

**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.

<b>Laboratory contact details</b>	EUPHRESKO-GRAFDEPI Via Carlo Giuseppe Bertero, 22, 00156 ROMA, Italy
<b>Short description of the test</b>	Simultaneous detection of FD and BN phytoplasmas by multiplex nested-PCR
<b>Date, reference of the validation report</b>	2014-07-31 - Project EUPHRESKO GRAFDEPI Final Report - 1) <a href="http://www.euphresco.net/media/project_reports/grafdepi_final_report.pdf">http://www.euphresco.net/media/project_reports/grafdepi_final_report.pdf</a> 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
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Euphresco
<b>If yes, please specify</b>	GRAFDEPI
<b>Description of the test</b>	
<b>Organism(s)</b>	Grapevine flavescence dorée phytoplasma (PHYP64)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	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 <i>Vitis</i> 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
<b>Plant species tested</b>	Vitis sp.
<b>Method(s)</b>	Molecular Conventional PCR
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/079 Grapevine flavescence dorée phytoplasma (version 1)
<b>Name of the test</b>	Multiplex nested-PCR for simultaneous FD and BN detection (Clair et al. 2003)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Multiplex (>3) - Nested
<b>Other details on the test</b>	Multiplex with a direct PCR with primers: - FD9f1/r1 and STOL11f2/1r1 followed by a nested PCR with primers: - FD9f3b/FD9r2 - STOL11f3/STOL11r2
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	no
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Grapevine flavescence dorée phytoplasma(PHYP64)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	The analytical sensitivity was calculated 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)
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	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%
<b>Standard test(s)</b>	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)
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	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%
<b>Specify the test(s)</b>	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)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	The reproducibility was calculated 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%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	The repeatability was calculated 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%
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Interlaboratory comparison among 15 laboratories within the EUPHRESKO 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)
<b>Other information</b>	
<b>Any other information considered useful</b>	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:	<ul style="list-style-type: none"> <li>• <a href="#">EUPHRESKO-GRAFDEPI Samples for determination of performance criteria</a></li> </ul>

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