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, Laboratoire de la Santé des Végétaux - Unité de bactériologie, virologie OGM  
|                                     | 7 rue Jean Dixméras, 49044 Angers, France  |
| 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**

<table>
<thead>
<tr>
<th>Method for extraction / isolation / baiting of target organism from matrix</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular methods, e.g. hybridization, PCR and real time PCR</td>
<td>X</td>
</tr>
<tr>
<td>CTAB DNA extraction (modified from Doyle &amp; Doyle (1990))</td>
<td></td>
</tr>
<tr>
<td>end-point PCR (Lorenz et al., 1995)</td>
<td></td>
</tr>
<tr>
<td>RFLP analysis (Lorenz et al., 1995)</td>
<td></td>
</tr>
<tr>
<td>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</td>
<td></td>
</tr>
<tr>
<td>Plating methods: selective isolation</td>
<td></td>
</tr>
<tr>
<td>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</td>
<td></td>
</tr>
<tr>
<td>Pathogenicity test</td>
<td></td>
</tr>
</tbody>
</table>
### 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

<table>
<thead>
<tr>
<th>Analytical sensitivity (= limit of detection)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What is smallest amount of target that can be detected reliably?</strong></td>
</tr>
<tr>
<td>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)</td>
</tr>
<tr>
<td>For 'Ca.P. mali': 1.10-4</td>
</tr>
<tr>
<td>For 'Ca.P. prunorum': 1.10-4</td>
</tr>
<tr>
<td>For 'Ca. P. pyri': 1.10-5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnostic sensitivity</th>
</tr>
</thead>
<tbody>
<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>
</tr>
<tr>
<td>For 'Ca.P. mali': 97.44%</td>
</tr>
<tr>
<td>For 'Ca.P. prunorum': 100%</td>
</tr>
<tr>
<td>For 'Ca. P. pyri': 96.67%</td>
</tr>
</tbody>
</table>

| Specify the standard test |

<table>
<thead>
<tr>
<th>Analytical specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specificity value</strong></td>
</tr>
<tr>
<td>For 'Ca.P. mali': 94.87%</td>
</tr>
<tr>
<td>For 'Ca.P. prunorum': 95.83%</td>
</tr>
<tr>
<td>For 'Ca. P. pyri': 98.31%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of strains/populations of target organisms tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca. P. prunorum N°220-20</td>
</tr>
<tr>
<td>Ca. P. prunorum N°223-8</td>
</tr>
<tr>
<td>Ca. P. prunorum N°223-14</td>
</tr>
<tr>
<td>Ca. P. prunorum N°223-27</td>
</tr>
<tr>
<td>Apple proliferation (AP15) - 16SrX-A</td>
</tr>
<tr>
<td>Apple proliferation (AT) - 16SrX-A</td>
</tr>
<tr>
<td>ESFY - 16SrX-B</td>
</tr>
<tr>
<td>PEAR DECLINE – 16SrX-C</td>
</tr>
<tr>
<td>S3 - 16SrX-A</td>
</tr>
<tr>
<td>S4 – 16SrX-B</td>
</tr>
<tr>
<td>S5 – 16SrX-B</td>
</tr>
<tr>
<td>S9 – 16SrX-C</td>
</tr>
<tr>
<td>S12 – 16SrX-A</td>
</tr>
<tr>
<td>S13 – 16SrX-C</td>
</tr>
<tr>
<td>S15 – 16SrX-A</td>
</tr>
<tr>
<td>S16 – 16SrX-A</td>
</tr>
<tr>
<td>S18 – 16 SrX-C</td>
</tr>
<tr>
<td>S26 – 16SrX-B</td>
</tr>
<tr>
<td>S28 – 16SrX-B</td>
</tr>
<tr>
<td>S30 – 16SrX-B</td>
</tr>
<tr>
<td>Ca. P. prunorum E134/10-12</td>
</tr>
<tr>
<td>Ca. P. prunorum E136/10-4</td>
</tr>
<tr>
<td>Ca. P. prunorum E136/10-5</td>
</tr>
<tr>
<td>Ca. P. pyri E112/11-2</td>
</tr>
<tr>
<td>Ca. P. pyri 3509</td>
</tr>
<tr>
<td>Apple proliferation AP#1 (1)</td>
</tr>
<tr>
<td>Cross reacts with (specify the species)</td>
</tr>
<tr>
<td>---------------------------------------</td>
</tr>
<tr>
<td><strong>Diagnostic Specificity</strong></td>
</tr>
</tbody>
</table>
| Proportion of uninfected/uninfested samples testing negative compared to results from a standard test | For ‘Ca. P. mali’: 92.31%  
For ‘Ca. P. prunorum’: 91.67%  
For ‘Ca. P. pyri’: 100% |
| **Specify the standard test**        |                                       |
| **Reproducibility**                  |                                       |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | For ‘Ca. P. mali’: 98.31%  
For ‘Ca. P. prunorum’: 100%  
For ‘Ca. P. pyri’: 97.8% |
| **Repeatability**                    |                                       |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | For ‘Ca. P. mali’: 98.31%  
For ‘Ca. P. prunorum’: 100%  
For ‘Ca. P. pyri’: 97.8% |

**Number of non-target organisms tested**

| 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 |

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
<table>
<thead>
<tr>
<th>Test performance study?</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Include brief details of the test performance study and its output. If available, provide a 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 e.g. robustness, ease of performing the test, etc.</td>
<td></td>
</tr>
</tbody>
</table>