

**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 real-time 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 real-time 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 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.
<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 and identification
<b>Matrix(ces) tested</b>	Other Phloem (leaf midrib tissue, petioles, stems, roots)
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time 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>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 real time 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>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>	yes
<b>Manufacturer name</b>	Applied Biosystems
<b>Specify the kit used</b>	TaqMan Universal PCR Master Mix
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	Appendix 3 of EPPO PM7/133(1) (Christensen et al., 2004)
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Phytoplasma(1PHYPG)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	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 <sup>-7</sup> for 'Ca. P. fraxini': 10 <sup>-5</sup> for 'Ca. P. aurantifolia': 10 <sup>-7</sup>

<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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.
<b>Specificity value</b>	99.8% The test will not detect all phytoplasmas in the group of palm lethal yellowing phytoplasmas
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	No. of non-targets tested: 43 (including at least 8 different bacteria species and 16 different plant host species)
<b>Specificity value</b>	100%
<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 real-time PCR instruments: 2 No. of different days: 6 Percentage of identical results (positive replicates) is 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 (high, medium and low target concentration) No. of replicates tested: 3 Percentage of identical results (positive replicates) is 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 (13 different plant species (leaf veins, root veins) and 3 different vectors). Full validation report is available on the EURL webpage: <a href="https://eurlplanthealth.nl/files/view/a154e011-d307-4248-90ed-69d91ce457b8/20240822_phytoplasmas_real-time-pcr_validation-report_nib.pdf">https://eurlplanthealth.nl/files/view/a154e011-d307-4248-90ed-69d91ce457b8/20240822_phytoplasmas_real-time-pcr_validation-report_nib.pdf</a>

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