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 Dixméras, 49000 Angers, France
Short description of the test	Detection of Xylophilus ampelinus by real time PCR in leaves
Date, reference of the validation report	2019-01-01 -
Link to other validation data	- Detection of Xylophilus ampelinus by Conventional PCR in leaves
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?	
Description of the test	
Organism(s)	Xylophilus ampelinus (XANTAM)
Detection / identification	detection
Matrix(ces) tested	Leaves Petioles
Plant species tested	Vitis sp.
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
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 7/096 Xylophilus ampelinus (version 1)
Name of the test	Real-time PCR targeting 16sRNA (Dreo et al., 2007)
Is the test modified compared to the reference test	no
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Xylophilus ampelinus(XANTAM)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Real-time PCR Dreo et al., 2007: 9.10^3 bact/mL with cut-off value of 35 Ct and 9.10^2 bact/mL without cut-off value
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Real-time PCR Dreo et al., 2007 (11 strains) - LNPV 05.02 CFBP 20.98 Vitis vinifera France 1979 - LNPV 29.32 CFBP 27.31 Vitis vinifera South Africa 1972 - LNPV 29.36 CFBP 22.66 Vitis vinifera France 1983 - LNPV 29.37 CFBP 18.42 Vitis vinifera France 1977 - LNPV 29.40 CFBP 23.99 Vitis vinifera France 1984 - LNPV 29.46 CFBP 19.28 Vitis vinifera Spain 1978 - LNPV 44.15 ⦸ Vitis vinifera France 2013 - LNPV 29.45 CFBP 18.34 Vitis vinifera France 1975 - LNPV 29.30 CFBP 20.59 Vitis vinifera France 1981 - LNPV 29.42 CFBP 11.92 Vitis vinifera Greece 1966 - LNPV 29.48 CFBP 19.27 Vitis vinifera Spain 1978
Specificity value	Inclusivity: Real-time PCR Dreo et al., 2007: 100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Real-time PCR Dreo et al., 2007 (21 strains) - Xanthomonas arboricola pv. pruni LNPV 25.74 CFBP 39.01 Apricot (Prunus armeniaca) USA 1987 - Xanthomonas arboricola pv. juglandis LNPV 08.62 NCPPB 362 Chestnut (Juglans regia) UK 1955 - Xanthomonas fuscans subsp. aurantifolii LNPV 26.47 CFBP 29.04 Limon (Citrus limon) Argentina NA - Xanthomonas axonopodis pv. aurantifolii LNPV 26.80 CFBP 35.29 Limon (Citrus limon) Uruguay 1983 - Xanthomonas axonopodis pv. phaseoli LNPV 10.14 ⦸ Bean (Phaseolus vulgaris) France 1994 - Xanthomonas fragariae LNPV 25.53 CFBP 21.57 Strawberry (Fragaria sp.) USA 1960 - Xanthomonas hortorum pv. carotae LNPV 17.76 ⦸ Carot (Daucus carota) France 1997 - Xanthomonas campestris pv. campestris LNPV 04.55 ⦸ Oleander (Nerium oleander) France 1990 - Xanthomonas hortorum pv. hederae LNPV 23.03 ⦸ Yvi (Hedera helix) France

	2000 - Xanthomonas translucens pv. graminis LNPV 06.28 CFBP 20.58 Ray grass (Lolium perenne) ⦸ 1981 - Xanthomonas translucens pv. translucens LNPV 06.29 CFBP 25.44 Barley (Hordeum vulgare) India 1970 - Xanthomonas oryzae pv. oryzae LNPV 08.65 BCCM/LMG 806 Rice (Oryza sativa) Philippines NA - Agrobacterium vitis ⦸ CFBP 55.23 Grapevine (Vitis vinifera) Australia 1977 - Acidovorax valerianellae LNPV 28.51 ⦸ lettuce (Valerianella incusta) France 2004 - Pseudomonas syringae pv. siryngae LNPV 08.17 ⦸ Apricot (Prunus armeniaca) France 1994 - Xanthomonas axonopodis pv. viticola ⦸ CFBP 58.68 Grapevine (Vitis vinifera) Brazil 1998 - Xylella fastidiosa subsp. fastidiosa LNPV 24.34 ATCC 35879 Grapevine (Vitis vinifera) Florida, USA NA - Flavescence dorée ⦸ ⦸ Grapevine (Vitis vinifera) France ⦸ - Bois noir ⦸ ⦸ Grapevine (Vitis vinifera) France ⦸ - Grapevine saprophytic bacterium 1 France ⦸ Vigne (Vitis vinifera) France ⦸ - Grapevine saprophytic bacterium 2 france⦸ ⦸ Vigne (Vitis vinifera) France
Specificity value	Exclusivity: Real-time PCR Dreo et al., 2007: 90% Real-time PCR Dreo et al., 2007: cross-reaction with Xanthomonas axonopodis pv. phaseoli (LNPV 10.14) et X. hortorum pv. carotae (LNPV 17.76)
Cross-reacts with	Xanthomonas axonopodis pv. phaseoli Xanthomonas hortorum pv. carotae
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Real-time PCR Dreo et al., 2007: 100%
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Real-time PCR Dreo et al., 2007: 100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Real-time PCR Dreo et al., 2007: 100%
Test performance study	
Test performance study?	no
The following complementary files are available online:	• rapport validation méthode X.amp 2019

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