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	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
Short description of the test	Detection of grapevine phytoplasmas of the 16SrV and 16SrXII-A groups
Date, reference of the validation report	2021-05-18 - Pelletier at al., 2009. Triplex real-time PCR assay for sensitive and simultaneous detection of grapevine phytoplasmas. Vitis 48(2), 87-95.
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?	
Description of the test	
Organism(s)	Grapevine flavescence dorée phytoplasma(PHYP64) 'Candidatus Phytoplasma solani'(PHYPSO)
Detection / identification	detection
Method(s)	Molecular real time PCR
Method: Molecular real time 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?	yes
EPPO Diagnostic Protocol name	PM 7/079 Grapevine flavescence dorée phytoplasma (version 2)
Name of the test	Multiplex real-time PCR according to Pelletier et al. (2009)
As or adapted from an IPPC diagnostic protocol	no
Other information	
Reaction type	Triplex - Probe
Are the performance characteristics included in the EPPO diagnostic protocol?	**yes**

Performance Criteria :	
Organism 1.:	**Grapevine flavescence dorée phytoplasma(PHYP64)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	In our condition, FD: to a dilution of 5^7 of a FD infected sample in water (100 times more sensitive than nested PCR)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% for each target
Standard test(s)	For FD: 4 samples agreement/4
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	samples for FD: FD (CAM-05) type FD1/V. faba (Gironde, France) FD (PEY-05) type FD2/V. faba (Gironde, France) FD (VI04-Lig2) type FD3/V. vinifera (Veneto, Italy) FD (VI04-C28) type FD3/V. vinifera (Veneto, Italy)
Specificity value	100% for each target
Analytical specificity - exclusivity	
Number of non-target organisms tested	Healthy C. roseus Healthy V. faba Healthy V. vinifera cv Pinot noir Healthy V. vinifera cv Gewurztraiminer Healthy V. vinifera cv Chardonnay Healthy V. vinifera cv Riesling Healthy V. vinifera cv Cabernet Franc Healthy V. vinifera cv Cabernet sauvignon 16SrI- Aster yellow (AY Whitcomb)/ C. roseus (USA) 16SrI - Clover phyllody (KVF)/C. roseus (France) 16SrII - Tomato big bud (TBB)/C. roseus (Australia) 16SrII - Whitches' broom disease of lime (WBDL)/C. roseus (Oman Sultanate) 16SrIII - Peach western X (Peach WX)/C. roseus (USA) 16SrVI - Brinjal little leaf (BLL)/C. roseus (India) 16SrVII - Ash yellows (Ash 12)/C. roseus (USA) 16SrX - Apple proliferation (AP-15)/C. roseus (Italy) 16SrX - European stone fruit yellows (ESFY)/C. roseus (Italy) 16SrX - Pear decline (PD)/C. roseus (Germany)
Specificity value	other phytoplasmas of the 16SrV group can be detected: PGY (PGYA et PGYC), GY (V04-11-1), AldY (ALY), RS, Spa W
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% for each target
Specify the test(s)	For FD: 29 samples agreement/29
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For FD: 98.72%

Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For FD: 99 to 100%
Organism 2.:	**'Candidatus Phytoplasma solani'(PHYPSO)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	In our condition, BN: to a dilution of 5^4 of a BN infected sample in water (5 times more sensitive than nested PCR)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% for each target
Standard test(s)	For BN: 11 samples agreement/11
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Samples for BN: Stolbur (P7)/C. roseus (Lebanon) Stolbur (Moliere)/C. roseus (France) Stolbur (Charente-1)/C. roseus (Charente, France) Stolbur (Charente-2)/C. roseus (Charente, France) Stolbur (LG)/C. roseus (Lot et Garonne, France) Stolbur (C)/C. roseus (France) Stolbur (PO)/C. roseus (Pyrénées Orientales, France) Stolbur (Red-Pepper)/C. roseus (Serbia) VK (GGY)C. roseus (Pfalz, Germany) VK (19-25)/C. roseus (Pfalz, Germany) BN (CH1)C. roseus (Italy)
Specificity value	100% for each target
Analytical specificity - exclusivity	
Number of non-target organisms tested	Healthy C. roseus Healthy V. faba Healthy V. vinifera cv Pinot noir Healthy V. vinifera cv Gewurztraiminer Healthy V. vinifera cv Chardonnay Healthy V. vinifera cv Riesling Healthy V. vinifera cv Cabernet Franc Healthy V. vinifera cv Cabernet sauvignon 16SrI- Aster yellow (AY Whitcomb)/ C. roseus (USA) 16SrI - Clover phyllody (KVF)/C. roseus (France) 16SrII - Tomato big bud (TBB)/C. roseus (Australia) 16SrII - Whitches' broom disease of lime (WBDL)/C. roseus (Oman Sultanate) 16SrIII - Peach western X (Peach WX)/C. roseus (USA) 16SrVI - Brinjal little leaf (BLL)/C. roseus (India) 16SrVII - Ash yellows (Ash 12)/C. roseus (USA) 16SrX - Apple proliferation (AP-15)/C. roseus (Italy) 16SrX - European stone fruit yellows (ESFY)/C. roseus (Italy) 16SrX - Pear decline (PD)/C. roseus (Germany)
Specificity value	other phytoplasmas of the 16SrV group can be detected: PGY (PGYA et PGYC), GY (V04-11-1), AldY (ALY), RS, Spa W
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% for each target

Specify the test(s)	For BN: 30 samples agreemnt/30
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For BN: 94.87%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For BN: 92.31 to 100%
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	Ring-tested during the GRAFDEPI (Euphresco projet), 6 laboratories tested this method on a total of 15 participants. Results obtained for FD detection: - Accuracy: 96.27% - Diagnostic sensitivity: 97.75% - Diagnostic specificity: 93.33% - Repeatability: 94.93% - Reproducibility: 93.27% Loiseau, M. (2015). European interlaboratory comparison of detection methods for “flavescence dorée” phytoplasma: preliminary results. Phytopathogenic Mollicutes, 5(1s), S35-S37.
Other information	
Any other information considered useful	other validation data available on request at the Plant Health Laboratory of ANSES (ANSES-LSV, France)

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