

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 | Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy |
| Short description of the test | detection of <i>Xylella fastidiosa</i> in composite olive samples |
| Date, reference of the validation report | 2021-09-26 - Diagnostic Procedures to Detect <i>Xylella fastidiosa</i> in Nursery Stocks and Consignments of Plants for Planting. Agriculture 2021, 11, 922. https://doi.org/10.3390/agriculture11100922 |
| 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 | XF-ACTORS |
| Description of the test | |
| Organism(s) | <i>Xylella fastidiosa</i> (XYLEFA) |
| Detection / identification | detection |
| Method(s) | Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) 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 <i>Xylella fastidiosa</i> (version 4) |
| Is the test modified compared to the reference test | no |
| Kit | |
| Is a kit used | yes |
| Manufacturer name | QIAGEN |
| Specify the kit used | DNeasy mericon Food Kit |

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| Kit used following the manufacturer's instructions? | yes "Modified DNeasy Mericon™ Food Standard Protocol" (Qiagen) |
| Other information | |
| Other details on the test | Total DNA were extracted from composite olive samples, prepared as reported in the attached additional file, by using "Modified DNeasy Mericon™ Food Standard Protocol" (Qiagen) |
| Method: Molecular Extraction DNA RNA (2) | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | no |
| Kit | |
| Is a kit used | yes |
| Manufacturer name | PROMEGA |
| Specify the kit used | Maxwell® RSC PureFood GMO and Authentication Kit |
| Kit used following the manufacturer's instructions? | |
| Other information | |
| Other details on the test | Total DNA were extracted from composite olive samples, prepared as reported in the attached additional file, by using "Maxwell® RSC PureFood GMO and Authentication Kit" protocol (Promega) |
| Method: Molecular Extraction DNA RNA (3) | |
| 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) |
| As or adapted from an IPPC diagnostic protocol | no |
| Is the test modified compared to the reference test | no |
| Kit | |
| Is a kit used | yes |
| Manufacturer name | |
| Specify the kit used | |
| Kit used following the manufacturer's instructions? | |
| Other information | |
| Other details on the test | Total DNA were extracted from composite olive samples, prepared as reported in the attached additional file, by using CTAB-based extraction protocol, Modified DNeasy® Mericon™ Food Standard Protocol and the Maxwell® RSC PureFood GMO and Authentication Kit" (Promega) |

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| Method: Molecular real time PCR | |
| Reference of the test description | |
| As or adapted from an EPPD diagnostic protocol | yes |
| EPPD Diagnostic Protocol name | PM 7/024 Xylella fastidiosa (version 3) |
| Name of the test | Real-time PCR - simplex (Harper et al., 2010; erratum 2013) |
| Is the test modified compared to the reference test | yes BSA was not included in the amplification MIX |
| Other information | |
| Performance Criteria : | |
| Organism 1.: | Xylella fastidiosa(XYLEFA) |
| Analytical sensitivity | |
| What is smallest amount of target that can be detected reliably? | 4 infected olive leaves in 20 gr of leaf petioles/midribs recovered from Xylella-free olive plants |
| Diagnostic sensitivity | |
| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | 100% using the 3 different DNA extraction procedures |
| Standard test(s) | standard tests reported by appendix 3 and 5 of PM 7/24 (3) |
| Diagnostic Specificity | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | 100% using the 3 different DNA extraction procedures |
| Specify the test(s) | standard tests reported by appendix 3 and 5 of PM 7/24 (3) |
| Repeatability | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 100% using the 3 different DNA extraction procedures |
| Test performance study | |
| Test performance study? | no |
| Other information | |
| Any other information considered useful | These validation data were obtained by IPSP-CNR in collaboration with the Department of Soil, Plant and Food Science of the University of Bari (ITAY). For any additional detail, see the attached file. |
| The following complementary files are available online: | |
| | • paper 2021 |