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.

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 Meloidogyne enterolobii by Molecular real-time PCR in juveniles
Date, reference of the validation report	2024-08-21 - Method for the identification of Meloidogyne enterolobii by real-time PCR (ANSES/LSV/MA071 – partially)
Link to other validation data	- Method for the Detection of Meloidogyne enterolobii in a Soil Extract (Version 1) Detection of Meloidogyne enterolobii in a soil extract. Molecular extraction method.
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)	Meloidogyne enterolobii (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 Meloidogyne enterolobii (version 2)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the	no

reference test	
Kit	
Is a kit used	no
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	
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 M. enterolobii (8 replicates x 3 PCR trials x 2 modalities = 48 tests).
Repeatability	
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 M. enterolobii 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 Meloidogyne enterolobii 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	The report is not publicly available, but can be provided on request (eurl.nematodes@anses.fr). It is resticted to those registered to the EURL website (see link below): https://sitesv2.anses.fr/en/minisite/plant-parasitic-nematodes/method-and-test-validation-reports . The report has been published to Zenodo with restricted access with the following citation: European Union Reference Laboratory for Plant Parasitic Nematodes. (2024). Method for the identification of Meloidogyne enterolobii by Real-Time PCR (Version 1). Zenodo. https://doi.org/10.5281/zenodo.14653321

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