

**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 direct and nested PCR
<b>Date, reference of the validation report</b>	2013-01-01 - 101 ; 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>	Other_project
<b>If yes, please specify</b>	Project financed by the Italian Ministry of Agriculture (ARNADIA) for the definition of 'Italian reference protocols'.
<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 Conventional 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 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
<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 Conventional PCR</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>	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.
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Nested
<b>Other details on the test</b>	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)
<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 bark samples collected in early spring and 1/100 leaf midribs samples collected in late summer
<b>Diagnostic sensitivity</b>	

<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	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 symptomatic 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 compared. Diagnostic sensitivity: 86% (in both sampling periods) Diagnostic sensitivity: 81%
<b>Standard test(s)</b>	- TaqMan real time PCR (Baric et al., 2004; Pignatta et al., 2008; Minguzzi et al., 2010)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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 Prunus salicina samples, 3 symptomatic Prunus domestica samples, 2 symptomatic peach samples; 2 apple samples positive for 'Ca. P. mali'; 1 pear sample positive for 'Ca. P. pyri'.
<b>Specificity value</b>	Analytical specificity: 100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	One DNA extract from an apricot sample infected by Pseudomonas syringae pv. syringae
<b>Specificity value</b>	Analytical specificity: 100% - no cross reaction
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Three samples of healthy plum, peach and apricot (certified material) tested in two sampling periods: early spring and late summer. Diagnostic specificity: 100% in both sampling periods
<b>Specify the test(s)</b>	- TaqMan real time PCR (Baric et al., 2004; Pignatta et al., 2008; Minguzzi et al., 2010)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	The reproducibility was calculated only in late summer, analyzing in seven laboratories all samples included in diagnostic specificity and sensitivity tests. Reproducibility: 68.7%
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	The repeatability was calculated in three laboratories analyzing three samples collected in two different periods (early spring and late summer) at seven dilution levels (1/1-1/1.000.000). The dilutions were in DNA from an healthy apple sample. Repeatability: 100% in both sampling periods
<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>	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'.

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