

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 | National Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 111, 1000 Ljubljana, Slovenia |
| Short description of the test | Detection of <i>Xylella fastidiosa</i> by real-time PCR in plant material |
| Date, reference of the validation report | 2016-02-26 - Dreo, Tanja, 2016. Validation data on the modified real-time PCR for detection of <i>Xylella fastidiosa</i> adapted from Francis et al. (2006) (No. D0002/16). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana. |
| Validation process according to EPPO Standard PM7/98? | no |
| Is the lab accredited for this test? | no |
| Was the validated data generated in the framework of a project? | |
| If yes, please specify | |
| Description of the test | |
| Organism(s) | <i>Xylella fastidiosa</i> (XYLEFA) |
| Detection / identification | detection |
| Matrix(ces) tested | Leaves, Shoots Plant material, mainly mixtures of midribs, petioles and vascular tissues. |
| Plant species tested | <i>Asparagus acutifolius</i> , <i>Coffea</i> , <i>Lavandula</i> , <i>Nerium oleander</i> , <i>Olea europaea</i> , <i>Polygala myrtifolia</i> , <i>Quercus cerris</i> , <i>Salvia rosmarinus</i> , <i>Spartium junceum</i> |
| Method(s) | Molecular Extraction DNA RNA Molecular real time PCR |
| Method: Molecular Extraction DNA RNA | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | yes |
| New test being considered for inclusion in the next version of the EPPO diagnostic protocol? | |
| EPPO Diagnostic Protocol name | PM 7/024 <i>Xylella fastidiosa</i> (version 2) |

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| Name of the test | |
| As or adapted from an IPPC diagnostic protocol | no |
| Is the test modified compared to the reference test | |
| 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 | DNA extraction from plant material using QuickPick™ SML Plant DNA kit (Bionobile). |
| Method: Molecular real time PCR | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | yes |
| New test being considered for inclusion in the next version of the EPPO diagnostic protocol? | |
| EPPO Diagnostic Protocol name | PM 7/024 Xylella fastidiosa (version 2) |
| Name of the test | Taqman real-time PCR tests (based on Francis et al., 2006) |
| As or adapted from an IPPC diagnostic protocol | no |
| Is the test modified compared to the reference test | |
| Kit | |
| Is a kit used | |
| Other information | |
| Reaction type | Simplex - Probe |
| Other details on the test | Modified real-time PCR adapted from Francis, M., Lin, H., Rosa, J.C.-L., Doddapaneni, H., Civerolo, E.L., 2006. Genome-based PCR Primers for Specific and Sensitive Detection and Quantification of Xylella fastidiosa. European Journal of Plant Pathology 115, 203-213. doi:10.1007/s10658-006-9009-4 |
| Are the performance characteristics included in the EPPO diagnostic protocol? | yes |
| Performance Criteria : | |
| Organism 1.: | Xylella fastidiosa(XYLEFA) |
| Analytical sensitivity | |

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| What is smallest amount of target that can be detected reliably? | On the DNA extracted from pure cultures of <i>X. fastidiosa</i> : 2.6, 3.2 and 3.5 (log (cells/mL) of <i>Xylella fastidiosa</i> subsp. multiplex, <i>Xylella fastidiosa</i> , and <i>Xylella fastidiosa</i> subsp. pauca CoDiRO strain, respectively. On plant material: 94 % (determined on log 5 cells/mL of plant extracts; the lowest concentration tested) |
| <u>Diagnostic sensitivity</u> | |
| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | No data available. |
| Standard test(s) | |
| <u>Analytical specificity - inclusivity</u> | |
| Number of strains/populations of target organisms tested | 4 |
| Specificity value | 100% |
| <u>Analytical specificity - exclusivity</u> | |
| Number of non-target organisms tested | 15 |
| Specificity value | 100% No cross reactions were observed. |
| Cross reacts with | |
| <u>Diagnostic Specificity</u> | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | No data available. |
| Specify the test(s) | |
| <u>Reproducibility</u> | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 0,97 |
| <u>Repeatability</u> | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | No data available. |
| <u>Test performance study</u> | |
| Test performance study? | no |
| Brief details of the test performance study and its output. It available, link to published article/report | |
| <u>Other information</u> | |
| Any other information considered useful | |
| The following complementary files are available online: | <ul style="list-style-type: none"> • Validation data on the modified real-time PCR for detection of <i>Xylella fastidiosa</i> adapted from Francis et al. (2006) (No. D0002/16) |

