

**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>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixm�ras, 49044 Angers, France
<b>Short description of the test</b>	Detection of fruit trees phytoplasmas by PCR followed by RFLP analysis
<b>Date, reference of the validation report</b>	2013-01-01 - Leguay A. and Loiseau M., janvier 2013. Evaluation des m�thodes de d�tection des phytoplasmes des arbres fruitiers. ANSES-LSV
<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>	
<b>If yes, please specify</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	'Candidatus Phytoplasma prunorum'(PHYPPR) 'Candidatus Phytoplasma mali'(PHYPMA) 'Candidatus Phytoplasma pyri'(PHYPPY)
<b>Detection / identification</b>	
<b>Matrix(ces) tested</b>	Woody cuttings branch of all relevant fruit trees, in particular, Malus sp., Pyrus sp. and Prunus sp..
<b>Plant species tested</b>	Malus sp., Prunus sp., Pyrus sp.
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular PCR-RFLP
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	
<b>EPPO Diagnostic Protocol name</b>	PM 7/062 Candidatus Phytoplasma mali (version 2)
<b>Name of the test</b>	
<b>As or adapted from an IPPC diagnostic protocol</b>	

Is the test modified compared to the reference test	
<b>Kit</b>	
Is a kit used	
<b>Other information</b>	
Other details on the test	CTAB DNA extraction (modified from Doyle & Doyle (1990))
<b>Method: Molecular PCR-RFLP</b>	
<b>Reference of the test description</b>	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/062 Candidatus Phytoplasma mali (version 2)
Name of the test	Conventional PCR for the generic detection of phytoplasmas (Lorenz et al., 1995) + RFLP (Schneider et al. (1995))
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
<b>Kit</b>	
Is a kit used	
<b>Other information</b>	
Reaction type	
Other details on the test	End-point PCR (Lorenz et al., 1995) & RFLP analysis (Lorenz et al., 1995) The test was not yet included in the EPPO DP PM7/062 when the validation sheet was filled. The validation data were included later in PM7/062.
Are the performance characteristics included in the EPPO diagnostic protocol?	yes
<b>Performance Criteria :</b>	
Organism 1.:	'Candidatus Phytoplasma prunorum'(PHYPPR)
<b>Analytical sensitivity</b>	
What is smallest amount of target that can be detected reliably?	Last level at 100% positive results: (levels tested between 1.10 <sup>-1</sup> and 1.10 <sup>-8</sup> for one ESFY positive DNA extract diluted in healthy DNA extract) For 'Ca.P. prunorum': 1.10 <sup>-4</sup>
<b>Diagnostic sensitivity</b>	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	For 'Ca.P. prunorum': 100%

<b>Standard test(s)</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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) Apple proliferation AP#1 (3) Apple proliferation N°2 TM1 AP Ca. P. mali pommier Ca. P. mali pommier Ca. P. mali pommier Ca. P. pyri poirier Ca. P. pyri poirier Ca. P. pyri poirier
<b>Specificity value</b>	For 'Ca.P. prunorum': 95.83%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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 Ragazzino n°11 16 SrV 2 Healthy apple 4 Healthy pear 2 Healthy peach 2 Healthy cherry 2 Healthy apricot Prunus
<b>Specificity value</b>	No repeatable cross reaction observed.
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	For 'Ca.P. prunorum': 91.67%
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	For 'Ca.P. prunorum': 100%
<b>Organism 2.:</b>	<b>'Candidatus Phytoplasma mali'(PHYPMA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Last level at 100% positive results: (levels tested between 1.10 <sup>-1</sup> and 1.10 <sup>-8</sup> for AP positive DNA extract diluted in healthy DNA extract) For 'Ca.P.

	mali': 1.10-4
<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>	For 'Ca.P. mali': 97.44%
<b>Standard test(s)</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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) Apple proliferation AP#1 (3) Apple proliferation N°2 TM1 AP Ca. P. mali pommier Ca. P. mali pommier Ca. P. mali pommier Ca. P. mali pommier Ca. P. pyri poirier Ca. P. pyri poirier Ca. P. pyri poirier
<b>Specificity value</b>	For 'Ca.P. mali': 94.87%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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 Ragazzino n°11 16 SrV 2 Healthy apple 4 Healthy pear 2 Healthy peach 2 Healthy cherry 2 Healthy apricot Prunus
<b>Specificity value</b>	No repeatable cross reaction observed.
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	For 'Ca.P. mali': 92.31%
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	For 'Ca.P. mali': 98.31%

<b>Organism 3.:</b>	<b>'Candidatus Phytoplasma pyri'(PHYPPY)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Last level at 100% positive results: (levels tested between 1.10 <sup>-1</sup> and 1.10 <sup>-8</sup> for one PD positive DNA extract diluted in healthy DNA extract) For 'Ca. P. pyri': 1.10 <sup>-5</sup>
<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>	For 'Ca. P. pyri': 96.67%
<b>Standard test(s)</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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) Apple proliferation AP#1 (3) Apple proliferation N°2 TM1 AP Ca. P. mali pommier Ca. P. mali pommier Ca. P. mali pommier Ca. P. mali pommier Ca. P. pyri poirier Ca. P. pyri poirier Ca. P. pyri poirier Ca. P. pyri poirier
<b>Specificity value</b>	For 'Ca. P. pyri': 98.31%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	healthy quince Erwinia amylovora Xanthomonas arboricola pv pruni Pseudomonas syringae morsprunorum Pseudomonas syringae syringae Pseudomonas syringae syringae Erwinia amylovora Sharka Aster Yellow Apricot (AYA) - 16Srl-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
<b>Specificity value</b>	No repeatable cross reaction observed.
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	For 'Ca. P. pyri': 100%
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	For 'Ca. P. pyri': 97.8%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful</b>	

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