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, 49044 Angers, France
Short description of the test	Detection of 'Candidatus Phytoplasma palmae' with an internal control by real time PCR adapted from Cordova et al. (2014)
Date, reference of the validation report	2024-04-15 - RV MA074 v1 - 2024 - version 02
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)	‘Candidatus Phytoplasma palmae' (PHYPPA)
Detection / identification	detection and identification
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
EPPO Diagnostic Protocol name	PM 7/079 Grapevine flavescence dorée phytoplasma (version 2)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes CTAB extraction was optimized to harmonize centrifugation speeds, reduce the process and discard use of toxic reagent beta-mercaptoethanol.
Kit	
Is a kit used	no
Other information	
Other details on the test	CTAB method
Method: Molecular real time PCR	

Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Cordova et al. (2014)
Is the test modified compared to the reference test	yes Adition of an internal control (CyOXID primers from Papayiannis et al, 2021) and optimization for routine analysis in lab conditions
Kit	
Is a kit used	no
Other information	
Reaction type	Duplex
Other details on the test	Target 16SrDNAof 'Ca. P. palmae' and cythochrome oxydase gene of plant as internal control.
Performance Criteria :	
Organism 1.:	**‘Candidatus Phytoplasma palmae'(PHYPPA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	For Cocos nucifera matrix, 15 copies of the 16SrDNA/µL. For Palm matrix, 110 copies of the 16SrDNA/µL
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	23 DNA extracts of different arecacae (Cocos nucifera, Washingtonia sp., Phoenix dactylifera, P. canariensis, P. sylvestris, W. filifera, Trachycarpus fortunei, Adonidia merillii, Red Malayan Dwarf, Sabal palmetto) contaminated by phytoplasmas of the 16SrIV group (subgroups present in the Caraïbeans), ‘Ca. P. palmae’ from different areas in the Carïbeans (Guadeloupe, Florida and Louisiana).
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	17 DNA extracts of healthy arecacea, 8 DNA extracts of plants contaminated phylogenetically close phytoplasma (16SrIV-C group) or that can involved lethal yellowing type syndrom of palm and a DNA extract of Cocos nucifera contamined by Bacillus megaterium.
Specificity value	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	89%
Test performance study	

Test performance study?	no
Other information	
Any other information considered useful	More information can be obtained on request from Anses, Plant Health Laboratory.

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