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	EUPHRESCO-GRAFDEPI Via Carlo Giuseppe Bertero, 22, 00156 ROMA, Italy
Short description of the test	Simplex real time PCR for the detection of FD and BN phytoplasmas with an internal control - Hren et al., 2007
Date, reference of the validation report	2014-07-31 - Project EUPHRESCO GRAFDEPI Final Report - 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 PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	Euphresco
If yes, please specify	GRAFDEPI
Description of the test	
Organism(s)	Grapevine flavescence dorée phytoplasma(PHYP64)
Detection / identification	detection
Matrix(ces) tested	Other 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 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 1 / 4 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
Plant species tested	Vitis sp.
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?	
EPPO Diagnostic Protocol name	PM 7/079 Grapevine flavescence dorée phytoplasma (version 2)
Name of the test	Real-time PCR according to Hren et al. (2007)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	Simplex
Other details on the test	A TaqMan real time PCR methodology performed with specific primers and probes for the detection of FD and BN phytoplasmas and an internal control (Human 18S rRNA). Detection of the single targets must be performed in separate reactions.
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?	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: 1/2700 (for all samples)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Ten laboratories performed this protocol within GRAFDEPI ringtest with a total of 240 results. 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: 97.28% False negative: (4/240) 1.7%
Standard test(s)	Other protocols included in the ringtest: - Simoultaneous detection of FD and BN phytoplasmas by multiplex nested-PCR (Dairè et al., 1997; Angelini et al., 2001; Clair et al., 2003) - 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) - 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)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	
Specificity value	
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared	Ten laboratories performed this protocol within GRAFDEPI ringtest with a total of 240 results. 13

to results from a standard test	non target samples: 4 healthy grapevines and 9 were other phytoplasmas of 16SrV group and phytoplasmas from other groups. Diagnostic specificity: 94.12%
Specify the test(s)	Other protocols included in the ringtest: - Simoultaneous detection of FD and BN phytoplasmas by multiplex nested-PCR (Dairè et al., 1997; Angelini et al., 2001; Clair et al., 2003) - 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) - 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)
Reproducibility	
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: 84.9%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The repeatability was calculated in five laboratories analysing 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: 91.04%
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, 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	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:	• EUPHRESCO-GRAFDEPI Samples for determination of performance criteria

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