

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.

Laboratory contact details	ILVO Institute for Agricultural and Fisheries Research Burg. Van Gansberghelaan 96, 9820 Merelbeke - Melle, Belgium
Short description of the test	Identification of <i>Meloidogyne graminicola</i> by molecular real time PCR He et al., 2021 in juveniles
Date, reference of the validation report	2023-10-31 - Validation report for the molecular identification of <i>Meloidogyne graminicola</i>
Link to other validation data	<ul style="list-style-type: none"> - 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 - 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 - 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 - 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 - 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 - 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
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	EURL
If yes, please specify	EU-funded project EURLs-EURCs 2021-2022 (grant SI2.870859)
Description of the test	

Organism(s)	Meloidogyne graminicola (MELGGC)
Detection / identification	identification
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Kit	
Is a kit used	no
Other information	
Other details on the test	Based on the use of Worm lysis buffer (see details in the report). Final volume 50 microliter evaluated.
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	He et al. 2021
Is the test modified compared to the reference test	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
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Meloidogyne graminicola(MELGGC)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	10 J2 and 10 fold dilutions: 100%
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Population from Brazil (30 ng/uL), Italy and the Philippines (2 J2) amplified
Specificity value	100%
Analytical specificity - exclusivity	

Number of non-target organisms tested	M. minor, M. hapla, M. chitwoodi, M. fallax, M. arenaria, M. enterolobii, M. incognita, M. javanica, M. naasi, M. oryzae (10J2), M. salasi
Specificity value	
Cross reacts with	Meloidogyne oryzae Meloidogyne salasi Meloidogyne naasi
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	10 J2 and 10 fold dilutions (5 replicates, 2 operators): 100%
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	10 J2 and 10 fold dilutions (5 replicates): 100%
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	Report available on the EURL website for the EU NRLs or on request to the EURL.

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