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	Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	Detection of 'Candidatus Phytoplasma mali' by SYBR Green real time PCR
Date, reference of the validation report	2013-01-01 - 1) www.strateco.it 2)Pasquini et al., 2013. Petria 23(3),461-490
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?	Other_project
If yes, please specify	Project financed by the Italian Ministry of Agriculture (ARNADIA)
Description of the test	
Organism(s)	'Candidatus Phytoplasma mali'(PHYPMA)
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	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Pasquini G., Ferretti L., Bertaccini A., Bianco P.A., Casati P., Costantini E., Martini M., Marzachì C., Palmano S., Paltrinieri S., 2013. Protocollo diagnostico per 'Candidatus Phytoplasma mali' (AP). Petria 23 (3), 461-490
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN

Specify the kit used	DNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	Commercial kit (DNeasy Plant Mini kit Qiagen) from leaf midribs or phloem tissue, previously powdered with liquid nitrogen. An alternative protocol has been used in the case of not availability of liquid nitrogen for the initial powdering of plant material. (Pasquini et al., 2013)
Method: Molecular real time 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	1) Galetto L., D. Bosco, C. Marzachì, 2005. Universal and group-specific real-time PCR diagnosis of flavescence dorée (16Sr-V), bois noir (16Sr-XII) and apple proliferation (16Sr-X) phytoplasmas from field-collected plant hosts and insect vectors. Annals of Applied Biology, 147, 191-201. 2) Pasquini G., Ferretti L., Bertaccini A., Bianco P.A., Casati P., Costantini E., Martini M., Marzachì C., Palmano S., Paltrinieri S., 2013. Protocollo diagnostico per 'Candidatus Phytoplasma mali' (AP). Petria 23 (3), 461-490
Other information	
Other details on the test	SYBR Green real time PCR performed with primers AP phytoplasma-specific
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**'Candidatus Phytoplasma mali'(PHYPMA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	The analytical sensitivity was calculated analyzing three samples at seven diluition levels (1/1-1/1.000.000). The dilutions were in DNA from an healthy apple sample. Last dilution level with 100% positive results for all three samples: 1/100.000
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	20 'target' apple samples infected by 'Ca. P. mali' coming from different Italian areas. Diagnostic sensitivity: 83%
Standard test(s)	- Direct PCR with primers fAT/rAS (Smart et al., 1996) - Direct universal PCR with primers P1(Deng and Hiruki, 1991)/16S-Sr (Lee et al., 1994), followed by a nested 16SrX group specific with primers

	fO1/rO1 (Lorenz et al., 1995)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	20 'target' apple samples infected by 'Ca. P. mali' coming from different Italian areas
Specificity value	Analytical specificity: 100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Five 'non target' samples were included: phytobacteria commonly spread on pome fruits and other phytoplasmas belonging to 16SrX group: - Extracted DNA from a pear infected by Pseudomonas syringae pv. syringae - Extracted DNA from an apple infected by Erwinia amilovora - two samples of plums infected by 'Candidatus Phytoplasma prunorum' (ESFY phytoplasma) - one sample of pear infected by 'Candidatus Phytoplasma pyri' (PD phytoplasma)
Specificity value	Analytical specificity: 100% No cross reaction occurred
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Five samples of apple tree uninfected (certified material) Diagnostic specificity: 100%
Specify the test(s)	- Direct PCR with primers fAT/rAS (Smart et al., 1996) - Direct universal PCR with primers P1(Deng and Hiruki, 1991)/16S-Sr (Lee et al., 1994), followed by a nested 16SrX group specific with primers fO1/rO1 (Lorenz et al., 1995)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The reproducibility was calculated analyzing in six laboratories all samples included in diagnostic specificity and sensitivity tests. Reproducibility: 90.9%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The repeatability was calculated in three laboratories analyzing three samples at seven diluition levels (1/1-1/1.000.000). The dilutions were in DNA from an healthy apple sample. Repeatability: 100%
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	A ringtest was organized with the official phytosanitary Italian laboratories within a Project financed by the Italian Ministry of Agriculture (ARNADIA) for the definition of 'Italian reference protocols'.

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