

**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>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
<b>Short description of the test</b>	Identification of Grapevine flavescence dorée phytoplasma - Nested-PCR 16SrV map adapted from Arnaud et al. (2007) followed by sequence analysis
<b>Date, reference of the validation report</b>	2023-11-13 - RV FDmapVF 6 V01 - Novembre 2023
<b>Link to other validation data</b>	- Loiseau (2023). Interlaboratory test Validation of methods for the identification of Flavescence dorée phytoplasma sensu stricto Report - 22FD - version N°01 Identification of Grapevine flavescence dorée phytoplasma - Nested-PCR 16SrV map adapted from Rossi et al. (2019) and Malembic-Maher et al. (2020) followed by sequence analysis
<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>	FLADOVIGILANT
<b>Description of the test</b>	
<b>Organism(s)</b>	Grapevine flavescence dorée phytoplasma (PHYP64)
<b>Detection / identification</b>	identification
<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>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Arnaud et al. (2007)

<b>Is the test modified compared to the reference test</b>	yes The sequence of the forward primer of the second PCR, FD9-F6, different because one SNP (T/C) has been evidenced for some genotypes. PCR conditions adapted for routine analysis.
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Nested
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Grapevine flavescence dorée phytoplasma(PHYP64)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Last level at 100% positive results: $1 \times 10^{-1}$ Last level with positive result(s): $2 \times 10^{-3}$
<b><u>Diagnostic sensitivity</u></b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	87.1%
<b>Standard test(s)</b>	triplex real-time PCR adapted from Pelletier et al. (2009)
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	15 samples positive for FD (VmpA-II and VmpA-III, M54 from different European countries, M38, M50, M51 and a variant, M122, M12, M36)
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	15 non target samples including Palatinate Grapevine Yellows (M53 and M46), 'Candidatus Phytoplasma rubi', 'Ca. P. solani', Alder Yellows phytoplasma, North American Grapevine Yellows, 'Ca. P. australiense', 'Ca. P. australasia', 'Ca. P. asteris'-related strain and healthy grapevine.
<b>Specificity value</b>	100%
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	82.2%
<b>Specify the test(s)</b>	triplex real-time PCR adapted from Pelletier et al. (2009)
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	93%
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a</b>	Between 86 and 100%

given level of the pest (see PM 7/98)	
<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>	<p>The samples subject to the Grafdepi TPS were DNA samples: 30 samples infected by grapevine flavesence dorée phytoplasma, 4 healthy grapevines and 26 samples infected by other phytoplasmas. Diagnostic sensitivity: 87.1% Twenty-two false negative results (FN) were generated with this method. Those FN are not reproducible i.e. they do not correspond to the same samples between participants. Thus, it is not a problem of inclusivity of the method but more a problem of reproducibility. Diagnostic specificity: 82.7% Eleven false positive results (FP) have been obtained after PCR and 19 after sequencing. However, those FP are not reproducible. Thus, it is not a problem of exclusivity of the method but it is more probably linked to problems of microcontaminations inherent in nested-PCR methods and/or problems in interpretation of the sequences. Three per cent of the participants' responses were inconclusive.</p>
<b>Other information</b>	
<b>Any other information considered useful</b>	More information can be obtained on request to Anses Plant health laboratory.

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