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	Walloon Agricultural Research Centre (CRA-W) Département Sciences du Vivant Unité Santé des plantes et forêts Bâtiment Marchal Rue de Liroux, 4, 5030 Gembloux, Belgium
Short description of the test	Detection and identification of Phytoplasma Phytoplasma by Molecular Conventional PCR in Leaves
Date, reference of the validation report	2015-02-27 - Detection and identification of Ca. P. phytoplasma in leaves
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Phytoplasma (1PHYPG)
Detection / identification	detection and identification
Method(s)	Molecular Conventional PCR
Method: Molecular Conventional PCR	
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?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Lorenz et al. 1995 (used in appendix 5 of PM 7/62(3)) and Lee et al. 1993, used in Appendix 2 of PM7/133(1))
Is the test modified compared to the reference test	yes PCR primers based on 2 existing tests: Forward : fU5L TTCGGCAATGGAGGAAACTCTGACC (adapted from Lorenz et al. 1995, used in appendix 5 of PM 7/62(3)) Reverse: R16R2 TGACGGGCGGTGTGTACAAACCCCG (Lee et al. 1993, used in Appendix 2 of PM7/133(1))

Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Other details on the test	Reaction volume of 25 µL containing 11.9 µL of water, 5µL of 5x GoTaq G2 Flexi Green buffer (Promega), 2µL of dNTP (2.5mM), 1 µL of each primer (at 20µM), 2µL of MgCl2 (25mM), 0.125µL of GoTaq G2 Flexy taq polymerase (Promega) and 2µL of target DNA extract. The cycling protocol is 2min at 95°C followed by 35 cycles of 94°C for 1min, 68°C for 45sec and 72°C for 2min. A final elongation at 72°C for 10min is then performed
Performance Criteria :	
Organism 1.:	**Phytoplasma(1PHYPG)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	At 100-fold dilution, all samples could be reliably detected when diluted in their original matrix
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Standard test(s)	qPCR from Hodgetts et al. (2009) (Appendix 4 of PM 7/133(1))
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Ca. P. asteris (Potato purple-top phytoplasma) Ca. P. pruni (Peach Western X) Ca. P. ulmi (Elm yellows l) Ca P. vitis (Flavescence dorée FD CAM05) Ca. P. fraxini (Ash yellows 4) Ca. P. mali (AT) Ca. P. mali (14/0346/VI.11) Ca. P. prunorum (14/0346/VI.3) Ca. P. solani (Stolbur C) Plum leptonecrosls (PLNV6)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Catharanthus roseus, Malus domestica, Prunus domestica, Ulmus sp., Vitis vinifera, Prunus persica, Prunus cerasus, Fragaria x ananassa, Ribes rubrum, Morus sp., Pyrus communis, Cydonia oblonga, Solanum tuberosum, Bacillus megaterium
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	qPCR from Hodgetts et al. (2009) (Appendix 4 of PM 7/133(1))
Reproducibility	

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Number of isolates tested: 2 (for one phytoplasma isolate 2 different dilutions were evaluated) Number of operators: 2 Number of PCR instruments: 2 Percentage of identical results (positive replicates) was 100%.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Number of isolates tested: 2 (for one phytoplasma isolate 2 different dilutions were evaluated) Number of replicates tested: 3 Percentage of identical results (positive replicates) was 100%.
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	The test was used as part of a proficiency test organized by the EURL-Virology (PT-2022-02-Phytoplasma) and yielded 100% concordant result. This PT and other analyses carried out since the initial validation extend the specificity of the test to include the following species/strains: - Ca. P. solani (bois noir) - Ca. P. aurentifolia (NIB F 122 & NIB F 123) - Ca. P. phoenicium (NIB F 121) - Ca. P. fraxini (NIB F 119) - Ca. P. australiense (NIB F 120) In addition, in silico analysis suggests that the test should work on all phytoplasma species whose NCBI accession numbers are listed in Bertaccini et al. 2022 (https://doi.org/10.1099/ijsem.0.005353) The test occasionally showed cross-reactity with other Canditatus species in non-plant matrices (e.g. Ca. Sulcia muelleri)

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