

**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>	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy
<b>Short description of the test</b>	Detection of <i>Meloidogyne graminicola</i> by SCAR conventional PCR in rice roots.
<b>Date, reference of the validation report</b>	2017-03-09 - Fanelli et al. 2017 Eur J Plant Pathol 149, 467-476
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Meloidogyne graminicola</i> (MELGGC)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular Conventional PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Fanelli et al. 2017
<b>Is the test modified compared to the reference test</b>	no
<b>Other information</b>	
<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>	De Luca et al., 2004 and Fanelli et al., 2017.
<b>Other information</b>	
<b>Other details on the test</b>	Total DNA was extracted from individual IJs or pool of 5-10 IJs as described in De Luca et al., 2004 and Fanelli et al., 2017.
<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>	Bellafiore, S., Jouglu, C., Chapuis, E., Besnard, G., Suong, M., Vu, P. N., De Waele, D., Gantet, P., & Thi, X. N. (2015). Intraspecific variability of the facultative meiotic parthenogenetic root-knot nematode ( <i>Meloidogyne graminicola</i> ) from rice fields in Vietnam. <i>Comptes Rendus Biologies</i> , 338, 471-483.
<b>Is the test modified compared to the reference test</b>	yes Lysis buffer and DNA extraction technique as reported in De Luca et al., 2004, PCR kit (GoTaq Flexi Promega) PCR cycling (an initial denaturation at 94°C for 3 min, followed by 35 cycles: 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min and a final step at 72°C for 7 min).
<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>	One individual infective juvenile (1 J2).
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	100% Italian <i>M. graminicola</i> population 100% for Philippines population
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target</b>	Two populations: one from Italy (18 specimens) and

<b>organisms tested</b>	one from Phylippine (13 specimens).
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Non-target organisms tested 4 J2 of <i>Meloidogyne oryzae</i> , 3J2 of <i>Meloidogyne naasi</i> , 3 J2 of <i>Meloidogyne javanica</i> and 25 J2 of <i>Meloidogyne incognita</i> .
<b>Specificity value</b>	97% Only one cross-reaction occurred in <i>Meloidogyne oryzae</i> (1J2).
<b>Cross reacts with</b>	<i>Meloidogyne oryzae</i>
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% (evaluated with 5 replicates by 1 operator on 5 different days and with 2 different PCR equipment).
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% (evaluated on 10 replicates).
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	De Luca, F., Fanelli, E., Di Vito, M., Reyes, A., & De Giorgi, C. (2004). Comparison of the sequences of the D3 expansion of the 26S ribosomal genes reveals different degrees of heterogeneity in different populations and species of <i>Pratylenchus</i> from the Mediterranean region. <i>European Journal of Plant Pathology</i> , 110, 949-957. Fanelli, Elena & Cotroneo, Alba & Carisio, Loredana & Troccoli, Alberto & Grosso, Silvio & Boero, Cristina & Capriglia, Francesco & De Luca, Francesca. (2017). Detection and molecular characterization of the rice root-knot nematode <i>Meloidogyne graminicola</i> in Italy. <i>European Journal of Plant Pathology</i> . 10.1007/s10658-017-1196-7. Fanelli, E., Gaffuri, F., Troccoli, A., Sacchi, S., & De Luca, F. (2022). New occurrence of <i>Meloidogyne graminicola</i> (Nematoda: Meloidogyninae) from rice fields in Italy: Variability and phylogenetic relationships. <i>Ecology and Evolution</i> , 12, e9326. <a href="https://doi.org/10.1002/ece3.9326">https://doi.org/10.1002/ece3.9326</a>

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