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 prunorum - European stone fruit yellows (ESFY) phytoplasma	
Short description	Detection of 'Candidatus Phytoplasma prunorumi' 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., 2013. Petria 23(3),491-516	
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 non culturable Mollicutes. Journal of Microbiol. Methods, 14, 53-61. - Lee I.M., M. Martini, C. Marcone and 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. International Journal of Systematic Evolutionary Microbiology, 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 (7), 771-776. - Pasquini G., Bertaccini A., Bianco P.A., Casati P., Costantini E., Martini M., Marzachì C., Palmano S., Paltrinieri S., 2013. Protocollo diagnostico per 'Candidatus Phytoplasma prunorum'. Petria 23 (3), 491-516	
Is the test the same as described in the EPPO DP?		
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	apricot, plum, peach, apple and pear species.	
Matrices tested (if relevant)	leaf midribs and bark	
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., 1994), 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 peach sample. Last dilution level with 100% positive results for all three samples: 1/1000 bark samples collected in early spring and 1/100 leaf midribs samples collected in late summer	
<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	24 'target' samples were analyzed in two different sampling periods: early spring (as bark matrix) and late summer (as leaf midribs) 21 stone fruit samples positive for 'Ca. P. prunorum': 7 symptomatic apricot samples, 9 symptomatic Japanese plum samples, 3 symptomatic European plum samples, 2 symtpomatic peach samples; 2 apple samples positive for 'Ca. P. mali';1 pear sample positive for 'Ca. P. pyri'. Within the ringtest two different methodologies were been compared. Diagnostic sensitivity: 86% (in both sampling periods) Diagnostic sensitivity: 81%	
Specify the standard test	- TaqMan real time PCR (Baric et al., 2004; Pignatta et al., 2008; Minguzzi et al., 2010)	

Analytical specificity	
Specificity value	Analitycal specificity: 100%
Number of strains/populations of target organisms tested	24 'target' samples were analyzed in two different sampling periods: learly spring (as bark matrix) and late saummer (as leaf midribs) 21 stone fruit samples positive for 'Ca. P. prunorum': 7 symptomatic apricot samples, 9 symptomatic Prunus salicina samples, 3 symptomatic Prunus domestica samples, 2 symtpomatic peach samples; 2 apple samples positive for 'Ca. P. mali';1 pear sample positive for 'Ca. P. pyri'.
Number of non-target organisms tested	One DNA extract from an apricot sample infected by Pseudomonas syringae pv. syringae
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	Three samples of healthy plum, peach and apricot (certified material) tested in two sampling period: early spring and late summer. Diagnostic specificity: 100% in both sampling periods
Specify the standard test	- TaqMan real time PCR (Baric et al., 2004; Pignatta et al., 2008; Minguzzi et al., 2010)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The reproducibility was calculated only in late summer, analyzing in seven laboratories all samples included in diagnostic specificity and sensitivity tests. Reproducibility: 68.7%
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 collected in two different periods (early spring and late summer) at seven diluition levels (1/1-1/1.000.000). The dilutions were in DNA from an healthy apple sample. Repeatability: 100% in both sampling periods
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 Italian phytosanitary 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.**	