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 graminicola by Molecular real time PCR in juveniles
Date, reference of the validation report	2024-08-21 - Identification of Meloidogyne graminicola by real-time PCR Htay et al 2016 on isolated juveniles
Link to other validation data	- Identification of Meloidogyne graminicola by real-time PCR Mattos et al., 2019 on isolated juveniles identification of Meloidogyne graminicola by Molecular real time PCR in juveniles
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?	EURL
If yes, please specify	EU funded project EURLs-EURCs 2023-2024 (grant Project 101143591)
Description of the test	
Organism(s)	Meloidogyne graminicola(MELGGC)
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	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	Ibrahim et al. 1994
Is the test modified compared to the reference test	yes

Kit	
Is a kit used	no
Other information	
Other details on the test	-Based on the use of a lysis buffer (see details in the report and EPPO diagnostic protocol). 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	Htay et al 2016
Is the test modified compared to the reference test	yes The reference test is in conventional PCR, which was adapted for a real-time PCR
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Other details on the test	The test was developed by Htay et al., 2016, and further adapted by INIAV during an EURL TPS (Report 22MG), and validated by the EURL for Plant Parasitic Nematode
Performance Criteria :	
Organism 1.:	**Meloidogyne graminicola(MELGGC)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	1 nematode (J2) 100%
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Population from Italy amplified (1, 2, 5 and 10 J2)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	22 populations (2 of M. minor, 3 of M. hapla, 2 of M. chitwoodi, 2 of M. fallax, 2 of M. arenaria, 2 of M. artiellia, 2 of M. enterolobii, 2 of M. incognita, 2 of M. javanica, 2 of M. naasi, one of M. hispanica, and one of M. oryzae.
Specificity value	cross-reaction with M. oryzae (Ct < 27). Other species Ct > 35 or no amplification
Cross reacts with	Meloidogyne oryzae

Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	8 replicates were analyzed in 2 different trials, performed on different days and/or using two real - time PCR machines: 100% for 1, 2, and 5 J2 of M. graminicola (8 replicates x 2 PCR trials x 3 modalities = 48 tests)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Evaluated using 8 replicates in 3 PCR trials: 100% for 1, 2, and 5 J2 of M. graminicola (8 replicates x 3 PCR trials x 3 modalities = 72 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	TEST PERFORMANCE STUDY REPORT 22MG Identification of Meloidogyne graminicola by molecular conventional PCR Htay et al 2016 in juveniles
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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