

**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.

<b>Laboratory contact details</b>	Anses Plant Health Laboratory - Nematology Unit Domaine de la Motte au Viconte BP 35327, 35653 Le Rheu, France
<b>Short description of the test</b>	identification of Meloidogyne graminicola by Molecular real time PCR in juveniles
<b>Date, reference of the validation report</b>	2024-08-21 - Identification of Meloidogyne graminicola by real-time PCR Htay et al 2016 on isolated juveniles
<b>Link to other validation data</b>	- 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
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	EURL
<b>If yes, please specify</b>	EU funded project EURLs-EURCs 2023-2024 (grant Project 101143591)
<b>Description of the test</b>	
<b>Organism(s)</b>	Meloidogyne graminicola (MELGGC)
<b>Detection / identification</b>	identification
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Ibrahim et al. 1994
<b>Is the test modified compared to the reference test</b>	yes

<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	-Based on the use of a lysis buffer (see details in the report and EPPO diagnostic protocol). Final volume 100 microliter evaluated.
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Htay et al 2016
<b>Is the test modified compared to the reference test</b>	yes The reference test is in conventional PCR, which was adapted for a real-time PCR
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	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
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Meloidogyne graminicola(MELGGC)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	1 nematode (J2) 100%
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	Population from Italy amplified (1, 2, 5 and 10 J2)
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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.
<b>Specificity value</b>	cross-reaction with M. oryzae (Ct < 27). Other species Ct > 35 or no amplification
<b>Cross reacts with</b>	Meloidogyne oryzae

<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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)
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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)
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	TEST PERFORMANCE STUDY REPORT 22MG Identification of Meloidogyne graminicola by molecular conventional PCR Htay et al 2016 in juveniles
<b>Other information</b>	
<b>Any other information considered useful</b>	Report available on the EURL website for the NRLs or available on request to the EURL.

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