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 detailsNetherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, NetherlandShort description of the testThis validation data is for generic detection and identification of phytoplasmas. Phytoplasmas car be detected using conventional nested PCR. The conventional (nested) PCR product is purified and finally sequenced using HTS. For identification set validation sheet 571.Date, reference of the validation report2022-07-21 - 2020.molbio.012Link to other validation data- 2020.molbio.012 This validation data is for generic detection of phytoplasmas. Phytoplasmas can be detected using real time PCR. For identification see validation neeted PCR. The conventional (nested) PCR product is purified and finally sequenced using real time PCR. For identification see validation data is for generic detection and identification of phytoplasmas. Phytoplasmas can be detected using real time PCR. The conventional (nested) PCR product is purified and finally sequenced using HTS. - 2020.molbio.004 v1, 2021.molbio.009 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		
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Is the lab accredited for this test? yes	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	Was the validated data generated in the framework of a project?	no

Description of the test

Organism(s) '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' (PHYPPN) 'Candidatus Phytoplasma solani' (PHYPPSO) 'Candidatus Phytoplasma trifolii' (PHYPTR) 'Candidatus Phytoplasma ulmi' (PHYPTR) 'Candidatus Phytoplasma ulmi' (PHYPTR)

	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 aurantifolia'(PHYPAF)	
Organism 2.:	'Candidatus Phytoplasma brasiliense'(PHYPBR)	
Organism 3.:	'Candidatus Phytoplasma fraxini'(PHYPFR)	
Organism 4.:	'Candidatus Phytoplasma oryzae'(PHYPOR)	
Organism 5.:	'Candidatus Phytoplasma phoenicium'(PHYPPH)	
Organism 6.:	'Candidatus Phytoplasma pruni'(PHYPPN)	
Organism 7.:	'Candidatus Phytoplasma pyri'(PHYPPY)	
Organism 8.:	'Candidatus Phytoplasma solani'(PHYPSO)	
Organism 9.:	'Candidatus Phytoplasma trifolii'(PHYPTR)	
Organism 10.:	'Candidatus Phytoplasma ulmi'(PHYPUL)	
Organism 11.:	'Candidatus Phytoplasma ziziphi'(PHYPZI)	
Organism 12.:	Grapevine flavescence dorée phytoplasma(PHYP64)	
Organism 13.:	'Candidatus Phytoplasma americanum'(PHYPAE)	
Organism 14.:	'Candidatus Phytoplasma palmicola'(PHYPPL)	
Organism 15.:	'Candidatus Phytoplasma palmae'(PHYPPA)	
Organism 16.:	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:	 <u>Validation report</u> <u>Validation report annex</u> <u>Validation report addendum</u> 	

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