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.

<table>
<thead>
<tr>
<th>Laboratory contact details</th>
<th>Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short description of the test</td>
<td>Detection of fruit trees phytoplasmas by PCR followed by RFLP analysis</td>
</tr>
<tr>
<td>Validation process according to EPPO Standard PM7/98?</td>
<td>yes</td>
</tr>
<tr>
<td>Is the lab accredited for this test?</td>
<td>no</td>
</tr>
<tr>
<td>Was the validated data generated in the framework of a project?</td>
<td></td>
</tr>
<tr>
<td>If yes, please specify</td>
<td></td>
</tr>
<tr>
<td>Description of the test</td>
<td></td>
</tr>
<tr>
<td>Organism(s)</td>
<td>'Candidatus Phytoplasma prunorum' (PHYPPR) 'Candidatus Phytoplasma mali' (PHYPMA) 'Candidatus Phytoplasma pyri' (PHYPY)</td>
</tr>
<tr>
<td>Detection / identification</td>
<td></td>
</tr>
<tr>
<td>Matrix(ces) tested</td>
<td>Woody cuttings branch of all relevant fruit trees, in particular, Malus sp., Pyrus sp. and Prunus sp.</td>
</tr>
<tr>
<td>Plant species tested</td>
<td>Malus sp., Prunus sp., Pyrus sp.</td>
</tr>
<tr>
<td>Method(s)</td>
<td>Molecular extraction DNA RNA Molecular PCR-RFLP</td>
</tr>
<tr>
<td>Method: Molecular Extraction DNA RNA</td>
<td></td>
</tr>
<tr>
<td>Reference of the test description</td>
<td>As or adapted from an EPPO diagnostic protocol yes</td>
</tr>
<tr>
<td>As or adapted from an IPPC diagnostic protocol</td>
<td></td>
</tr>
<tr>
<td>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</td>
<td></td>
</tr>
<tr>
<td>EPPO Diagnostic Protocol name</td>
<td>PM 7/062 Candidatus Phytoplasma mali (version 2)</td>
</tr>
<tr>
<td>Name of the test</td>
<td></td>
</tr>
<tr>
<td>As or adapted from an IPPC diagnostic protocol</td>
<td></td>
</tr>
<tr>
<td><strong>Is the test modified compared to the reference test</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Kit</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Is a kit used</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Other information</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Other details on the test</strong></td>
<td>CTAB DNA extraction (modified from Doyle &amp; Doyle (1990))</td>
</tr>
<tr>
<td><strong>Method:</strong> Molecular PCR-RFLP</td>
<td></td>
</tr>
<tr>
<td><strong>Reference of the test description</strong></td>
<td></td>
</tr>
<tr>
<td><strong>As or adapted from an EPPO diagnostic protocol</strong></td>
<td>yes</td>
</tr>
<tr>
<td><strong>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</strong></td>
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</tr>
<tr>
<td><strong>EPPO Diagnostic Protocol name</strong></td>
<td>PM 7/062 Candidatus Phytoplasma mali (version 2)</td>
</tr>
<tr>
<td><strong>Name of the test</strong></td>
<td>Conventional PCR for the generic detection of phytoplasmas (Lorenz et al., 1995) + RFLP (Schneider et al. (1995))</td>
</tr>
<tr>
<td><strong>As or adapted from an IPPC diagnostic protocol</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Is the test modified compared to the reference test</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Kit</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Is a kit used</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Other information</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Reaction type</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Other details on the test</strong></td>
<td>End-point PCR (Lorenz et al., 1995) &amp; 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.</td>
</tr>
<tr>
<td><strong>Are the performance characteristics included in the EPPO diagnostic protocol?</strong></td>
<td>yes</td>
</tr>
<tr>
<td><strong>Performance Criteria :</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Organism 1.:</strong></td>
<td>'Candidatus Phytoplasma prunorum'(PHYPPR)</td>
</tr>
<tr>
<td><strong>Analytical sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td><strong>What is smallest amount of target that can be detected reliably?</strong></td>
<td>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</td>
</tr>
<tr>
<td><strong>Diagnostic sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</strong></td>
<td>For ‘Ca.P. prunorum’: 100%</td>
</tr>
<tr>
<td>Standard test(s)</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analytical specificity - inclusivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of strains/populations of target organisms tested</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specificity value</th>
</tr>
</thead>
<tbody>
<tr>
<td>For ‘Ca.P. prunorum’: 95.83%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analytical specificity - exclusivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of non-target organisms tested</strong></td>
</tr>
<tr>
<td>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</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specificity value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No repeatable cross reaction observed.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cross reacts with</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Candidatus Phytoplasma mali’(PHYPMA)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnostic Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</strong></td>
</tr>
<tr>
<td>For ‘Ca.P. prunorum’: 91.67%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specify the test(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</strong></td>
</tr>
<tr>
<td>For ‘Ca.P. prunorum’: 100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</strong></td>
</tr>
<tr>
<td>For ‘Ca.P. prunorum’: 100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism 2:</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Candidatus Phytoplasma mali’(PHYPMA)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analytical sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What is smallest amount of target that can be detected reliably?</strong></td>
</tr>
</tbody>
</table>
| 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.
### 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 test(s)

### 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 – 16SrX-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
- Apple proliferation AP#1
- 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
- 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 (WBFLB)
- 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.

### Cross reacts with

- None

### Diagnostic Specificity

**Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test**

For ‘Ca. P. mali’: 92.31%

### Specify the test(s)

- None

### Reproducibility

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)

For ‘Ca. P. mali’: 98.31%

### Repeatability

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)

For ‘Ca. P. mali’: 98.31%
<table>
<thead>
<tr>
<th><strong>Organism 3.</strong></th>
<th>'Candidatus Phytoplasma pyri'(PHYPPY)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td><strong>What is smallest amount of target that can be detected reliably?</strong></td>
<td>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</td>
</tr>
<tr>
<td><strong>Diagnostic sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</strong></td>
<td>For ‘Ca. P. pyri’: 96.67%</td>
</tr>
<tr>
<td><strong>Standard test(s)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Analytical specificity - inclusivity</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Specificity value</strong></td>
<td>For ‘Ca. P. pyri’: 98.31%</td>
</tr>
<tr>
<td><strong>Analytical specificity - exclusivity</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Number of non-target organisms tested</strong></td>
<td>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) - 16SrlII-B PEACH-WX - 16SrlIII-A ESPAGNE III - 16SrlVI ASHY-4 - 16SrlVII-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</td>
</tr>
<tr>
<td><strong>Specificity value</strong></td>
<td>No repeatable cross reaction observed.</td>
</tr>
<tr>
<td><strong>Cross reacts with</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Diagnostic Specificity</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</strong></td>
<td>For ‘Ca. P. pyri’: 100%</td>
</tr>
<tr>
<td><strong>Specify the test(s)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Reproducibility</strong></td>
<td>5 / 6</td>
</tr>
<tr>
<td><strong>Repeatability</strong></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>---</td>
</tr>
<tr>
<td>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</td>
<td>For ‘Ca. P. pyri’: 97.8%</td>
</tr>
<tr>
<td><strong>Test performance study</strong></td>
<td></td>
</tr>
<tr>
<td>Test performance study?</td>
<td>no</td>
</tr>
<tr>
<td>Brief details of the test performance study and its output. Is available, link to published article/report</td>
<td></td>
</tr>
<tr>
<td><strong>Other information</strong></td>
<td></td>
</tr>
<tr>
<td>Any other information considered useful</td>
<td></td>
</tr>
</tbody>
</table>

Creation date: 2016-01-26 00:00:00 - Last update: 2020-09-03 17:15:58