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	National Institiute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
Short description of the test	Validation report on the testing of phytoplasmas listed in Annex II, Part A of Commission Implementing Regulation (EU) 2021/2285 by real-time PCR.
Date, reference of the validation report	2024-08-22 - Validation report on the testing of phytoplasmas listed in Annex II, Part A of Commission Implementing Regulation (EU) 2021/2285 by real-time PCR.
Link to other validation data	- Validation report on the testing of phytoplasmas listed in Annex II, Part A of Commission Implementing Regulation (EU) 2021/2285 by PCR and nested PCR. Validation report on the testing of phytoplasmas listed in Annex II, Part A of Commission Implementing Regulation (EU) 2021/2285 by PCR and nested PCR.
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?	EURL
If yes, please specify	EURL-Virology (European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas)
Description of the test	
Organism(s)	Phytoplasma (1PHYPG)
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	no
As or adapted from an IPPC diagnostic protocol	no

Reference of the test	Mehle et al., 2013
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	
Other details on the test	Total DNA extracts were eluted in 200 µL elution buffer.
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?	no
EPPO Diagnostic Protocol name	PM 7/133 Generic detection of phytoplasmas (version 1)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	Applied Biosystems
Specify the kit used	TaqMan Universal PCR Master Mix
Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex
Other details on the test	Appendix 3 of EPPO PM7/133(1) (Christensen et al., 2004)
Performance Criteria :	
Organism 1.:	**Phytoplasma(1PHYPG)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Dilutions of: - gBlock FJ914644 (‘Ca. P. aurantifolia’) in a homogenate of healthy potato material - ‘Ca. P. fraxini’ in DNA from leaves of healthy Vitis vinifera - ‘Ca. P. aurantifolia’ in DNA from roots of healthy Malus domestica. LOD: for gBlock FJ914644: 10^-7 for ‘Ca. P. fraxini’: 10^-5 for ‘Ca. P. aurantifolia’: 10^-7
Analytical specificity - inclusivity	

Number of strains/populations of target organisms tested	No. of targets tested: 455 isolates/samples from 11 different 16Sr phytoplasma groups; of which 7 isolates from 4 different 16Sr phytoplasma groups are listed in Annex II, Part A of Commission Implementing Regulation (EU) 2021/2285 In addition: in-silico comparison of the primers-probe sequence with 66 representative sequences of phytoplasmas from Annex II, Part A of Commission Implementing Regulation (EU) 2021/2285 + gBlocks testing.
Specificity value	99.8% The test will not detect all phytoplasmas in the group of palm lethal yellowing phytoplasmas
Analytical specificity - exclusivity	
Number of non-target organisms tested	No. of non-targets tested: 43 (including at least 8 different bacteria species and 16 different plant host species)
Specificity value	100%
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	No. of isolates tested: 3 (for one phytoplasma isolate 3 different dilutions were evaluated) No. of operators: 2 No. of real-time PCR instruments: 2 No. of different days: 6 Percentage of identical results (positive replicates) is 100%.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	No. of samples tested: 3 (high, medium and low target concentration) No. of replicates tested: 3 Percentage of identical results (positive replicates) is 100%.
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	The test was successfully used for the detection of phytoplasmas in various matrices (13 different plant species (leaf veins, root veins) and 3 different vectors). Full validation report is available on the EURL webpage: https://eurlplanthealth.nl/files/view/a154e011-d307-4248-90ed-69d91ce457b8/20240822_phytoplasmas_real-time-pcr_validation-report_nib.pdf

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