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	INRA Angers 42 rue Georges MOREL, 49070 Beaucouzé, France
Short description of the test	detection of Xylella fastidiosa by Recombinase polymerase amplification (RPA) in leaf petioles, Shoots
Date, reference of the validation report	2021-12-16 - INRAE_RPA_Xf
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?	Other_project
If yes, please specify	XF actors
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Leaves, Shoots healthy plants : Cistus collected in Corsica at altitude; Prunus, Lavander, oleander, curry plant, olive tree, grapevine collected in orchards surrounding the INRAE center (Angers, France); Citrus X clementina, Citron X limon, Citrus X sinensis, Citrus X reticulata, Citrus X maxima and myrtle leaf milkwort were bought in garden centers.
Plant species tested	Cistus monspeliensis, Citrus maxima, Citrus reticulata, Citrus x aurantium var. clementina, Citrus x aurantium var. sinensis, Citrus x limon, Helichrysum italicum, Lavandula, Nerium oleander, Olea europaea, Polygala myrtifolia, Prunus cerasus, Prunus dulcis, Quercus ilex, Quercus sp., Vitis vinifera
Method(s)	Molecular other
Method: Molecular other	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the	yes

next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Li, R., Russell, P., Zhang, S., Davenport, B., & Eads, A. Development of a rapid and reliable isothermal AmplifyRP® XRT+ diagnostic assay for specific detection of Xylella fastidiosa.
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	AGDIA
Specify the kit used	AmplifyRP® XRT+ for Xf (XCS 34501)
Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex - Probe
Other details on the test	Recombinase polymerase amplification (RPA) is a single tube, isothermal amplification which gives a result on 20 minutes
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	plant crude extracts spiked with Xf: -100% detection at 10^6 cells/mL for Quercus sp. and Vitis vinifera ; -100% detection at 10^5 cells/mL for Prunus dulcis, Prunus cerasifera, Polygala myrtifolia, Citrus sp , Helichrysum italicum, Nerium oleander, Lavandula sp. ; -100% detection at 10^4 cells/mL for Olea europea -Inhibitions of RPA observed with Holm oak and Cistus monspeliensis (60% and 11% detection at 10^6 cells/mL respectively) -bacterial DNA :100% detection at 25,2fg.µL-1 (10 copies/µl or 250 copies/reaction)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	not available
Standard test(s)	qPCR from Harper
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	22 target strains: X. fastidiosa subsp. fastidiosa (4 strains), X. fastidiosa subsp. multiplex (12 strains), X. fastidiosa subsp. pauca (3 strains), X. fastidiosa subsp. morus (1 strain), X. fastidiosa subsp. sandyi (2 strains)
Specificity value	100% positive reactions
Analytical specificity - exclusivity	

Number of non-target organisms tested	30 non-target species (Xanthomonas arboricola pv juglandis CFBP 2528, Xanthomonas arboricola pv. pruni CFBP 2535, Xanthomonas axonopodis pv axonopodis 9.3 CFBP 4924, Xanthomonas campestris pv campestris CFBP 5241, Xanthomonas citri pv aurantifolii 9.6 CFBP 2901, Xanthomonas citri pv citri 9.5 CFBP 2525, Xanthomonas citri pv viticola 9.5 CFBP 7660, Xanthomonas gardneri CFBP 2625, Xanthomonas hortorum pv. pelargonii CFBP 2533, Xanthomonas hyacinthi CFBP 1156, Xanthomonas oryzae pv oryzae CFBP 2532, Xanthomonas translucens pv translucens CFBP 2054, Xanthomonas vasicola pv holcicola CFBP 2543, Xylophilus ampelinus CFBP 1192, Stenotrophomonas maltofilia 13100, Pseudomonas amygdali CFBP 3205, Agrobacterium rubi CFBP 6448, Agrobacterium tumefaciens CFBP 2413, Agrobacterium vitis CFBP 5523, Clavibacter michiganensis subsp. insidiosus CFBP 2404, Dickeya dianthicola CFBP 1200, Ensifer meliloti CFBP 5561, Erwinia amylovora CFBP 1232, Pantoea agglomerans CFBP 3845, Pantoea stewartii subsp. stewartii CFBP 3167, Pseudomonas cerasi CFBP 8305, Pseudomonas syringae pv. persicae CFBP 1573, Pseudomonas syringae pv. syringae CFBP 1392, Rhizobium nepotum CFBP 7436, Xanthomonas alfalfae subsp. citrumelonis 9.2 CFBP 3371 : no positive reaction
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	qPCR from Harper
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	not evaluated
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	10^6 cells/mL: 11% for Cistus 10^4 cells/mL: 20% Quercus ilex, 87% Prunus dulcis, 89% Prunus cerasifera, 22% Polygala myrtifolia, 67% Vitis vinifera, 60% Quercus sp., 47% citrus, 67% Helichrysum italicum, 83% Nerium oleander, 67% Lavandula sp.; 10^3 cells/mL: 20% for Olive tree
Test performance study	
Test performance study?	no

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