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	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
Short description of the test	Detection of fruit trees phytoplasmas by PCR followed by RFLP analysis
Date, reference of the validation report	2013-01-01 - 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 PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	'Candidatus Phytoplasma prunorum' (PHYPPR) 'Candidatus Phytoplasma mali' (PHYPMA) 'Candidatus Phytoplasma pyri' (PHYPPY)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular PCR-RFLP
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/062 Candidatus Phytoplasma mali (version 2)
Other information	
Other details on the test	CTAB DNA extraction (modified from Doyle & Doyle (1990))
Method: Molecular PCR-RFLP	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
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))
Other information	
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**
Performance Criteria :	
Organism 1.:	**'Candidatus Phytoplasma prunorum'(PHYPPR)**
Analytical sensitivity	
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 one ESFY positive DNA extract diluted in healthy DNA extract) For 'Ca.P. prunorum': 1.10-4
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. prunorum': 100%
Analytical specificity - inclusivity	
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) 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
Specificity value	For 'Ca.P. prunorum': 95.83%
Analytical specificity - exclusivity	
Number of non-target organisms tested	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
Specificity value	No repeatable cross reaction observed.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	For ‘Ca.P. prunorum’: 91.67%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For ‘Ca.P. prunorum’: 100%
Organism 2.:	**'Candidatus Phytoplasma mali'(PHYPMA)**
Analytical sensitivity	
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 AP positive DNA extract diluted in healthy DNA extract) For ‘Ca.P. mali’: 1.10-4
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%
Analytical specificity - inclusivity	
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) 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
Specificity value	For ‘Ca.P. mali’: 94.87%
Analytical specificity - exclusivity	
Number of non-target organisms tested	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

Specificity value	No repeatable cross reaction observed.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	For ‘Ca.P. mali’: 92.31%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For ‘Ca.P. mali’: 98.31%
Organism 3.:	**'Candidatus Phytoplasma pyri'(PHYPPY)**
Analytical sensitivity	
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 one PD positive DNA extract diluted in healthy DNA extract) 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. pyri’: 96.67%
Analytical specificity - inclusivity	
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) 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
Specificity value	For ‘Ca. P. pyri’: 98.31%
Analytical specificity - exclusivity	
Number of non-target organisms tested	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
Specificity value	No repeatable cross reaction observed.

Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	For ‘Ca. P. pyri’: 100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For ‘Ca. P. pyri’: 97.8%
Test performance study	
Test performance study?	no

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