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	Triplex real-time PCR for simultaneous detection of FD and BN phytoplasmas and an internal control for grapevine. - Pelletier et al., 2009
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
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
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
Other details on the test	A TaqMan real time PCR methodology performed with specific primers and probes for the simoultaneous detection of FD and BN phytoplasmas and a grapevine internal control (Grapevine chloroplast trnL-F spacer). The detection of the three targets is carried out in a single reaction.
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 calculated in five laboratories (but the results of one of them has been removed because a problem in the double detection of FAM and VIC) 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). Two values are provided: The last dilution level with 100% positive results: 1/100 (sample B); 1/2700 (samples A and C) 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	Eight laboratories performed this protocol within GRAFDEPI ringtest but the results of two Partners have been removed for different technical problems. The total result analysed were 144. 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: 97.75% False negative: (2/144) 1.4%
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) - 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 - (under patent IPADLAB)
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Eight laboratories performed this protocol within GRAFDEPI ringtest but the results of two Partners have been removed for different technical problems. The total result analysed were 144.13 non target samples: 4 healthy grapevines and 9 were other phytoplasmas of 16SrV group and phytoplasmas from other groups. Diagnostic specificity: 93.33%
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) - 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 - (under patent IPADLAB)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The reproducibility was calculated in five laboratories (but the results of one of them have been removed because of a problem in the double detection of FAM and VIC) 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). Reproducibility: 93.27%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The repeatability was calculated in five laboratories (but the results of one of them have been removed because of a problem in the double detection of FAM and VIC) 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: 94.93%

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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