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>Target Organism</th>
<th>Xylella fastidiosa subsp. pauca strain CoDiRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short description</td>
<td>Detection of Xylella fastidiosa subsp. pauca ceppo CoDiRo from plant olive extracts by Conventional PCR according to Minsavage et al. (1994)</td>
</tr>
<tr>
<td>Laboratory contact details</td>
<td>Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy</td>
</tr>
<tr>
<td>Validation process according to EPPO Standard PM 7/98:</td>
<td>Yes</td>
</tr>
<tr>
<td>Reference of the test description</td>
<td>0</td>
</tr>
<tr>
<td>Is the test the same as described in the EPPO DP?</td>
<td>Yes</td>
</tr>
<tr>
<td>Is the lab accredited for this test?</td>
<td>No</td>
</tr>
<tr>
<td>Plant species tested (if relevant)</td>
<td>Olea europea</td>
</tr>
<tr>
<td>Matrices tested (if relevant)</td>
<td>leaves and petioles extracts</td>
</tr>
</tbody>
</table>

**List of methods used**

<table>
<thead>
<tr>
<th>Method for extraction / isolation / baiting of target organism from matrix</th>
<th>X</th>
<th>DNA extraction by following LoConsole et al. (2014) (procedure B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular methods, e.g. hybridization, PCR and real time PCR</td>
<td>X</td>
<td>Conventional PCR according to Minsavage et al. (1994)</td>
</tr>
<tr>
<td>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plating methods: selective isolation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathogenicity test</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fingerprint methods:</strong> protein profiling, fatty acid profiling &amp; DNA profiling</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Morphological and morphometrical methods intended for identification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biochemical methods:</strong> e.g. enzyme electrophoresis, protein profiling</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Analytical sensitivity (= limit of detection)**

What is smallest amount of target that can be detected reliably? 10⁻⁴ CFU/ml

**Diagnostic sensitivity**

Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 47%

Specify the standard test PCR according to Minsavage et al. (1994)

**Analytical specificity**

Specificity value

Number of strains/populations of target organisms tested

Number of non-target organisms tested

Cross reacts with (specify the species)

**Diagnostic Specificity**

Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test 100%

Specify the standard test PCR according to Minsavage et al. (1994)

**Reproducibility**

Provide the calculated % of agreement for a given level of the pest (see PM 7/98) (Concordance) 85%

**Repeatability**

Provide the calculated % of agreement for a given level of the pest (see PM 7/98) (Accordance) 91%

**Test performance study**

Test performance study? Yes

Include brief details of the test performance study and its output. It available, provide a link to published article/report 1. Two series of olive extracts spiked with ten fold dilution of Xylella fastidiosa CODiRo strain suspensions from 10⁻⁷ to 10 cfu/ml plus two healthy samples (16 samples in total) were tested in three different laboratories (CREA-PAV; CNR-IPSP;
To check the diagnostic sensitivity and specificity, the accuracy, the repeatability and reproducibility, olive extract samples spiked with Xylella fastidiosa CODiRo strain suspensions at $10^6$ cfu/ml (three repetitions), $10^4$ cfu/ml (three repetitions), $10^3$ cfu/ml (three repetitions), healthy olive extracts (three repetitions) for a total of 12 samples, were tested by the following TPS participants:

1. CREA-DC (N. Pucci; S. Loreti)
2. SELGE/CNR-IPSP/ DiSSPA-Uniba (M. Saponari, G. Loconsole; O. Potere)
3. PPS Piemonte (C. Morone, G. Mason)
4. PPS Friuli Venezia Giulia (G. Bianchi)
5. PPS Lombardia (F. Gaffuri)
6. PPS Emilia Romagna (A. Alessandrini; R. Gozzi)
7. PPS Trentino Alto Adige (V. Gualandri; L. Tessari)
8. PPS Marche (S. Nardi; S. Talevi)
9. PPS Liguria (M. Guelfi)
10. CIHEAM-IAMB (A.M. D’Onghia; M. Digiaro)
11. CRSFA (F. Palmisano)
12. Centro di Sperimentazione Agraria e Forestale, Laimburg (A. Gallmetzer; A. Kraus)
13. Uni-MI (P. Casati)
14. Uni-CT (V. Catara)
15. PPS Toscana (D. Rizzo)
16. PPS Veneto (A. Saccardi; D. Pasqua di Bisceglie)

Olive extract samples spiked with Xylella fastidiosa CODiRo strain suspensions at $10^6$ cfu/ml (three repetitions), $10^4$ cfu/ml (three repetitions), $10^3$ cfu/ml (three repetitions), healthy olive extracts (three repetitions) for a total of 12 samples, were tested by the following TPS participants:

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2. SELGE/CNR-IPSP/ DiSSPA-Uniba (M. Saponari, G. Loconsole; O. Potere)
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**Other information**

**Any other information considered useful e.g. robustness, ease of performing the test, etc.**

Accuracy: 92%