**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.

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| **Laboratory contact details** | National Institiute of Biology, Department of Biotechnology and Systems Biology<br>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) |
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| **Description of the test** | |
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| **Organism(s)** | Phytoplasma (1PHYPG) |
| **Detection / identification** | detection |
| **Method(s)** | Molecular Extraction DNA RNA<br>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 |

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| **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 |

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| **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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