

**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 mali' by SYBR Green real time PCR
<b>Date, reference of the validation report</b>	2013-01-01 - 1) www.strateco.it 2)Pasquini et al., 2013. Petria 23(3),461-490
<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)
<b>Description of the test</b>	
<b>Organism(s)</b>	'Candidatus Phytoplasma mali'(PHYPMA)
<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 G., Ferretti L., Bertaccini A., Bianco P.A., Casati P., Costantini E., Martini M., Marzachì C., Palmano S., Paltrinieri S., 2013. Protocollo diagnostico per 'Candidatus Phytoplasma mali' (AP). Petria 23 (3), 461-490
<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>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	1) Galetto L., D. Bosco, C. Marzachì, 2005. Universal and group-specific real-time PCR diagnosis of flavescence dorée (16Sr-V), bois noir (16Sr-XII) and apple proliferation (16Sr-X) phytoplasmas from field-collected plant hosts and insect vectors. Annals of Applied Biology, 147, 191-201. 2) Pasquini G., Ferretti L., Bertaccini A., Bianco P.A., Casati P., Costantini E., Martini M., Marzachì C., Palmano S., Paltrinieri S., 2013. Protocollo diagnostico per 'Candidatus Phytoplasma mali' (AP). Petria 23 (3), 461-490
<b>Other information</b>	
<b>Other details on the test</b>	SYBR Green real time PCR performed with primers AP phytoplasma-specific
<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 mali'(PHYPMA)</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 apple sample. Last dilution level with 100% positive results for all three samples: 1/100.000
<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>	20 'target' apple samples infected by 'Ca. P. mali' coming from different Italian areas. Diagnostic sensitivity: 83%
<b>Standard test(s)</b>	- Direct PCR with primers fAT/rAS (Smart et al., 1996) - 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>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	20 'target' apple samples infected by 'Ca. P. mali' coming from different Italian areas
<b>Specificity value</b>	Analytical specificity: 100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Five 'non target' samples were included: phyto bacteria commonly spread on pome fruits and other phytoplasmas belonging to 16SrX group: - Extracted DNA from a pear infected by Pseudomonas syringae pv. syringae - Extracted DNA from an apple infected by Erwinia amilovora - two samples of plums infected by 'Candidatus Phytoplasma prunorum' (ESFY phytoplasma) - one sample of pear infected by 'Candidatus Phytoplasma pyri' (PD phytoplasma)
<b>Specificity value</b>	Analytical specificity: 100% No cross reaction occurred
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Five samples of apple tree uninfected (certified material) Diagnostic specificity: 100%
<b>Specify the test(s)</b>	- Direct PCR with primers fAT/rAS (Smart et al., 1996) - 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>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	The reproducibility was calculated analyzing in six laboratories all samples included in diagnostic specificity and sensitivity tests. Reproducibility: 90.9%
<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 at seven dilution levels (1/1-1/1.000.000). The dilutions were in DNA from an healthy apple sample. Repeatability: 100%
<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 phytosanitary Italian laboratories within a Project financed by the Italian Ministry of Agriculture (ARNADIA) for the definition of 'Italian reference protocols'.