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 prunorum' by real time PCR
Date, reference of the validation report	2013-01-01 - 102 ; 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?	no
Description of the test	
Organism(s)	'Candidatus Phytoplasma prunorum' (PHYPPR)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular real time 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 et al., 2013. 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 real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
Other information	
Reaction type	Duplex - Probe
Other details on the test	TaqMan real time PCR specific for the detection and identification of 'Ca. P. prunorum' and an internal control (18S gene)
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 for bark samples collected in collected in early spring and 1/100 leaf midribs samples collected in late summer
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	ffff
Other information	
Any other information considered useful	-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. 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., Marzachì C., Palmano S., Paltrinieri S., 2013. Protocollo diagnostico per 'Candidatus Phytoplasma prunorum'. Petria 23 (3), 491-516

Creation date: 2015-02-13 00:00:00 - Last update: 2020-10-08 18:14:34