

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 Institute 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 PCR and nested 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 PCR and nested 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 real-time PCR. Validation report on the testing of phytoplasmas listed in Annex II, Part A of Commission Implementing Regulation (EU) 2021/2285 by real-time 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
Method(s)	Molecular Extraction DNA RNA Molecular Conventional PCR
Method: Molecular Extraction DNA RNA	
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	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 Conventional 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)
Name of the test	Conventional nested PCR using the primers P1/P7 and R16F2n/R16R2
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Nested
Performance Criteria :	
Organism 1.:	Phytoplasma(1PHYPG)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Dilutions of: -gBlock KP053907 (palm lethal yellowing phytoplasma) in a homogenate of of palm tree leaves (Trachycarpus fortunei) - '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 KP053907: 10 ⁻⁶ (first PCR); for 'Ca. P. fraxini': 10 ⁻³ (first PCR), 10 ⁻⁶ (second PCR); for 'Ca. P. aurantifolia': 10 ⁻⁵ (first PCR), 10 ⁻⁶ (second PCR).
Analytical specificity - inclusivity	
Number of strains/populations of target	No. of targets tested: 10 isolates/samples + 1

organisms tested	gBlock, together representing 6 different 16Sr phytoplasma groups; of which 5 isolates and the gBlock represent phytoplasmas listed in Annex II, Part A of Commission Implementing Regulation (EU) 2021/2285, from 4 different 16Sr phytoplasma groups
Specificity value	First PCR: 90.9 % (one sample failed due too low phytoplasma titer) Second PCR: 100 %
Analytical specificity - exclusivity	
Number of non-target organisms tested	No. of non-targets tested: 9 (including 8 different plant species, in one of these Friedmanniella sp. was confirmed)
Specificity value	First PCR: 100 % Second PCR: 88.9 % (cross-reaction with bacteria Friedmanniella sp)
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 PCR instruments: 3 No. of different days: 6 Percentage of identical results (positive replicates): 100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	No. of samples tested: 3 (for one phytoplasma isolate 3 different dilutions were evaluated) No. of replicates tested: 2 (evaluated 5 times) Percentage of identical results (positive replicates): 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 (6 different plant species – leaf or root veins). Full validation report is available on the EURL webpage: https://eurlplanthealth.nl/files/view/38f43b6f-e8fb-4b24-ad95-6547a56c2620/20240822_phytoplasma_nested-pcr_validation-report_nib.pdf

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