EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

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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
Short description	Simoultaneous detection of FD and BN phytoplasmas by multiplex nested-PCR
Laboratory contact details	EUPHRESCO-GRAFDEPI Via Carlo Giuseppe Bertero, 22, 00156 ROMA, Italy
Date and reference of the validation report	Project EUPHRESCO GRAFDEPI Final Report 2014-07-31 - 1)http://www.euphresco.net/media/project_reports/grafdepi_final_report.pdf 2)The Euphresco Grafdepi Group, 2015. European interlaboratory comparison of detection methods for “flavescence dorée” phytoplasma: preliminary results. Phytopathogenic Mollicutes doi: 10.5958/2249-4677.2015.00015.8 Vol. 5 (1-Supplement), January 2015, S35-S37
Validation process according to EPPO Standard PM 7/98:	Yes
Reference of the test description	PM 7/079(1) 1) Daire, X., Clair, D., Reinert, W., & Boudon-Padieu, E., 1997. European Journal of Plant Pathology, 103(6), 507-514. 2) Angelini, E, Clair, D., Borgo, M., Bertaccini, A., & Boudon-Padieu, E., 2001. Vitis, 40(2), 79-86. 3) Clair, D., Larrue, J., Aubert, G., Gillet, J., Cloquemin, G., & Boudon-Padieu, E., 2003. Vitis, 42(3), 151-157.
Is the test the same as described in the EPPO DP?	Yes
Is the lab accredited for this test?	No
	The samples had been provided by different partners of Project GRAFDEPI and belonged to different plant host species. The homogenising and preparation were performed by ANSES-LSV (France). The samples consisted in DNA extracts. The batches’ selection was based on methodology proposed in PM7/98 for the evaluation of the performance criteria of analytical methods. Positive samples were from different parts of Europe in order to have a wide diversity of strains for testing the inclusivity of methods. Phytoplasmas in the same group and/or infecting grapevines were also chosen to test different degrees of specificity/exclusivity of methods. 13 samples were negative for the Flavescence dorée phytoplasma. 4 of

Plant species tested (if relevant)	them were healthy Vitis sp.. The other ones were other phytoplasmas of 16SrV group and phytoplasmas from other groups, mixed with DNA extract of healthy grapevine to reach the volume necessary for the ring-test. 11 samples were positive for the Flavescence dorée phytoplasma. They were DNA extracts of Vitis sp. tested positive by PCR for Flavescence dorée phytoplasmas pure or mixed with different quantities of healthy grapevine or mixed with DNA extracts positive for the 16SrXII group phytoplasmas. When it was possible, supernumerary fractions were produced for each sample to validate their status and for testing the homogeneity of the division during the preparation of tubes for the participants. Then, these supernumerary fractions were randomly chosen in the series of tubes. DNA extracts were amplified in real-time triplex PCR (Pelletier et al., 2009). See Appendix.	
Matrices tested (if relevant)		
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	Multiplex with a direct PCR with primers: - FD9f1/r1 and STOL11f2/1r1 followed by a nested PCR with primers: - FD9f3b/FD9r2 - STOL11f3/STOL11r2
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		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	The analytical sensitivity was calulated in five laboratories analyzing three samples at five dilution levels (1/10; 1/100; 1/300; 1/900; 1/2700) in five repetitions. Samples (DNA extracts) came from IPEP (Serbia), ACW (Switzerland) and ANSES (France). The homogenising and preparation were performed by ANSES-LSV (France) Two values are provided: The last dilution level with 100% positive results: less than 1/10 (for all samples) The last dilution level with, at least, one positive result for each sample: 1/900 (Sample A) 1/2700 (Samples B and C)	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	13 laboratories were involved in performing this method within the ringtest. The results of one Partner have been removed because all samples were positive although the test was repeated and the controls were compliant. Determined in 11 samples positive for Flavescence dorée phytoplasma. They were DNA extracts of Vitis sp. tested positive by PCR for FD pure or mixed with different quantities of healthy grapevine or mixed with DNA extracts positive for the 16SrXII group phytoplasmas. Within the ringtest 7 diagnostic methods were compared. Diagnostic sensitivity: 83.72% False negative: (28/288) 9.7%	
Specify the standard test	Other protocols included in the ringtest: - Detection of Flavescence dorée phytoplasma by universal direct PCR and nested 16SrV-group specific PCR - Detection and identification of Flavescence dorée phytoplasma by direct and nested PCR followed by RFLP with Taq I (Martini et al., 1999) - Simplex real time PCR for the detection of FD and BN phytoplasmas with an internal control for grapevine (Angelini et al., 2007) - Simplex real time PCR for the detection of FD and BN phytoplasmas with an internal control - (Hren et al., 2007) - Triplex real-time PCR for simultaneous FD and BN phytoplasmas detection with an internal control for grapevine. (Pelletier et al., 2009) - Triplex real time PCR for simultaneous FD and BN phytoplasmas detection with an internal control - (under patent IPADLAB)	
<u>Analytical specificity</u>		
Specificity value		
Number of		

