

**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>	Anses Plant Health Laboratory - Nematology Unit Domaine de la Motte au Viconte BP 35327, 35653 Le Rheu, France
<b>Short description of the test</b>	detection and identification of <i>Globodera pallida</i> , <i>Globodera rostochiensis</i> by Molecular real time PCR from isolated nematode cysts or isolated juveniles
<b>Date, reference of the validation report</b>	2021-10-21 - EURL diagnostic protocol EURL-Globo- qPCR_Version 02
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	Internal study at Anses - Plant Health Laboratory
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Globodera pallida</i> (HETDPA) <i>Globodera rostochiensis</i> (HETDRO)
<b>Detection / identification</b>	detection and identification
<b>Matrix(ces) tested</b>	Other Isolated nematode cysts or isolated juveniles
<b>Plant species tested</b>	
<b>Method(s)</b>	Molecular real time PCR
<b>Method: Molecular real time 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>	Gamel et al., 2017 (modified: duplex, <i>G. rostochiensis</i> probe labelled FAM-BHQ1)
<b>Is the test modified compared to the reference test</b>	no

<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Duplex
<b>Other details on the test</b>	G. rostochiensis probe labelled FAM-BHQ1 (compared to the publication Gamel et al. 2017)
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Globodera pallida(HETDPA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	For cyst mixture: 10 juveniles of G. pallida within 50 cysts of G. rostochiensis: 100% For juveniles: 2 juveniles of G. pallida
<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>	
<b>Standard test(s)</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	14 populations of G. pallida (including 7 South American populations)
<b>Specificity value</b>	100 % for G. pallida
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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
<b>Specificity value</b>	100 % for G. pallida
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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
<b>Repeatability</b>	

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	For cysts mixture: 100% for 10 juveniles of <i>G. pallida</i> + 50 cysts of <i>G. rostochiensis</i> 100% for 1 cyst of <i>G. pallida</i> + 50 cysts of <i>G. rostochiensis</i> For juveniles: 100% for 1 juvenile of <i>G. pallida</i> (same results with 2 and 5 juveniles)
<b>Organism 2.:</b>	<b><i>Globodera rostochiensis</i>(HETDRO)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	For cyst mixture: 10 juveniles of <i>G. rostochiensis</i> within 50 cysts of <i>G. pallida</i> : 83% (but 100% in simplex reaction) For juveniles: 2 juveniles of <i>G. rostochiensis</i>
<b><u>Diagnostic sensitivity</u></b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	
<b>Standard test(s)</b>	
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	13 populations of <i>G. rostochiensis</i> (5 from South America and 8 from Europe)
<b>Specificity value</b>	100 % for <i>G. rostochiensis</i>
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	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
<b>Specificity value</b>	100 % for <i>G. rostochiensis</i>
<b>Cross reacts with</b>	
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	
<b>Specify the test(s)</b>	
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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>
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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)
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful</b>	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"> <li>• <a href="#">Validation_sheet_Globo_Game1</a></li> </ul>

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