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 direct and nested 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., Petria 23(3), 461-490	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	N/R - Deng S., C. Hiruki, 1991. Amplification of 16S rRNA genes from culturable and nonculturable mollicutes. J. Microbiol. Methods, 14, 53-61. - Lee I.M., M. Martini, C. Marcone, S.F. Zhu, 2004. Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of 'Candidatus Phytoplasma ulmi' for the phytoplasma associated with elm yellows. Int J Syst Evol Microbiol, 54, 337-347. - Lorenz K.H., B. Schneider, U. Ahrens, E. Seemuller, 1995. Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. Phytopathology, 85, 771-776. - 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	Direct universal PCR with primers P1(Deng and Hiruki, 1991)/16S-Sr (Lee et al., 2004), followed by a nested 16SrX group specific with primers fO1/rO1 (Lorenz et al., 1995)
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		

<u>Analytical sensitivity (= limit of detection)</u>	
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: 1/1000
<u>Diagnostic sensitivity</u>	
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	23 'target' samples: 20 apple samples infected by 'Ca. P. mali' coming from different Italian areas, 2 plum samples infected by 'Candidatus Phytoplasma prunorum' (ESFY phytoplasma) and one sample of pear infected by 'Candidatus Phytoplasma pyri' (PD phytoplasma) Diagnostic sensitivity: 83%
Specify the standard test	Other methodologies included in the ringtesT: - Direct universal PCR with primers fAT/rAS (Smart et al., 1996) - SYBR Green real time PCR (Galetto et al., 2005)
<u>Analytical specificity</u>	
Specificity value	Analytical specificity: 100%
Number of strains/populations of target organisms tested	23 'target' samples: 20 apple samples infected by 'Ca. P. mali' coming from different Italian areas, 2 plum samples infected by 'Candidatus Phytoplasma prunorum' (ESFY phytoplasma) and one sample of pear infected by 'Candidatus Phytoplasma pyri' (PD phytoplasma)
Number of non-target organisms tested	Two 'non target' samples were included: phytobacteria commonly spread on pome fruits: - Extracted DNA from a pear infected by Pseudomonas syringae pv. syringae - Extracted DNA from an apple infected by Erwinia amilovora

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	Other methodologies included in the ringtesT: - Direct universal PCR with primers fAT/rAS (Smart et al., 1996) - SYBR Green real time PCR (Galetto et al., 2005)
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: 89.1%
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.**	