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	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	This validation data is for generic detection and identification of phytoplasmas. Phytoplasmas can be detected using conventional nested PCR. The conventional (nested) PCR product is purified and finally sequenced using HTS. For identification see validation sheet 571.
Date, reference of the validation report	2022-07-21 - 2020.molbio.012
Link to other validation data	- 2020.molbio.012 This validation data is for generic detection and identification of phytoplasmas. Phytoplasmas can be detected using real time PCR or conventional nested PCR. The conventional (nested) PCR product is purified and finally sequenced using HTS. - 2020.molbio.012 This validation data is for generic detection of phytoplasmas. Phytoplasmas can be detected using real time PCR. For identification see validation sheet 555. - 2020.molbio.004 v1, 2021.molbio.009 v3 This test can be used for the untargeted detection and identification of molecularly characterized ssRNA(+), ssRNA(-), dsRNA, cssRNA, dsDNA(-RT), ssDNA viruses and viroids in symptomatic plant samples.
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)	'Candidatus Phytoplasma fragariae' (PHYPFG) 'Candidatus Phytoplasma aurantifolia' (PHYPAF) 'Candidatus Phytoplasma brasiliense' (PHYPBR) 'Candidatus Phytoplasma fraxini' (PHYPFR) 'Candidatus Phytoplasma oryzae' (PHYPOR) 'Candidatus Phytoplasma phoenicium' (PHYPPH) 'Candidatus Phytoplasma pruni' (PHYPPN) 'Candidatus Phytoplasma pyri' (PHYPPY) 'Candidatus Phytoplasma solani' (PHYPSO) 'Candidatus Phytoplasma trifolii' (PHYPTR) 'Candidatus Phytoplasma ulmi' (PHYPUL)

	'Candidatus Phytoplasma ziziphi' (PHYPZI) Grapevine flavescence dorée phytoplasma (PHYP64) 'Candidatus Phytoplasma americanum' (PHYPAE) ‘Candidatus Phytoplasma palmicola’ (PHYPPL) 'Candidatus Phytoplasma palmae' (PHYPPA) Phytoplasma (1PHYPG)
Detection / identification	detection and identification
Method(s)	Molecular Extraction DNA RNA Molecular Conventional PCR Molecular HTS Molecular other
Method: Molecular Extraction DNA RNA	
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?	yes
EPPO Diagnostic Protocol name	PM 7/133 Generic detection of phytoplasmas (version 1)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	DNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	yes
Other information	
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	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 12 DP 12: Phytoplasmas (version

	2016)
Name of the test	Conventional nested PCR
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Nested
Method: Molecular HTS	
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	Addendum – New Supporting information for PM 7/151 Considerations for the use of high throughput sequencing in plant health diagnostics, appendix 1.
Is the test modified compared to the reference test	no
Other information	
Other details on the test	The same pipeline for viruses and viroids is used to detect phytoplasmas. See data validation 512 and Addendum – New Supporting information for PM 7/151 Considerations for the use of high throughput sequencing in plant health diagnostics, appendix 1.
Method: Molecular other	
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	QIAquick PCR Purification Kit (Qiagen)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	QIAquick PCR Purification Kit (Qiagen)

Kit used following the manufacturer's instructions?	yes
Other information	
Other details on the test	Purification of PCR-product before sequencing (HTS)
Performance Criteria :	
Organism 1.:	**'Candidatus Phytoplasma fragariae'(PHYPFG)**
Organism 2.:	**'Candidatus Phytoplasma aurantifolia'(PHYPAF)**
Organism 3.:	**'Candidatus Phytoplasma brasiliense'(PHYPBR)**
Organism 4.:	**'Candidatus Phytoplasma fraxini'(PHYPFR)**
Organism 5.:	**'Candidatus Phytoplasma oryzae'(PHYPOR)**
Organism 6.:	**'Candidatus Phytoplasma phoenicium'(PHYPPH)**
Organism 7.:	**'Candidatus Phytoplasma pruni'(PHYPPN)**
Organism 8.:	**'Candidatus Phytoplasma pyri'(PHYPPY)**
Organism 9.:	**'Candidatus Phytoplasma solani'(PHYPSO)**
Organism 10.:	**'Candidatus Phytoplasma trifolii'(PHYPTR)**
Organism 11.:	**'Candidatus Phytoplasma ulmi'(PHYPUL)**
Organism 12.:	**'Candidatus Phytoplasma ziziphi'(PHYPZI)**
Organism 13.:	**Grapevine flavescence dorée phytoplasma(PHYP64)**
Organism 14.:	**'Candidatus Phytoplasma americanum'(PHYPAE)**
Organism 15.:	**‘Candidatus Phytoplasma palmicola’(PHYPPL)**
Organism 16.:	**'Candidatus Phytoplasma palmae'(PHYPPA)**
Organism 17.:	**Phytoplasma(1PHYPG)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Grapevine flavescence doree phytoplasma is detected at 10^3 dilution in the real time PCR (Hodgetts et al. 2009). GFDP is detected at 10^2 dilution in the conventional nested PCR (Lee at al., 1993 and Gundersen & Lee, 1996).
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	see annex 4 (validation report) and table 1 in (addendum). Ca. Phytoplasma aurantifolia Ca. Phytoplasma aurantifolia-related strain SPLL Ca. Phytoplasma brasiliense Ca. Phytoplasma fraxini-reference strain Ca. Phytoplasma oryzae Ca. Phytoplasma phoenicium Ca. Phytoplasma pruni Ca. Phytoplasma pyri Ca. Phytoplasma solani Ca. Phytoplasma trifolii Ca. Phytoplasma ulmi Ca. Phytoplasma ulmi Ca. Phytoplasma ziziphi Grapevine flavescence doree phytoplasma

	Grapevine flavescence doree phytoplasma GFDP Map-FD1 Grapevine flavescence doree phytoplasma GFDP Map-FD2 Grapevine flavescence doree phytoplasma GFDP Map-FD2 PEY05 Grapevine flavescence doree phytoplasma GFDP Map-FD3 Ca. Phytoplasma americanum Ca. Phytoplasma palmae Ca. Phytoplasma palmicola
Specificity value	100
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	see PM 7/079 (2) for grapevine flavescence doree phytoplasma.
The following complementary files are available online:	• Validation report • Validation report annex • Validation report addendum • 2020.molbio.012 Detection and identification of Candidatus Phytoplasma fragariae V1.0

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