**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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| **Laboratory contact details** | Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit<br>7 rue Jean Dixméras, 49044 Angers, France |
| **Short description of the test** | Detection of Xylella fastidiosa by real-time PCR in plant material (Harper et al., 2010, Erratum 2013) |
| **Date, reference of the validation report** | 2015-09-30 - Rapport de caractérisation et de validation de méthode d'analyse - Détection de Xylella fastidiosa par PCR en temps réel sur plantes hôtes MA039ver01 |
| **Validation process according to EPPO Standard PM7/98?** | yes |
| **Is the lab accredited for this test?** | no |
| **Was the validated data generated in the framework of a project?** | |
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| **Description of the test** | |
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| **Organism(s)** | Xylella fastidiosa (XYLEFA) |
| **Detection / identification** | detection |
| **Method(s)** | Molecular Extraction DNA RNA<br>Molecular real time PCR |
| **Method: Molecular Extraction DNA RNA** | |
| ***Reference of the test description*** | |
| **As or adapted from an EPPO diagnostic protocol** | yes |
| **EPPO Diagnostic Protocol name** | PM 7/024 Xylella fastidiosa (version 4) |
| ***Kit*** | |
| **Is a kit used** | yes |
| **Manufacturer name** | BIONOBILE |
| **Specify the kit used** | QuickPick™ SML Plant DNA |
| Kit used following the manufacturer's instructions? | |
| ***Other information*** | |
| **Other details on the test** | QuickPick™ Plant DNA kit (Bio-Nobile) Automated protocol with KingFisher™ mL (Themo Scientific) |
| **Method: Molecular real time PCR** | |

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| ***Reference of the test description*** | |
| **As or adapted from an EPPO diagnostic protocol** | yes |
| **EPPO Diagnostic Protocol name** | PM 7/024 Xylella fastidiosa (version 4) |
| **Name of the test** | Real-time PCR - simplex (Harper et al., 2010; erratum 2013) |
| **Is the test modified compared to the reference test** | no |
| ***Other information*** | |
| **Performance Criteria :** | |
| **Organism 1.:** | **Xylella fastidiosa(XYLEFA)** |
| **Analytical sensitivity** | |
| **What is smallest amount of target that can be detected reliably?** | - Grapevine: ˜ 10^3 bact./mL - Orange tree: ˜ 10^2 bact./mL - Olive tree: ˜ 10^5 bact./mL With a probability of detection of 100% |
| **Diagnostic sensitivity** | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98** | - Grapevine: 94% - Orange tree: 100% - Olive tree: 67% |
| **Standard test(s)** | - Spiked matrices with bacterial concentration from 10^2 to 10^5 bact./mL - Grapevine spiked with X. f. subsp. fastidiosa (CFBP7970) - Orange tree spiked with X. f. subsp. pauca (CFBP8072) - Olive tree spiked with X. f. subsp. multiplex (CFBP8173) 15 samples per matrix 30 DNA extraction per matrix 60 amplifications per matrix |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | Inclusivity tested with19 target strains: 100% - X.f. subsp. fastidiosa (CFBP8069 - LSV 0056 / CFBP7970 - LSV 2434 / CFBP8082 - LSV 4040 / CFBP8071 - LSV 4041 / CFBP8083 - LSV 4042 / CFBP8073-LSV4209 / CFBP8351 - LSV4626) - X.f. subsp. pauca (CFBP8072 - LSV 4103) - X.f. subsp. sandyi (CFBP8077 - LSV 4236 / CFBP 8356 - LSV4627 / LSV4628 / LSV4639 / LSV4659) - X.f. subsp. multiplex (CFBP8068 - LSV 0054 / CFBP8070 - LSV 4038/ CFBP8173 - LSV 4039 / CFBP8075 - LSV 4230/ CFBP8076 - LSV 4231 / CFBP8078 - LSV 4311) Bacterial suspension concentration of about 10^7 bact./mL |
| **Specificity value** | 100% |
| **Analytical specificity - exclusivity** | |
| **Number of non-target organisms tested** | Exclusity tested with 29 non-target strains: 100% - 1 Xylophilus ampelinus (CFBP2098) - 2 Xanthomonas arboricola pv. pruni (LSV2574/LSV 2573) - 1 Xanthomonas arboricola pv. juglandis (LSV0862) - 1 Xanthomonas axonopodis pv. citri (LSV2647) - 1 Xanthomonas axonopodis pv. aurantifolia (LSV2680) - 2 Xanthomonas |

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| | axonopodis pv. phaseoli (LSV1014/LSV3161) - 1 Xanthomonas axonopodis pv. fragariae (LSV3151) - 1 Xanthomonas fragariae (LSV2553) - 1 Xanthomonas hortorum pv. carotae (LSV1776) - 1 Xanthomonas campestris pv. campestris (LSV0455) - 1 Xanthomonas campestris pv. juglandis (LSV1158) - 1 Xanthomonas hortorum pv. hedera (LSV2303) - 1 Xanthomonas translucens pv. graminis (LSV0628) - 1 Xanthomonas translucens pv. hordei (LSV0629) - 1 Xanthomonas oryzae pv. oryzae (LSV0865) - 1 Ca. Liberibacter asiaticus - 1 Ca. L. africanus - 6 saprophytic bacteria saprophytes isolated from Coffea spp. - 4 bactéries saprophytes isolées de Citrus sinensis Bacterial suspension concentration of about 10^7 bact./mL |
| **Specificity value** | 100% no cross reaction |
| **Diagnostic Specificity** | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | - Grapevine: 100% - Orange tree: 100% - Olive tree: 100% |
| **Specify the test(s)** | Spiked matrices with bacterial concentration from 10^3 to 10^5 bact./mL - Grapevine spiked with X. f. subsp. fastidiosa (CFBP7970) - Orange tree spiked with X. f. subsp. pauca (CFBP8072) - Olive tree spiked with X. f. subsp. multiplex (CFBP8173) 15 samples per matrix 30 DNA extraction per matrix 60 amplifications per matrix |
| **Reproducibility** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 98% |
| **Repeatability** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | - Grapevine: 96% - Orange tree: 100% - Olive tree: 100% |
| **Test performance study** | |
| **Test performance study?** | yes |
| **Brief details of the test performance study and its output.It available, link to published article/report** | A test performance study was performed in 2014 for the Real time PCR Harper et al., 2010 method but with another DNA extraction method (DNeasy® Plant mini kit (Qiagen) Analytical sensitivity (with a probability of detection of 100%): - Orange tree: ˜ 10^2 bact./mL - Grapevine: ˜ 10^6 bact./mL - Peach tree: ˜ 10^4 bact./mL - Olive tree: ˜ 10^5 bact./mL - Coffee tree: ˜ 10^4 bact./mL Diagnostic sensitivity: 97% Diagnostic specificity: 97% Reproducibility: 84% Repeatability: 91% |
| **Other information** | |
| **Any other information considered useful** | For information, a proficiency test was performed in 2015 for this method. |

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