strains/populations of target organisms tested	
Number of non-target organisms tested	
Cross reacts with (specify the species)	
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	The method was performed by 13 laboratories within the ringtest, but the result of one partner has been removed because all samples were positive although the test was repeated and the controls were compliant. The total analysed results was 288. 13 non target samples: 4 healthy grapevines and 9 were other phytoplasmas of 16SrV group and phytoplasmas from other groups. Diagnostic specificity: 92.38%
Specify the standard test	Other protocols included in the ringtest: - Detection of Flavescence dorée phytoplasma by universal direct PCR and nested 16SrV-group specific PCR - Detection and identification of Flavescence dorée phytoplasma by direct and nested PCR followed by RFLP with Taq I (Martini et al., 1999) - Simplex real time PCR for the detection of FD and BN phytoplasmas with an internal control for grapevine (Angelini et al., 2007) - Simplex real time PCR for the detection of FD and BN phytoplasmas with an internal control - (Hren et al., 2007) - Triplex real-time PCR for simultaneous FD and BN phytoplasmas detection with an internal control for grapevine. (Pelletier et al., 2009) - Triplex real time PCR for simultaneous FD and BN phytoplasmas detection with an internal control - (under patent IPADLAB)
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The reproducibility was calulated in five laboratories analyzing three samples at five dilution levels (1/10; 1/100; 1/300; 1/900; 1/2700) in five repetitions. Samples (DNA extracts) came from IPEP (Serbia), ACW (Switzerland) and ANSES (France). The homogenising and preparation were performed by ANSES-LSV (France). Reproducibility: 60.19%
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The repeatability was calulated in five laboratories analyzing three samples at five dilution levels (1/10; 1/100; 1/300; 1/900; 1/2700) in five repetitions. Samples (DNA extracts) came from IPEP (Serbia), ACW (Switzerland) and ANSES (France). The homogenising and preparation were performed by ANSES-LSV (France) Repeatability: 92.53%
<u>Test performance study</u>	
Test performance	Yes

study?	Yes
Include brief details of the test performance study and its output.It available, provide a link to published article/report	Interlaboratory comparison among 15 laboratories within the EUPHRESCO Project GRAFDEPI (CRA-PAV, Italy; AGES, Austria; CRA-W, Belgium, PPRS, Turkey; INIAV, Portugal; ACW, Switzerland; ILVO, Belgium; DIPSA, Bologna Italy; DISAA, Milan Italy; IPEP, Serbia; NIB, Slovenia; IRTA, Spain; ANSES, France; Cra-VIT, Italy)
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	The ringtest was carried out by 15 laboratories and it is not possible to state if any of them is accredited for this test.
The following complementary files are available online:	• Samples for determination of performance criteria