

**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>                                                    | Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit<br>7 rue Jean Dixm ras, 49000 Angers, France                                                                                                                                                           |
| <b>Short description of the test</b>                                                 | Detection of <i>Xylella fastidiosa</i> by immunofluorescence in plant material                                                                                                                                                                                             |
| <b>Date, reference of the validation report</b>                                      | 2015-09-30 - Rapport de caract risation et de validation de m thode d'analyse - D tection de <i>Xylella fastidiosa</i> par PCR en temps r el sur plantes h tes MA039                                                                                                       |
| <b>Validation process according to EPPO Standard PM7/98?</b>                         | yes                                                                                                                                                                                                                                                                        |
| <b>Is the lab accredited for this test?</b>                                          | no                                                                                                                                                                                                                                                                         |
| <b>Was the validated data generated in the framework of a project?</b>               |                                                                                                                                                                                                                                                                            |
| <b>Description of the test</b>                                                       |                                                                                                                                                                                                                                                                            |
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| <b>Organism(s)</b>                                                                   | <i>Xylella fastidiosa</i> (XYLEFA)                                                                                                                                                                                                                                         |
| <b>Detection / identification</b>                                                    | detection                                                                                                                                                                                                                                                                  |
| <b>Method(s)</b>                                                                     | Serological IF                                                                                                                                                                                                                                                             |
| <b>Method: Serological IF</b>                                                        |                                                                                                                                                                                                                                                                            |
| <b>Reference of the test description</b>                                             |                                                                                                                                                                                                                                                                            |
| <b>Other information</b>                                                             |                                                                                                                                                                                                                                                                            |
| <b>Other details on the test</b>                                                     | In-house antiserum produced in collaboration with INRA Unit  de recherche Biopolym res, Interactions, Assemblages - Nantes in 2012                                                                                                                                         |
| <b>Are the performance characteristics included in the EPPO diagnostic protocol?</b> | no                                                                                                                                                                                                                                                                         |
| <b>Performance Criteria :</b>                                                        |                                                                                                                                                                                                                                                                            |
| <b>Organism 1.:</b>                                                                  | <b><i>Xylella fastidiosa</i>(XYLEFA)</b>                                                                                                                                                                                                                                   |
| <b>Analytical sensitivity</b>                                                        |                                                                                                                                                                                                                                                                            |
| <b>What is smallest amount of target that can be detected reliably?</b>              | - Grapevine ( <i>Vitis vinifera</i> ): ~ 10 <sup>6</sup> bact./mL - Peach tree ( <i>Prunus persica</i> ): ~ 10 <sup>5</sup> bact./mL - Coffee tree ( <i>Coffea arabica</i> ): ~ 10 <sup>4</sup> bact./mL - Coffee tree ( <i>C. canephora</i> ): ~ 10 <sup>4</sup> bact./mL |
| <b>Diagnostic sensitivity</b>                                                        |                                                                                                                                                                                                                                                                            |

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| <b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b> | - Grapevine ( <i>Vitis vinifera</i> ): 40% - Peach tree ( <i>Prunus persica</i> ): 50% - Orange tree ( <i>Citrus sinensis</i> ): 78% - Coffee tree ( <i>Coffea arabica</i> ): 60% - Coffee tree ( <i>C. canephora</i> ): 30%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Standard test(s)</b>                                                                                                              | - Spiked matrices with bacterial concentration from $10^2$ to $10^6$ bact./mL - Grapevine spiked with <i>X. f. subsp. fastidiosa</i> (CFBP7970) - Orange tree spiked with <i>X. f. subsp. pauca</i> (CFBP8072) - Peach tree spiked with <i>X. f. subsp. multiplex</i> (CFBP8173 and CFBP8070) - Coffee tree ( <i>Coffea arabica</i> ) spiked with <i>X. f. subsp. pauca</i> (CFBP8072) - Coffee tree ( <i>C. canephora</i> ) spiked with <i>X. f. subsp. fastidiosa</i> (CFBP 8074) 20 samples per matrix (orange) 12 samples per matrix (grapevine) 12 samples per matrix (peach tree) 20 samples per matrix ( <i>C. arabica</i> ) 20 samples per matrix ( <i>C. canephora</i> )                                                                                                                                                                                                                                                                                                                                |
| <b>Analytical specificity - inclusivity</b>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Number of strains/populations of target organisms tested</b>                                                                      | Inclusivity tested with 10 target strains: 100% - <i>X.f. subsp. fastidiosa</i> (CFBP8069 -LSV0056/ CFBP8071 -LSV4041/ CFBP8083 -LSV4042/ CFBP7970-LSV2434/ CFBP8082 -LSV4040) - <i>X.f. subsp. pauca</i> (CFBP8072 - LSV4103) - <i>X.f. subsp. sandyi</i> (CFBP8077 -LSV4236) - <i>X.f. subsp. multiplex</i> (CFBP8173 -LSV4039/ CFBP8068-LSV0054/ CFBP8070-LSV4038)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Specificity value</b>                                                                                                             | 100%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Analytical specificity - exclusivity</b>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Number of non-target organisms tested</b>                                                                                         | Exclusivity tested with 17 non-target strains: 100% - 1 <i>Xylophilus ampelinus</i> (CFBP2098) - 2 <i>Xanthomonas arboricola</i> pv. <i>pruni</i> (LSV2574/LSV 2573) - 1 <i>Xanthomonas arboricola</i> pv. <i>juglandis</i> (LSV0862) - 1 <i>Xanthomonas axonopodis</i> pv. <i>citri</i> (LSV2647) - 1 <i>Xanthomonas axonopodis</i> pv. <i>aurantifolia</i> (LSV2680) - 2 <i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i> (LSV1014/LSV3161) - 1 <i>Xanthomonas axonopodis</i> pv. <i>fragariae</i> (LSV3151) - 1 <i>Xanthomonas fragariae</i> (LSV2553) - 1 <i>Xanthomonas hortorum</i> pv. <i>carotae</i> (LSV1776) - 1 <i>Xanthomonas campestris</i> pv. <i>campestris</i> (LSV0455) - 1 <i>Xanthomonas campestris</i> pv. <i>juglandis</i> (LSV1158) - 1 <i>Xanthomonas hortorum</i> pv. <i>hedera</i> (LSV2303) - 1 <i>Xanthomonas translucens</i> pv. <i>graminis</i> (LSV0628) - 1 <i>Xanthomonas translucens</i> pv. <i>hordei</i> (LSV0629) - 1 <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> (LSV0865) |
| <b>Specificity value</b>                                                                                                             | 100% No cross reaction                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Diagnostic Specificity</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | - Coffee tree ( <i>Coffea arabica</i> ): 100% - Coffee tree ( <i>C. canephora</i> ): 100%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Repeatability</b>                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

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| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b> | - Grapevine (Vitis vinifera): 67% - Peach tree (Prunus persica): 83% |
| <b>Test performance study</b>                                                            |                                                                      |
| <b>Test performance study?</b>                                                           | no                                                                   |

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