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	Detection of Grapevine flavescence dorée phytoplasma and 'Candidatus Phytoplasma solani' with an internal control by real time PCR in petioles
Date, reference of the validation report	2023-01-24 - RV MA006 v3 - 2023
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Grapevine flavescence dorée phytoplasma (PHYP64) 'Candidatus Phytoplasma solani' (PHYPSO)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
EPPO Diagnostic Protocol name	PM 7/079 Grapevine flavescence dorée phytoplasma (version 2)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes CTAB extraction was optimized to harmonize centrifugation speeds, reduce the process and discard use of toxic reagent beta-mercaptoethanol.
Kit	
Is a kit used	no

Other information	
Other details on the test	CTAB method
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the 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
Is the test modified compared to the reference test	yes Optimization of the test with Tm of 60°C and use of cheaper reagent.
Kit	
Is a kit used	no
Other information	
Reaction type	Triplex
Other details on the test	Target map genes of both phytoplasmas and leucine tRNA gene of grapevine as internal control.
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: 2x10^-2 corresponding to 17.5 copies of the target gene/µL
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	15 grapevine DNA extracts contaminated by phytoplasmas of the 16SrV-C and D subgroups: FD1 (4xM50, 1xvariant M50), FD2 (1xM38, 4xM54), 3xFD3, PGY (1xM36, 1xM88)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	DNA extract of different healthy grapevine cultivars (Chenin, Gamay…), DNA extracts of plant material infected by other phytoplasmas: 'Candidatus Phytoplasma solani' (16SrXII), 'Ca. Phytoplasma asteris' (16SrI-B), 'Ca. P. fraxini' (16SrVII-A), 'Ca. P. rubi' (16SrV-E), Grapevine yellows GY-U (16SrIII-B), 'Ca. P. ziziphi' (16SrV-B).
Specificity value	100%

Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	93%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	93%
Organism 2.:	**'Candidatus Phytoplasma solani'(PHYPSO)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Last level at 100% positive results: 2x10^-2 corresponding to 4 to 5 copies of the target gene/µL
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	15 grapevine DNA extracts contaminated by 'Candidatus Phytoplasma solani' from different French growing areas: Alsace, Auvergne, Rhône-Alpes, Franche Comté, Grand Est, Limousin, Lorraine, Provence Alpes Côtes d'Azur, Pays de Loire, Poitou Charentes.
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	DNA extract of different healthy grapevine cultivars (Chenin, Gamay…), DNA extracts of plant material infected by other phytoplasmas: 'Candidatus Phytoplasma asteris' (16SrI-B), 'Ca. P. fraxini' (16SrVII-A), 'Ca. P. rubi' (16SrV-E), Grapevine yellows GY-U (16SrIII-B), 'Ca. P. ziziphi' (16SrV-B), Flavescence dorée phytoplasma (16SrV-C and -D)
Specificity value	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	93%
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	More information can be obtained on request from Anses, Plant Health Laboratory.

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