EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

Target Organism	Grapevine flavescence dorée phytoplasma Stolbur phytoplasma	
Short description	Detection of grapevine phytoplasmas of the 16SrV and 16SrXII-A groups	
Laboratory contact details	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France	
Date and reference of the validation report	- 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 PM 7/98:	Yes	
Reference of the test description	PM 7/079(1) Pelletier at al., 2009. Triplex real-time PCR assay for sensitive and simultaneous detection of grapevine phytoplasmas. Vitis 48(2), 87-95.	
Is the test the same as described in the EPPO DP?	Modified Pelletier at al., 2009. Triplex real-time PCR assay for sensitive and simultaneous detection of grapevine phytoplasmas. Vitis 48(2), 87-95.	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Vitis sp.	
Matrices tested (if relevant)	petioles	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	real-time PCR
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		

Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
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) 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	
Specify the standard test	For FD: 4 samples agreement/4 For BN: 11 samples agreement/11	
Analytical specificity		
Specificity value	100% for each target	
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) 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)	
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)
Cross reacts with (specify the species)	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 standard test	For FD: 29 samples agreement/29 For BN: 30 samples agreemnt/30
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For FD: 98.72% For BN: 94.87%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For FD: 99 to 100% For BN: 92.31 to 100%
Test performance study	
Test performance study?	Yes
Include brief details of the test performance study and its output.It available, provide a 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 **e.g. robustness, ease of performing the test, etc.**	other validation data available on request at the Plant Health Laboratory of ANSES (ANSES-LSV, France)