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	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
Short description of the test	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
Date, reference of the validation report	2023-04-03 - Loiseau (2023). Interlaboratory test Validation of methods for the identification of Flavescence dorée phytoplasma sensu stricto Report - 22FD - version N°01
Link to other validation data	- RV FDmapVF 6 V01 - Novembre 2023 Identification of Grapevine flavescence dorée phytoplasma - Nested-PCR 16SrV map adapted from Arnaud et al. (2007) followed by sequence analysis
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	FLADOVIGILANT
Description of the test	
Organism(s)	Grapevine flavescence dorée phytoplasma (PHYP64)
Detection / identification	identification
Method(s)	Molecular Conventional PCR
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Rossi et al. (2019) and Malembic-Maher et al. (2020)

Is the test modified compared to the reference test	yes PCR conditions adapted for routine analysis.
Kit	
Is a kit used	no
Other information	
Reaction type	Nested
Performance Criteria :	
Organism 1.:	**Grapevine flavescence dorée phytoplasma(PHYP64)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Last level at 100% positive results: none Last level with positive result(s): 2x10^-3
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	61.6% for vectotype II and 75.8% for vectotype III
Standard test(s)	triplex real-time PCR adapted from Pelletier et al. (2009)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	15 samples positive for FD (VmpA-II and VmpA-III, M54 from different European countries, M38, M50, M51 and a variant, M122, M12, M36)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	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.
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	95.3% for vectotype II and 97.4% for vectotype III
Specify the test(s)	triplex real-time PCR adapted from Pelletier et al. (2009)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	81%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Between 78 and 98%

Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	The samples subject to the Grafdepi TPS were DNA samples: 30 samples infected by grapevine flavescence dorée phytoplasma, 4 healthy grapevines and 26 samples infected by other phytoplasmas. Diagnostic sensitivity: 61.6% for vectotype II and 75.8% for vectotype III Diagnostic specificity: 95.3% for vectotype II and 97.4% for vectotype III Forty false negative (FN) results were attributed to PCR inhibitors in the four DNA extracts (two samples of Catharanthus roseus contaminated by Palatinate Grapevine yellows PGY-A and PGY-C) or to the concentration of the target in those samples. It is worth noting that DNA extracts of these phytoplasmas from another source were also tested and, correct detection and identification were possible in all the laboratories. Except for these two samples, false positive (FP) and FN are not reproducible i.e. they do not correspond to the same samples between participants. Thus, it is not a problem of inclusivity or exclusivity of the method but more problems of reproducibility (FN) or problems of micro-contaminations and/or problems in the interpretation of the sequences (FP). Inconclusive results represent 3.6% of the participants' responses.
Other information	
Any other information considered useful	More information can be obtained on request to Anses Plant health laboratory.

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