

**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.

<b>Laboratory contact details</b>	National Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
<b>Short description of the test</b>	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.
<b>Date, reference of the validation report</b>	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.
<b>Link to other validation data</b>	- 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.
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	EURL
<b>If yes, please specify</b>	EURL-Virology (European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas)
<b>Description of the test</b>	
<b>Organism(s)</b>	Phytoplasma(1PHYPG)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Conventional PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no

<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Mehle et al., 2013
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	BIONOBILE
<b>Specify the kit used</b>	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA extracts were eluted in 200 µL elution buffer.
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/133 Generic detection of phytoplasmas (version 1)
<b>Name of the test</b>	Conventional nested PCR using the primers P1/P7 and R16F2n/R16R2
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Nested
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Phytoplasma(1PHYPG)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	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 <sup>-6</sup> (first PCR); for 'Ca. P. fraxini': 10 <sup>-3</sup> (first PCR), 10 <sup>-6</sup> (second PCR); for 'Ca. P. aurantifolia': 10 <sup>-5</sup> (first PCR), 10 <sup>-6</sup> (second PCR).
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target</b>	No. of targets tested: 10 isolates/samples + 1

<b>organisms tested</b>	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
<b>Specificity value</b>	First PCR: 90.9 % (one sample failed due too low phytoplasma titer) Second PCR: 100 %
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	No. of non-targets tested: 9 (including 8 different plant species, in one of these Friedmanniella sp. was confirmed)
<b>Specificity value</b>	First PCR: 100 % Second PCR: 88.9 % (cross-reaction with bacteria Friedmanniella sp)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	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: <a href="https://eurlplanthealth.nl/files/view/38f43b6f-e8fb-4b24-ad95-6547a56c2620/20240822_phytoplasma_nested-pcr_validation-report_nib.pdf">https://eurlplanthealth.nl/files/view/38f43b6f-e8fb-4b24-ad95-6547a56c2620/20240822_phytoplasma_nested-pcr_validation-report_nib.pdf</a>

Creation date: 2024-10-07 10:13:04 - Last update: 2024-10-09 09:50:03