaves, Shoots, Herbaceous cuttings, Woody cuttings
Date, reference of the validation report	2020-03-25 - MA039 version 5 report version1
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?	Euphresco
If yes, please specify	- Euphresco project 09/2016-08/2018 "Harmonized protocol for monitoring and detection of Xylella fastidiosa in its host plants and its vectors" - H2020 Ponte project 2016-2020
Description of the test	
Organism(s)	Xylella fastidiosa(XYLEFA)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
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?	no
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 4)
As or adapted from an IPPC diagnostic protocol	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 25 DP 25: Xylella fastidiosa (version 2018)
Name of the test	QuickPick SML Plant DNA kit (Bio-Nobile)
Is the test modified compared to the	no

reference test	
Kit	
Is a kit used	yes
Manufacturer name	BIONOBILE
Specify the kit used	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	yes
Other information	
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?	yes
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	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 25 DP 25: Xylella fastidiosa (version 2018)
Name of the test	Real-time PCR -simplex (Harper et al., 2010;errtum 2013)
Is the test modified compared to the reference test	yes On Olea europaea and Quercus ilex, after CTAB-based DNA extraction, used of DNA extract volume of 4 µL for PCR reaction on a total volume of 20 µL. A cut-off value of 38 is applied
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Probe
Other details on the test	- Olea europaea and Quercus ilex :CTAB-based DNA extraction with prior sonication step on macerate (1 min at 35 kHz) / DNA extract of 4 µL for PCR reaction - Citrus sinensis and Vitis vinifera : QuickPick SML Plant DNA kit with prior sonication step on macerate (1 min at 35 kHz) / / DNA extract of 2 µL for PCR reaction - Cut-off value of 38
Performance Criteria :	
Organism 1.:	Xylella fastidiosa(XYLEFA)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	With a detection rate of 100% and with a cut-off value of 38 Ct : - Olea europaea : 1.10 ⁴ cells/mL (1.10 ³ cells/mL with a detection rate of 70%) - Quercus ilex : 1.10 ³ cells/mL - Citrus sinensis :

	3.10 ² cells/mL - Vitis vinifera : 1.10 ³ cells/mL
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Not evaluated
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	55 target organisms Cf. attached file "Rapport de validation MA039v5 - annexe D"
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	18 non target organisms Species Reference Ref. LSV Host Country Year Xylophilus ampelinus CFBP 2098 LSV 0502 Vitis vinifera)cv. Grenache France 1979 Xanthomonas arboricola pv. pruni CFBP 3901 LSV 2574 Prunus armeniaca USA 1987 Xanthomonas arboricola pv. juglandis NCPPB 362 LSV 0862 Juglans regia Royaume unis 1955 Xanthomonas axonopodis pv. citri CFBP 2904 LSV 2647 Citrus limon Argentine NA Xanthomonas axonopodis pv.. aurantifolia CFBP 3529 LSV 2680 Citrus limon Uruguay 1983 Xanthomonas axonopodis pv.. phaseoli ☉ LSV 10.14 Phaseolus vulgaris France 1994 Xanthomonas axonopodis pv. phaseoli ☉ LSV 3161 Phaseolus vulgaris Chine 2006 Xanthomonas arboricola pv fragariae ☉ LSV 3151 Fragaria sp. France 2006 Xanthomonas fragariae CFBP 2157 LSV 2553 Fragaria sp. USA 1960 Xanthomonas campestris pv.carotae ☉ LSV 1776 Daucus carota France 1997 Xanthomonas campestris pv. campestris ☉ LSV 0455 Nerium oleander France 1990 Xanthomonas hortorum pv.hederiae ☉ LSV 2303 Hedera helix France 2000 Xanthomonas translucens pv.graminis CFBP 2058 LSV 0628 Lolium sp. NA 1981 Xanthomonas translucens pv translucens CFBP 2544 LSV 0629 Hordeum vulgare Inde 1970 Xanthomonas arboricola pv pruni CFBP 3900 LSV 2573 Prunus persica USA 1987 Xanthomonas oryzae pv.oryzae LMG 806 LSV 0865 Oryza sativa Philippines NA Ca. Liberibacter asiaticus ☉ ☉ NA NA NA Ca. Liberibacter africanus ☉ ☉ NA NA NA
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Not evaluated
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Olea europaea : 100% at 1.10 ⁴ cells/mL Quercus ilex : 100% at 1.10 ³ cells/mL Citrus sinensis : 100% at 3.10 ² cells/mL Vitis vinifera : 100% at 1.10 ³ cells/mL Olea europaea and Quercus ilex : Evaluated with 3 replicates per concentration by 1

	operators on 3 different days. 4 bacterial concentration tested : 10^3 cells/mL; 10^4 cells/mL, 10^5 cells/mL, 10^6 cells/mL and healthy samples. Citrus sinensis and Vitis vinifera : Evaluated with 3 replicates per concentration by 1 operators on 2 different days. 3 bacterial concentration tested : 10^2 cells/mL, 10^3 cells/mL; 10^4 cells/mL, 10^5 cells/mL and healthy samples.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Olea europaea : 100% at 1.10^4 cells/mL Quercus ilex : 100% at 1.10^3 cells/mL Citrus sinensis : 100% at 3.10^2 cells/mL Vitis vinifera : 100% at 1.10^3 cells/mL Olea europaea and Quercus ilex : Evaluated with 3 replicates per concentration by 1 operators on 3 different days. 4 bacterial concentration tested : 10^3 cells/mL; 10^4 cells/mL, 10^5 cells/mL, 10^6 cells/mL and healthy samples. Citrus sinensis and Vitis vinifera : Evaluated with 3 replicates per concentration by 1 operators on 2 different days. 3 bacterial concentration tested : 10^2 cells/mL, 10^3 cells/mL; 10^4 cells/mL, 10^5 cells/mL and healthy samples.
Test performance study	
Test performance study?	no
The following complementary files are available online:	
	<ul style="list-style-type: none"> • Rapport de validation MA039v5

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