

**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.

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| <b>Laboratory contact details</b>                                          | Council for Agricultural Research and Economics-<br>Research Centre for Plant Protection and<br>Certification<br>Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy                                                                                             |
| <b>Short description of the test</b>                                       | Detection of <i>Xylella fastidiosa</i> subsp. <i>pauca</i> ceppo<br>CoDiRo by LAMP-PCR from DNA extracted from<br>olive leaves and petioles                                                                                                                    |
| <b>Date, reference of the validation report</b>                            | 2015-10-28 - Loreti S., Pucci N., Loconsole G.,<br>Modesti V, Lucchesi S.,Potere O., Saponari M 2017.<br>Protocollo Diagnostico per XYLELLA FASTIDIOSA<br>subsp. PAUCA ceppo CoDiRO. In Protocolli<br>Diagnostici - ASPROPI- ISBN 9788899595722.pp.<br>241-278 |
| <b>Validation process according to EPPO<br/>Standard PM7/98?</b>           | yes                                                                                                                                                                                                                                                            |
| <b>Is the lab accredited for this test?</b>                                | no                                                                                                                                                                                                                                                             |
| <b>Was the validated data generated in the<br/>framework of a project?</b> |                                                                                                                                                                                                                                                                |
| <b>Description of the test</b>                                             |                                                                                                                                                                                                                                                                |
|                                                                            |                                                                                                                                                                                                                                                                |
| <b>Organism(s)</b>                                                         | <i>Xylella fastidiosa</i> subsp. <i>pauca</i> (XYLEFP)                                                                                                                                                                                                         |
| <b>Detection / identification</b>                                          | detection                                                                                                                                                                                                                                                      |
| <b>Method(s)</b>                                                           | Molecular Extraction DNA RNA<br>Molecular LAMP<br>Molecular LAMP (2)                                                                                                                                                                                           |
| <b>Method: Molecular Extraction DNA RNA</b>                                |                                                                                                                                                                                                                                                                |
| <b>Reference of the test description</b>                                   |                                                                                                                                                                                                                                                                |
| <b>Other information</b>                                                   |                                                                                                                                                                                                                                                                |
| <b>Other details on the test</b>                                           | DNA extraction by following Loconsole et al. (2014)<br>(procedure B)                                                                                                                                                                                           |
| <b>Method: Molecular LAMP</b>                                              |                                                                                                                                                                                                                                                                |
| <b>Reference of the test description</b>                                   |                                                                                                                                                                                                                                                                |
| <b>Kit</b>                                                                 |                                                                                                                                                                                                                                                                |
| <b>Is a kit used</b>                                                       | yes                                                                                                                                                                                                                                                            |
| <b>Manufacturer name</b>                                                   | ENBIOTECH                                                                                                                                                                                                                                                      |

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| <b>Specify the kit used</b>                                                                                                          | ENBIOTECH Xylella fastidiosa LAMP kit                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Kit used following the manufacturer's instructions?                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Other information</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Method: Molecular LAMP (2)</b>                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Reference of the test description</b>                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Kit</b>                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Is a kit used</b>                                                                                                                 | yes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Manufacturer name</b>                                                                                                             | QUALIPLANTE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Specify the kit used</b>                                                                                                          | Isothermal PCR kit (ref bK.1/Xfas)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Kit used following the manufacturer's instructions?                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Other information</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>                                                 | yes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Performance Criteria :</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Organism 1.:</b>                                                                                                                  | <b>Xylella fastidiosa subsp. pauca(XYLEFP)</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Analytical sensitivity</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>What is smallest amount of target that can be detected reliably?</b>                                                              | 10 <sup>2</sup> /10 <sup>3</sup> CFU/ml (Qualiplante SAS by using real-time PCR machine) 10 <sup>3</sup> CFU/ml (Enbiothec s.r.l. by using real-time PCR machine) 10 <sup>1-2</sup> CFU/ml (Enbiothec s.r.l. by using ic-gene)                                                                                                                                                                                                                                                                                                                                                                           |
| <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> | 90% LAMP-PCR (Enbiotech s.r.l.)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Standard test(s)</b>                                                                                                              | LAMP-PCR (Enbiotech s.r.l.)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Analytical specificity - exclusivity</b>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Number of non-target organisms tested</b>                                                                                         | LAMP-PCR (Enbiotech s.r.l. kit) tested on the following bacterial strains: 3 Xanthomonas arboricola pv. pruni, 1 X. arboricola pv. juglandis, 2 X. arboricola pv. fragariae, 1 X. arboricola pv. corylina, 1 X. arboricola pv. celebensis), 1 X. campestris pv. campestris, 1 X. campestris pv. populi, 2 X. hortorum pv. pelargonii), 3 Pseudomonas savastanoi pv. savastanoi, 1 P. marginalis, 4 P. syringae pv. syringae, 4 Brenneria (ssp. rubrifaciens, quercina, salicis, populi), 2 Pantoea stewartii, 1 Pantoea agglomerans, 1 Erwinia amylovora, 3 Agrobacterium tumefaciens, 2 Rhizobium vitis |
| <b>Specificity value</b>                                                                                                             | No cross-reaction with LAMP-PCR (Enbiotech s.r.l. kit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Diagnostic Specificity</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | 100% LAMP-PCR (Enbiotech s.r.l.)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

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| <b>Reproducibility</b>                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                          | (Concordance) LAMP-PCR (Enbiotech s.r.l.): 85%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Repeatability</b>                                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                          | (Accordance) LAMP-PCR (Enbiotech s.r.l.): 91%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Test performance study</b>                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Test performance study?</b>                                                                                    | yes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Brief details of the test performance study and its output. It available, link to published article/report</b> | <p>1. Two series of olive extracts spiked with ten fold dilution of Xylella fastidiosa CODiRo strain suspensions from <math>10^7</math> to <math>10^1</math> cfu/ml plus two healthy samples (16 samples in total) were tested in three different laboratories (CREA-PAV; CNR-IPSP; Plant Protection Service Lombardy) (NTC, healthy and infected olive extracts as control) for analytical sensitivity. 2. To check the diagnostic sensitivity and specificity, the accuracy, the repeatability and reproducibility, olive extract samples spiked with Xylella fastidiosa CODiRo strain suspensions at <math>10^6</math> cfu/ml (three repetitions), <math>10^4</math> cfu/ml (three repetitions), <math>10^3</math> 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. Digiario) 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)</p> |
| <b>Other information</b>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Any other information considered useful</b>                                                                    | <p>Accuracy: 92% (Enbiotech s.r.l.) Validation data were carried out by the Laboratories listed below, under the supervision of the reference laboratory CREA-PAV with the collaboration of CNR-IPSP:</p> <ul style="list-style-type: none"> <li>• CREA-PAV: Centro di Ricerca per la Patologia Vegetale CREA, Rome (Italy)</li> <li>• CNR-IPSP: Istituto per la Protezione Sostenibile delle Piante CNR, UOS Bari (Italy)</li> <li>• UNIBA: Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Bari (Italy);</li> <li>• Servizio Fitosanitario Regione Lombardia, Laboratorio Fitopatologico c/o Fondazione Minoprio 22070 Vertemate con Minoprio (CO) Italy</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |