EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

Target Organism	Candidatus Phytoplasma mali - Apple proliferation phytoplasma	
Short description	Detection of 'Candidatus Phytoplasma mali' by SYBR Green real time PCR	
Laboratory contact details	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy	
Date and reference of the validation report	2013 - 1) www.strateco.it 2)Pasquini et al., 2013. Petria 23(3),461-490	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	N/R 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	
Is the test the same as described in the EPPO DP?		
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	apple, pear and plum species	
Matrices tested (if relevant)	leaf midribs and phloem tissue	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	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)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	SYBR Green real time PCR performed with primers AP phytoplasma-specific

Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
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%	
Specify the standard test	- 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		
Specificity value	Analytical specificity: 100%	
Number of strains/populations of target organisms tested	20 'target' apple samples infected by 'Ca. P. mali' coming from different Italian areas	
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)	
Cross reacts with (specify the species)	Not 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 standard test	- 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
Include brief details of the test performance study and its output.It available, provide a 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'.
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	