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 Globodera pallida, Globodera rostochiensis 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)	Globodera pallida (HETDPA) Globodera rostochiensis (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, G. rostochiensis probe labelled FAM-BHQ1)	
Is the test modified compared to the reference test	no	
Kit		
ls 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	
<u>Reproducibility</u>		
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 G. pallida (including 7 South American populations) + 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. rostochiensis	
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 G. rostochiensis + 50 cysts of G. pallida (but 100% for 10 juveniles of G. rostochiensis + 50 cysts of G. pallida in simplex reaction) 100% for 1 cyst of G. rostochiensis + 50 cysts of G. pallida For juveniles: 100% with 2 and 5 juveniles of G. rostochiensis	
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 G. rostochiensis + 50 cysts of G. pallida (but 100% for 10 juveniles of G. rostochiensis + 50 cysts of G. pallida in simplex reaction) 100% for 1 cyst of G. rostochiensis + 50 cysts of G. pallida For juveniles: 100% for 1 juvenile of G. rostochiensis (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 G. artemisiae (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:	Validation_sheet_Globo_Gamel	

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