**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** | Fera<br>Sand Hutton, YO41 1LZ York, United Kingdom |
| **Short description of the test** | Subspecies identification of Xylella fastidiosa by molecular real time PCR |
| **Date, reference of the validation report** | 2018-08-14 - Fera_Hodgetts_Xf_subspecies |
| **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?** | Other_project |
| **If yes, please specify** | Defra Future Proofing Plant Health |
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| **Description of the test** | |
| | |
| **Organism(s)** | Xylella fastidiosa (XYLEFA) |
| **Detection / identification** | identification |
| **Matrix(ces) tested** | Leaves, Pure culture Isolates sourced from NCPPB and LMG. A limited number of artificially infected and naturally infected plant species were tested. |
| **Plant species tested** | Calicotome, Catharanthus roseus, Coffea arabica, Genista, Helichrysum, Laurus nobilis, Myrtus communis, Olea europaea, Polygala, Salvia rosmarinus |
| **Method(s)** | Molecular real time PCR |
| **Method: Molecular real time PCR** | |
| ***Reference of the test description*** | |
| **As or adapted from an EPPO diagnostic protocol** | no |
| **New test being considered for inclusion in the next version of the EPPO diagnostic protocol?** | yes |
| **As or adapted from an IPPC diagnostic protocol** | no |
| **Reference of the test** | Hodgetts, J., Glover, R., Cole, J., Hall, J. and Boonham, N. (2021), Genomics informed design of a suite of real-time PCR assays for the specific detection of each Xylella fastidiosa subspecies. J Appl Microbiol, 131: 855-872. |

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| **Is the test modified compared to the reference test** | yes The final primer concentration was 375 nM (instead of 300 nM) |
| ***Kit*** | |
| **Is a kit used** | no |
| ***Other information*** | |
| **Reaction type** | Simplex |
| **Other details on the test** | Taqman Fast Universal PCR Master Mix, no AmpErase UNG, Ultrapure bovine serum albumin |
| **Performance Criteria :** | |
| **Organism 1.:** | **Xylella fastidiosa(XYLEFA)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | X. f. subsp. fastidiosa = 124 fg X. f. subsp. multiplex = 182 fg X. f. subsp. pauca = 84.2 fg X. f. subsp. morus = 59.2 fg X. f. subsp. sandyi = 908 fg |
| **Diagnostic sensitivity** | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98** | 100% |
| **Standard test(s)** | Real-time PCR Harper et al., 2010 |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 8 target strains: X. f. subsp. fastidiosa (LMG 17159, LMG 15099, NCPPB 4605), X. f. subsp. multiplex (LMG 9063, NCPPB 4604), X. f. subsp. pauca (NCPPB 4595), X. f. subsp. morus (NCPPB 4589), X. f. subsp. sandyi (NCPPB 4606) |
| **Specificity value** | 100% |
| **Analytical specificity - exclusivity** | |
| **Number of non-target organisms tested** | 50 strains |
| **Specificity value** | 100% |
| **Diagnostic Specificity** | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | 100% |
| **Specify the test(s)** | Real-time PCR Harper et al., 2010 |
| **Reproducibility** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | High bacterial DNA concentration (5.92-18.2 ng DNA per reaction): 100%; Low bacterial DNA concentration (592-1820 fg DNA per reaction): 92% - 100%; Very low DNA concentration (59.2-182 fg DNA per reaction): test dependent (8% - 63%) |
| **Repeatability** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | High bacterial DNA concentration (5.92-18.2 ng DNA per reaction): 100%; Low bacterial DNA concentration (592-1820 fg DNA per reaction): |

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| | 100%; Very low bacterial DNA concentration (59.2-182 fg DNA per reaction): test dependent (0% - 62.5%) |
| **Test performance study** | |
| **Test performance study?** | no |
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| The following complementary files are available online: | • Specificity- List of bacterial species |

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