

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 - Nematology Unit Domaine de la Motte au Viconte BP 35327, 35653 Le Rheu, France |
| Short description of the test | identification of <i>Meloidogyne enterolobii</i> by Molecular real-time PCR in juveniles |
| Date, reference of the validation report | 2024-08-21 - Method for the identification of <i>Meloidogyne enterolobii</i> by real-time PCR (ANSES/LSV/MA071 - partially) |
| Validation process according to EPPO Standard PM7/98? | yes |
| Is the lab accredited for this test? | yes |
| Was the validated data generated in the framework of a project? | EURL |
| If yes, please specify | EU funded project EURLs-EURCs 2023-2024 (grant Project 101143591) and funded by ANSES - Plant Health Laboratory - Nematology Unit |
| Description of the test | |
| Organism(s) | <i>Meloidogyne enterolobii</i> (MELGMY) |
| Detection / identification | identification |
| 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? | yes |
| EPPO Diagnostic Protocol name | PM 7/103 <i>Meloidogyne enterolobii</i> (version 2) |
| As or adapted from an IPPC diagnostic protocol | no |
| Is the test modified compared to the reference test | no |
| Kit | |
| Is a kit used | no |

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| Other information | |
| Other details on the test | Based on the use of lysis buffer (see details in the report). Final volume 100 microliter evaluated. |
| 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 | Kiewnick et al. 2015 |
| Is the test modified compared to the reference test | no |
| Kit | |
| Is a kit used | no |
| Other information | |
| Reaction type | Simplex - Probe |
| Other details on the test | Minor modifications were made to the test compared with the publication, for reasons of harmonisation with practices already in place in the laboratory and after checking that this had no impact on the performance of the test. See details in the validation report. |
| Performance Criteria : | |
| Organism 1.: | Meloidogyne enterolobii(MELGMY) |
| Analytical sensitivity | |
| What is smallest amount of target that can be detected reliably? | 1 nematode (juvenile or female or male) 100% |
| Analytical specificity - inclusivity | |
| Number of strains/populations of target organisms tested | 6 populations of M. enterolobii, one from Senegal, two from the USA, and one from Switzerland, Guadeloupe and the Ivory Coast. |
| Specificity value | 100% |
| Analytical specificity - exclusivity | |
| Number of non-target organisms tested | 31 populations: 28 belonging to 12 species of Meloidogyne (M. arenaria, M. chitwoodi, M. fallax, M. hapla, M. incognita, M. javanica, M. oryzae, M. graminicola, M. minor, M. naasi, M. artiellia); and 3 of different Globodera species (G. pallida, G. rostochiensis, G. tabacum) |
| Specificity value | 100% |
| Reproducibility | |

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| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 8 replicates were analyzed in 3 different trials, performed on different days and/or using 2 different real-time PCR thermocyclers: 100% for 1 and 2 juveniles (J2) of <i>M. enterolobii</i> (8 replicates x 3 PCR trials x 2 modalities = 48 tests). |
| <u>Repeatability</u> | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 8 replicates: 100% for 1 and 2 juveniles (J2) of <i>M. enterolobii</i> were analyzed in 3 different trials (8 replicates x 3 PCR trials x 2 modalities = 48 tests) |
| Test performance study | |
| Test performance study? | yes |
| Brief details of the test performance study and its output. It available, link to published article/report | (2016) Assessment of a new qPCR tool for the detection and identification of the root-knot nematode <i>Meloidogyne enterolobii</i> by an international test performance study (TPS). Braun-Kiewnick, et al. Eur J Plant Pathol 144, 97–108. https://doi.org/10.1007/s10658-015-0754-0 |
| Other information | |
| Any other information considered useful | Report available on the EURL website for the NRLs or available on request to the EURL. |

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