

**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	Anses Plant Health Laboratory - Nematology Unit Domaine de la Motte au Viconte BP 35327, 35653 Le Rheu, France
Short description of the test	detection and identification of <i>Globodera pallida</i> , <i>Globodera rostochiensis</i> by Molecular real time PCR from isolated nematode cysts or isolated juveniles
Date, reference of the validation report	2021-10-21 - EURL diagnostic protocol EURL-Globo- qPCR_Version 02
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?	Other_project
If yes, please specify	Internal study at Anses - Plant Health Laboratory
Description of the test	
Organism(s)	<i>Globodera pallida</i> (HETDPA) <i>Globodera rostochiensis</i> (HETDRO)
Detection / identification	detection and identification
Method(s)	Molecular real time PCR
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?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Gamel et al., 2017 (modified: duplex, <i>G. rostochiensis</i> probe labelled FAM-BHQ1)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no

Other information	
Reaction type	Duplex
Other details on the test	G. rostochiensis probe labelled FAM-BHQ1 (compared to the publication Gamel et al. 2017)
Performance Criteria :	
Organism 1.:	Globodera pallida(HETDPA)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	For cyst mixture: 10 juveniles of G. pallida within 50 cysts of G. rostochiensis: 100% For juveniles: 2 juveniles of G. pallida
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	14 populations of G. pallida (including 7 South American populations)
Specificity value	100 % for G. pallida
Analytical specificity - exclusivity	
Number of non-target organisms tested	13 populations of G. rostochiensis (5 from South America and 8 from Europe) + 28 others populations: 7 of G. tabacum 1 of G. ellingtonae 1 of G. artemisiae 1 of G. mexicana 7 of H. schachtii 11 of Heterodera, including 5 species belonging to the Schachtii group
Specificity value	100 % for G. pallida
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For cysts mixture: 100% for 10 juveniles of G. pallida + 50 cysts of G. rostochiensis 100% for 1 cyst of G. pallida + 50 cysts of G. rostochiensis For juveniles: 100% with 2 and 5 juveniles of G. pallida
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For cysts mixture: 100% for 10 juveniles of G. pallida + 50 cysts of G. rostochiensis 100% for 1 cyst of G. pallida + 50 cysts of G. rostochiensis For juveniles: 100% for 1 juvenile of G. pallida (same results with 2 and 5 juveniles)
Organism 2.:	Globodera rostochiensis(HETDRO)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	For cyst mixture: 10 juveniles of G. rostochiensis within 50 cysts of G. pallida: 83% (but 100% in simplex reaction) For juveniles: 2 juveniles of G. rostochiensis
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	13 populations of G. rostochiensis (5 from South America and 8 from Europe)
Specificity value	100 % for G. rostochiensis
Analytical specificity - exclusivity	

Number of non-target organisms tested	14 populations of <i>G. pallida</i> (including 7 South American populations) + 28 others populations: 7 of <i>G. tabacum</i> 1 of <i>G. ellingtonae</i> 1 of <i>G. artemisiae</i> 1 of <i>G. mexicana</i> 7 of <i>H. schachtii</i> 11 of Heterodera, including 5 species belonging to the Schachtii group
Specificity value	100 % for <i>G. rostochiensis</i>
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For cysts mixture: 0% for 20 juveniles of <i>G. rostochiensis</i> + 50 cysts of <i>G. pallida</i> (but 100% for 10 juveniles of <i>G. rostochiensis</i> + 50 cysts of <i>G. pallida</i> in simplex reaction) 100% for 1 cyst of <i>G. rostochiensis</i> + 50 cysts of <i>G. pallida</i> For juveniles: 100% with 2 and 5 juveniles of <i>G. rostochiensis</i>
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	For cysts mixture: 46% for 20 juveniles of <i>G. rostochiensis</i> + 50 cysts of <i>G. pallida</i> (but 100% for 10 juveniles of <i>G. rostochiensis</i> + 50 cysts of <i>G. pallida</i> in simplex reaction) 100% for 1 cyst of <i>G. rostochiensis</i> + 50 cysts of <i>G. pallida</i> For juveniles: 100% for 1 juvenile of <i>G. rostochiensis</i> (same results with 2 and 5 juveniles)
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	No cross reaction with Ct < 35. Only <i>G. artemisiae</i> (1 non-european population tested) showed amplification with a Ct value > 35. Robustness: The method's performance has been tested with a lower and higher annealing temperature (59°C and 61°C) instead of 60°C. The results are equivalent to those presented above. Not yet accredited but planned for 2022-2023
The following complementary files are available online:	
	<ul style="list-style-type: none"> • Validation_sheet_Globo_Gamel

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