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 prunorum' by 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),491-516	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	N/R -Baric S., J. Dalla-Via, 2004. A new approach to apple proliferation detection: a highly sensitive real-time PCR assay. Journal of Microbiological Methods, 57, 135-145. - Pignatta D., C. Poggi Pollini, L. Giunchedi, M. Gobber, P. Morelli, F. Forno, L. Martedì, E. Ropelato, 2008. A Real-time PCR assay for the detection of European stone fruit yellows phytoplasma (ESFYP) in plant propagation material. Acta Horticulturae, 781, 499-503 - Minguzz i S., C. Ratti, C. Lanzoni, C. Rubies Autonell, N. Reggiani, C. Poggi Pollini, 2010. Detection and relative quantification of 'Candidatus Phytoplasma prunorum' by spot real-time RT-PCR TaqMan assay. Petria, 20 (2), 219-220; -Osman F., C. Leutenegger, D. Golino, A. Rowhani, 2007. Real-time RT-PCR (Taq-Man) assays for the detection of Grapevine leafroll associated virus 1-5 and 9. Journal of Virological Methods, 141, 22-29. - 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	TaqMan real time PCR specific for the detection and identification of 'Ca. P. prunorum' and an internal control (18S gene)
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 for bark samples collected in 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		
Specify the standard test		
<u>Analytical specificity</u>		
Specificity value		
Number of strains/populations of target organisms tested		
Number of non-target organisms tested		
Cross reacts with (specify the species)		
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a		

standard test	
Specify the standard test	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
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	ffff
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	