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.

Laboratory contact details	Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	Detection of 'Candidatus Phytoplasma prunorumi' by direct and nested PCR
Date, reference of the validation report	2013-01-01 - 101 ; 1) www.strateco.it 2)Pasquini et al., 2013. Petria 23(3),491-516
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	Other_project
If yes, please specify	Project financed by the Italian Ministry of Agriculture (ARNADIA) for the definition of 'Italian reference protocols'.
Description of the test	
Organism(s)	'Candidatus Phytoplasma prunorum' (PHYPPR)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular Conventional PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN

Specify the kit used	DNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	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)
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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.
Other information	
Reaction type	Simplex - Nested
Other details on the test	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)
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**'Candidatus Phytoplasma prunorum'(PHYPPR)**
Analytical sensitivity	
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
Diagnostic sensitivity	

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%
Standard test(s)	- TaqMan real time PCR (Baric et al., 2004; Pignatta et al., 2008; Minguzzi et al., 2010)
Analytical specificity - inclusivity	
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'.
Specificity value	Analitycal specificity: 100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	One DNA extract from an apricot sample infected by Pseudomonas syringae pv. syringae
Specificity value	Analitycal specificity: 100% - no cross reaction
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 test(s)	- 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
Brief details of the test performance study and its output.It available, 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'.

Creation date: 2015-02-13 00:00:00 - Last update: 2020-10-08 18:15:00