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	Identification of Xylella fastidiosa by multilocus sequence typing (MLST) (with BSA) (Yuan et al., 2010)
Date, reference of the validation report	2019-04-01 - Rapport de validation de méthode d'analyse – Apport de la BSA (0,3µg/µL BSA) sur la sensibilité de la PCR Yuan et l'identification de sous-espèce de Xylella fastidiosa par MLST
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?	no
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	identification
Matrix(ces) tested	Leaves, Shoots Plant DNA extract (from petioles, twigs and midribs) hosts in France with most of PCR Yuan inhibitors
Plant species tested	Helichrysum italicum, Lavandula x intermedia, Salvia rosmarinus
Method(s)	Molecular Extraction DNA RNA Molecular Sanger seq
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	no
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 Sanger seq	
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)
Name of the test	Multilocus sequence typing (MLST) (Yuan et al.,2010)
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.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Data from intra-laboratory study performed in 2018 (Anses): - On 3 plant matrices, hosts in France with most of PCR Yuan inhibitors (3 PCR/gene (1 repeat per concentration) during 3 days): range from 106 b/mL to 103 bacteria per ml on undiluted DNA , with and without BSA addition (0,3µg/µl) Conclusion: PCR Yuan sensitivity is variable according to the plant matrix, but use of BSA improves Detection threshold (100%) Helichrysum italicum without BSA 0.3µg/µL: Not reached (68% for 106 bacteria / mL) Undiluted DNA with BSA 0.3µg/µL: 10^4 bacteria / mL (89% for 10^3 bact /mL) Rosmarinus officinalis without BSA 0.3µg/µL: Not detected (86% for 10^6 bacteria / mL) Undiluted DNA with BSA 0.3µg/µL: 10^5 bac / mL (98% for 10^4 bacteria / mL) Lavandula x intermedia without BSA 0.3µg/µL: 10^6 bac / ml (89% for 10^5 bacteria / mL) Undiluted DNA with BSA 0.3µg/µL: 10^4 bac / ml (94% for 10^3 bacteria / mL)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	If criteria calculated for target concentration higher than or equal to the detection threshold: undiluted DNA: 100%; 1/10 diluted DNA: 100% for all genes and plant species.
Standard test(s)	Lavandula x intermedia, Rosmarinus officinalis, Helichrysum italicum spiked with a range from 106 b/mL to 103 bacteria per ml of Xylella fastidiosa subsp mutiplex ST7 (CFBP 8416) before DNA

	extraction step. For each gene, 3 PCR (1 repeat per concentration) with/without BSA for 3 days on undiluted DNA extracts were performed per plant matrix. 630 amplifications per matrix per gene
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Inclusivity tested with 14 target strains Strains: CFBP8068 (ST 41) (multiplex) CFBP8069 (ST 1) (fastidiosa) CFBP7970 (ST 2) (multiplex) CFBP8070 (ST10) (multiplex) CFBP8073 (ST 75) (multiplex) CFBP8074 (ST 74) (pauca) CFBP8075 (ST 27) (multiplex) CFBP8076 (ST 9) (multiplex) CFBP8077 (ST 5) (sandyi) CFBP8356 (ST 72) (sandyi) CFBP8084 (ST 29) (morus) CFBP8416 (ST 7) (multiplex) CFBP8418 (ST 6) (multiplex) CFBP8429 (ST 53) (pauca) CFBP8402 (ST 53) (pauca)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Exclusivity tested with 39 non-target strains (24 plant pathogenic bacteria and 16 saprophytes) Reference strains: CFBP 2351: Pseudomonas syringae pv morsprunorum CFBP 1573: Pseudomonas syringae pv persicae 90 650: Xanthomonas campestris pv campestris CFBP 2058: Xanthomonas translucens pv graminis NCPB 362 : Xanthomonas axonopodis pv juglandis 96 0963 : Xanthomonas carotae 2000 91: Xanthomonas hortorum pv hederae CFBP 2404: Clavibacter michiganensis pv insidiosus CFBP 2157 : Xanthomonas fragariae CFBP 3900 : Xanthomonas arboricola pv pruni CFBP 3529 : Xanthomonas axonopodis pv aurantifolia 2005 267.2 : Pseudomonas fluorescens 2005 0607 : Pseudomonas syringae pv mori 08 00358 : Agrobacterium tumefaciens 08 1161 : Pseudomonas savastanoi pv savastanoi 10 0592: Xanthomonas hortorum pv pelargoni CFBP 2894 : Pseudomonas syringae pv aesculi CFBP 5868 : Xanthomonas axonopodis pv viticola LSV.1.01 : Pseudomonas syringae pv syringae LSV.8.19 : Pseudomonas viridiflava CFBP 1232 : Erwinia amylovora LSV.05.02 : Xylophilus amplelinus 16/306.1 : Bois noir 16/309.1 : Flavescence dorée 6 saprophyte strains isolated from Westringia fruticosa 5 saprophyte strains isolated from Lavandula sp. 5 saprophyte strains isolated from Rosmarinus officinalis
Specificity value	100% Note: After electrophoresis step, bands were present with some strains but not with the expected size of band pairs and not for all genes of one strain. Sequencing of these non-specific amplifications confirmed the absence of cross reaction with the target Xf.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared	Lavandula x intermedia: 100% Rosmarinus officinalis:100% Helichrysum italicum: 100%

to results from a standard test	
Specify the test(s)	3 repeats per gene and matrix, with/without BSA
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	If criteria calculated for target concentration higher than or equal to the detection threshold: - Without BSA : between 56% (Helichrysum/leuA/malF/cysG/gltT) to 100% (others genes Helichrysum; all genes on Rosmarinus, Lavandula) - With 0,3µg/µL BSA: 100% (Helichrysum, Rosmarinus, Lavandula)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	If criteria calculated for target concentration higher than or equal to the detection threshold: -Without BSA : between 70,4% (Helichrysum/malF), 85,2% (Helichrysum/petC/holC/nuoL; Rosmarinus/leuA/petC/malF/cysG/gltT, to 100% (others genes on Helichrysum and Rosmarinus; all genes on Lavandula) - With 0,3µg/µL BSA: 100% (Helichrysum, Rosmarinus, Lavandula)
Test performance study	
Test performance study?	no

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