

**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>	Detection and identification of Flavescence dorée phytoplasma by direct and nested PCR followed by RFLP
<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>Method(s)</b>	Molecular PCR-RFLP
<b>Method: Molecular PCR-RFLP</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	- Martini, M.; Murari, E.; Mori, N.; Bertaccini, A.; 1999. Plant Disease 83, 925-930. - Deng, S.; Hiruki, C.; 1991. Journal of Microbiological Methods 14, 53 - 61) - Schneider B., Seemüller E., Smart C. D., Kirkpatrick B. C., 1995. In: Razin S. and Tully J. G. (ed.). Molecular and Diagnostic Procedures in

	Mycoplasmology 2: 369-380. New York: Academic Press. -Gibb, K. S.; Padovan, A. C.; Mogen, B. D.; 1995. Phytopathology 85, 169-174. - Padovan, A. C.; Gibb, K. S.; Bertaccini, A.; Vibio, M.; Bonfiglioli, R. G.; Magarey, P. A.; Sears, B. B.; 1995. Australian Journal of Grape and Wine Research 1, 25-31.
<b>Other information</b>	
<b>Reaction type</b>	Nested
<b>Other details on the test</b>	Detection and identification of Flavescence dorée phytoplasmas by a direct universal PCR with primers P1/P7, followed by a nested universal PCR with primers 16R758f (M1)/M23SR1804r (B6) and RFLP analysis of nested amplicons after digestion with TaqI restriction enzyme
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Grapevine flavescence dorée phytoplasma(PHYP64)</b>
<b>Analytical sensitivity</b>	
<b>What is 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: 1/2700 (for all samples)
<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>	Six laboratories were involved in performing this method within the ringtest. The results of one Partner have been removed because the RFLP analysis was not possible. A total of 120 results has been analysed. 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: 88.89% False negative: (8/120) 6.7%
<b>Standard test(s)</b>	Other protocols included in the ringtest: - Simultaneous 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 - Simplex real time PCR for the detection of FD and BN phytoplasmas with an

	<p>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)</p>
<b>Diagnostic Specificity</b>	
<p><b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b></p>	<p>Six laboratories were involved in performing this method within the ringtest. The results of one Partner have been removed because the RFLP analysis was not possible. A total of 120 results has been analysed. 13 non target samples: 4 healthy grapevines and 9 were other phytoplasmas of 16SrV group and phytoplasmas from other groups. Diagnostic specificity: 93.18%</p>
<p><b>Specify the test(s)</b></p>	<p>Other protocols included in the ringtest: - Simultaneous 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 - 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)</p>
<b>Reproducibility</b>	
<p><b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b></p>	<p>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: 67.73%</p>
<b>Repeatability</b>	
<p><b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b></p>	<p>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: 77.60%</p>
<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>	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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