

**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, 49000 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>Description of the test</b>	
<b>Organism(s)</b>	'Candidatus Phytoplasma prunorum' (PHYPPR) 'Candidatus Phytoplasma mali' (PHYPPMA) 'Candidatus Phytoplasma pyri' (PHYPPY)
<b>Detection / identification</b>	detection
<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>EPPO Diagnostic Protocol name</b>	PM 7/062 Candidatus Phytoplasma mali (version 2)
<b>Other information</b>	
<b>Other details on the test</b>	CTAB DNA extraction (modified from Doyle & Doyle (1990))
<b>Method: Molecular PCR-RFLP</b>	
<b>Reference of the test description</b>	

<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/062 Candidatus Phytoplasma mali (version 2)
<b>Name of the test</b>	Conventional PCR for the generic detection of phytoplasmas (Lorenz et al., 1995) + RFLP (Schneider et al. (1995))
<b>Other information</b>	
<b>Other details on the test</b>	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.
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>yes</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>'Candidatus Phytoplasma prunorum'(PHYPPR)</b>
<b>Analytical sensitivity</b>	
<b>What is the 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 ESFY positive DNA extract diluted in healthy DNA extract) For 'Ca.P. prunorum': 1.10 <sup>-4</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. prunorum': 100%
<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) - 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>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>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 the 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 <sup>-4</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. mali': 97.44%
<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 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>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>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 the 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>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. 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) - 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
<b>Specificity value</b>	No repeatable cross reaction observed.
<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>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

Creation date: 2016-01-26 00:00:00 - Last update: 2025-02-26 15:25:28