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 Meloidogyne graminicola by molecular conventional PCR Mattos et al., 2019 (M. oryzae primers) in juveniles
Date, reference of the validation report	2023-10-31 - Validation report for the molecular identification of Meloidogyne graminicola
Link to other validation data	- Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular conventional PCR Bellafiore et al. 2015 in juveniles - Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular conventional PCR Htay et al., 2016 in juveniles - Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular real time PCR Htay et al., 2016 in juveniles - Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular conventional PCR He et al., 2021 in juveniles - Validation report for the molecular identification of Meloidogyne graminicola Identification of Meloidogyne graminicola by molecular real time PCR He et al., 2021 in juveniles - 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
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 Conventional 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 or 5 microliter evaluated.
Method: Molecular Conventional 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?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Mattos et al., 2019
Is the test modified compared to the reference test	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.
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Other details on the test	M. oryzae primers 2.5 uL of DNA in final mix
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?	With 2.5 uL DNA in PCR mix 5 J2 (in 50µl WLB): 100% (10 replicates) 1 J2 (in 5µl WLB): 100 % (10 replicates)
Analytical specificity - inclusivity	

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