

**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, Belgium
<b>Short description of the test</b>	Identification of <i>Meloidogyne graminicola</i> by molecular conventional PCR Mattos et al., 2019 (M. oryzae primers) 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 conventional PCR Bellafiore 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 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 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 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> <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> </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 or 5 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>	Mattos et al., 2019
<b>Is the test modified compared to the reference test</b>	yes Adapted to "normal" concentrations: the publication uses abnormally high concentrations of dNTPs: 200 µM used instead of 2.5mM in publication, primer concentration 1µM instead of 4µM in publication and polymerase 1U/25µl instead of 1U/µl in publication. Different cycler program.
<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>	M. oryzae primers 2.5 uL of DNA in final mix
<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>	With 2.5 uL DNA in PCR mix 5 J2 (in 50µl WLB): 100% (10 replicates) 1 J2 (in 5µl WLB): 100 % (10 replicates)
<b>Analytical specificity - inclusivity</b>	

<b>Number of strains/populations of target organisms tested</b>	Only one population was tested: M. oryzae from Brazil (obtained via ANSES)
<b>Specificity value</b>	
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	M. graminicola (philippine and italian population), M naasi
<b>Specificity value</b>	
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	With 2.5 uL DNA in PCR mix 5 J2 (in 50µl WLB): 100% (10 replicates) 1 J2 (in 5µl WLB): 100 % (10 replicates)
<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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