

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)

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.

Target Organism	Candidatus Phytoplasma mali Candidatus Phytoplasma prunorum Candidatus Phytoplasma pyri	
Short description	Detection of fruit trees phytoplasmas by PCR followed by RFLP analysis	
Laboratory contact details	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49000 Angers, France	
Date and reference of the validation report	2013-01 - Leguay A. and Loiseau M., janvier 2013. Evaluation des méthodes de détection des phytoplasmes des arbres fruitiers. ANSES-LSV	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 PM7-62 and PM7-63	
Is the test the same as described in the EPPO DP?	No The protocol was published by Lorenz et al. (1995) and by Seemüller et al. (1996)	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	All relevant fruit trees, in particular, Malus sp., Pyrus sp. and Prunus sp..	
Matrices tested (if relevant)	branch	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	CTAB DNA extraction (modified from Doyle & Doyle (1990)) end-point PCR (Lorenz et al., 1995) RFLP analysis (Lorenz et al., 1995)
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		

Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	Last level at 100% positive results: (levels tested between 1.10 ⁻¹ and 1.10 ⁻⁸ for 3 different positive DNA extract diluted in healthy DNA extract; one PD, one ESFY and one AP) For 'Ca.P. mali': 1.10 ⁻⁴ For 'Ca.P. prunorum': 1.10 ⁻⁴ For 'Ca. P. pyri': 1.10 ⁻⁵	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	For 'Ca.P. mali': 97.44% For 'Ca.P. prunorum': 100% For 'Ca. P. pyri': 96.67%	
Specify the standard test		
Analytical specificity		
Specificity value	For 'Ca.P. mali': 94.87% For 'Ca.P. prunorum': 95.83% For 'Ca. P. pyri': 98.31%	
Number of strains/populations of target organisms tested	Ca. P. prunorum N°220-20 Ca. P. prunorum N°223-8 Ca. P. prunorum N°223-14 Ca. P. prunorum N°223-27 Apple proliferation (AP15) - 16SrX-A Apple proliferation (AT) - 16SrX-A ESFY - 16SrX-B PEAR DECLINE - 16SrX-C S3 - 16SrX-A S4 - 16SrX-B S5 - 16SrX-B S9 - 16SrX-C S12 - 16SrX-A S13 - 16SrX-C S15 - 16SrX-A S16 - 16SrX-A S18 - 16 SrX-C S26 - 16SrX-B S28 - 16SrX-B S30 - 16SrX-B Ca. P. prunorum E134/10-12 Ca. P. prunorum E136/10-4 Ca. P. prunorum E136/10-5 Ca. P. pyri E112/11-2 Ca. P. pyri 3509 Apple proliferation AP#1 (1)	

	<p>Apple proliferation AP#1 (3) Apple proliferation N°2 TM1 AP Ca. P. mali pommier Ca. P. pyri poirier Ca. P. pyri poirier Ca. P. pyri poirier Ca. P. pyri poirier</p>
Number of non-target organisms tested	<p>healthy quince Erwinia amylovora Xanthomonas arboricola pv pruni Pseudomonas syringae morsprunorum Pseudomonas syringae syringae Pseudomonas syringae syringae Erwinia amylovora Sharka Aster Yellow Apricot (AYA) - 16SrI-F Lime Witches' broom (WBDL) - 16SrII-B PEACH-WX - 16SrIII-A ESPAGNE III - 16SrVI ASHY-4 - 16SrVII-A BVK - 16SrXI STOLBUR - 16SrXII-A SURINAM VIRESCENCE - 16SrXV Peach Yellow Ragozzino n°11 16 SrV 2 Healthy apple 4 Healthy pear 2 Healthy peach 2 Healthy cherry 2 Healthy apricot Prunus</p>
Cross reacts with (specify the species)	No repeatable cross reaction observed.
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	<p>For 'Ca.P. mali': 92.31% For 'Ca.P. prunorum': 91.67% For 'Ca. P. pyri': 100%</p>
Specify the standard test	
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	<p>For 'Ca.P. mali': 98.31% For 'Ca.P. prunorum': 100% For 'Ca. P. pyri': 97.8%</p>
<u>Test performance study</u>	

Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	