

**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>	ILVO Institute for Agricultural and Fisheries Research Burg. Van Gansberghelaan 96, 9820 Merelbeke - Melle, Belgium
<b>Short description of the test</b>	Identification of <i>Meloidogyne graminicola</i> by molecular conventional PCR Htay et al., 2016 in juveniles
<b>Date, reference of the validation report</b>	2023-10-31 - Validation report for the molecular identification of <i>Meloidogyne graminicola</i>
<b>Link to other validation data</b>	<ul style="list-style-type: none"> <li>- Validation report for the molecular identification of <i>Meloidogyne graminicola</i> Identification of <i>Meloidogyne graminicola</i> by molecular real time PCR Htay et al., 2016 in juveniles</li> <li>- Validation report for the molecular identification of <i>Meloidogyne graminicola</i> Identification of <i>Meloidogyne graminicola</i> by molecular conventional PCR Mattos et al., 2019 (M. oryzae primers) in juveniles</li> <li>- TEST PERFORMANCE STUDY REPORT 22MG Identification of <i>Meloidogyne graminicola</i> by molecular conventional PCR Bellafigliore et al. 2015 in juveniles</li> <li>- TEST PERFORMANCE STUDY REPORT 22MG Identification of <i>Meloidogyne graminicola</i> by molecular conventional PCR Htay et al 2016 in juveniles</li> <li>- Validation report for the molecular identification of <i>Meloidogyne graminicola</i> Identification of <i>Meloidogyne graminicola</i> by molecular conventional PCR Mattos et al., 2019 (M. graminicola primers) in juveniles</li> <li>- Validation report for the molecular identification of <i>Meloidogyne graminicola</i> Identification of <i>Meloidogyne graminicola</i> by molecular conventional PCR Bellafigliore et al. 2015 in juveniles</li> <li>- Validation report for the molecular identification of <i>Meloidogyne graminicola</i> Identification of <i>Meloidogyne graminicola</i> by molecular conventional PCR He et al., 2021 in juveniles</li> <li>- Validation report for the molecular identification of <i>Meloidogyne graminicola</i> Identification of <i>Meloidogyne graminicola</i> by molecular real time PCR He et al., 2021 in juveniles</li> </ul>
<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 2021-2022 (grant SI2.870859)
<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 Conventional PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<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 Worm lysis buffer (see details in the report). Final volume 50 microliter evaluated.
<b>Method: Molecular Conventional 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>	no
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<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) 90% (1 well did not amplify which may be an experimental error) 2 nematodes (J2) 100%

<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	Population from Brazil, Italy and the Philippines amplified (10 J2)
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	M. minor, M. hapla, M. chitwoodi, M. fallax, M. arenaria, M. enterolobii, M. incognita, M. javanica, M. naasi (2 populations), M. oryzae and M. salasi
<b>Specificity value</b>	cross reaction M. oryzae and M salasi
<b>Cross reacts with</b>	Meloidogyne oryzae Meloidogyne salasi
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Evaluated by 2 operators (10 replicates for 1 J2 and 2 J2). similar results obtained. Results reproducible
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Same operator repeated experiment twice. 2 nematode extracted (10 replicates): 100% positives in the 2 experiments 1 nematode extracted (10 replicates): 90% positive in first experiment and 100% positive in second experiment
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	Report available on the EURL website for the EU NRLs or available on request to the EURL.

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