

**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.

<b>Laboratory contact details</b>	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
<b>Short description of the test</b>	Detection of 'Candidatus Phytoplasma prunorum' by real time PCR
<b>Date, reference of the validation report</b>	2013-01-01 - 102 ; 1) www.strateco.it 2)Pasquini et al., 2013. Petria 23(3),491-516
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	'Candidatus Phytoplasma prunorum' (PHYPPR)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Pasquini et al., 2013. Petria 23(3),491-516
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	DNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	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)
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Duplex - Probe
<b>Other details on the test</b>	TaqMan real time PCR specific for the detection and identification of 'Ca. P. prunorum' and an internal control (18S gene)
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>'Candidatus Phytoplasma prunorum'(PHYPPR)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	The analytical sensitivity was calculated analyzing three samples at seven dilution 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
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output.It available, link to published article/report</b>	ffff
<b>Other information</b>	
<b>Any other information considered useful</b>	-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 - Minguzzi 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. Realtime 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., Marzachi C., Palmano S., Paltrinieri S., 2013. Protocollo diagnostico per 'Candidatus Phytoplasma prunorum'. Petria 23 (3), 491-516
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