

**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 Meloidogyne graminicola by molecular real time PCR He et al., 2021 in juveniles
<b>Date, reference of the validation report</b>	2023-10-31 - Validation report for the molecular identification of Meloidogyne graminicola
<b>Link to other validation data</b>	<ul style="list-style-type: none"> <li>- Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular conventional PCR Mattos et al., 2019 (M. oryzae primers) in juveniles</li> <li>- Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular conventional PCR Bellafiore et al. 2015 in juveniles</li> <li>- Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular conventional PCR Htay et al., 2016 in juveniles</li> <li>- Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular real time PCR Htay et al., 2016 in juveniles</li> <li>- Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular conventional PCR He et al., 2021 in juveniles</li> <li>- Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular conventional PCR Mattos et al., 2019 (M. graminicola primers) 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 real time 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 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>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	He et al. 2021
<b>Is the test modified compared to the reference test</b>	yes Enzyme activation 3 min instead of 30 sec, 40 cycles instead of 39 cycles, melting curve between 60°C and 95°C at 0.1°C/sec instead of 0.3°C/15 sec
<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>	no
<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>	10 J2 and 10 fold dilutions: 100%
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	Population from Brazil (30 ng/uL), Italy and the Philippines (2 J2) amplified
<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, M. oryzae (10J2), M. salasi
<b>Specificity value</b>	
<b>Cross reacts with</b>	Meloidogyne oryzae Meloidogyne salasi Meloidogyne naasi
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	10 J2 and 10 fold dilutions (5 replicates, 2 operators): 100%
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	10 J2 and 10 fold dilutions (5 replicates): 100%
<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 on request to the EURL.

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