

**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>	Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands
<b>Short description of the test</b>	Qualitative detection of viability and identification of <i>Globodera pallida</i> en <i>G. rostochiensis</i>
<b>Date, reference of the validation report</b>	2014-07-01 - Qualitative detection of <i>Globodera</i> spp. viability version 2
<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>	no
<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>Method(s)</b>	Molecular real time RT PCR
<b>Method: Molecular real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/040 <i>Globodera rostochiensis</i> and <i>Globodera pallida</i> (version 4)
<b>Name of the test</b>	RNA-specific real-time PCR (Beniers et al., 2014)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes The test is based on the article of Beniers et al. 2014 but some changes have been made (another internal control is used and the extraction method is automated).
<b>Other information</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b><i>Globodera pallida</i>(HETDPA)</b>
<b>Analytical sensitivity</b>	

<b>What is smallest amount of target that can be detected reliably?</b>	1 viable juveniles or egg
<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>	G. pallida: 100%
<b>Standard test(s)</b>	Bulman & Marshall (1997), Van de Vossenberg (2014)
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	8 strains of G. pallida (Pa2 WUR 248, Pa2 Averis 2013, Pa3 Ecnavornay, Pa3 Rookmaker 2009, Pa3 Rookmaker 2013, Pa3 Averis 2013)
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	6 non-target organisms were tested (Heterodera betae, Heterodera glycines, Heterodera schachtii, Heterodera trifolii, Globodera tabacum, Cactodera cacti)
<b>Specificity value</b>	100% No cross reactions with other organisms
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	G. pallida: 100%
<b>Specify the test(s)</b>	Visual determination
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Reproducibility: 100%
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Repeatability: 100%
<b>Organism 2.:</b>	<b>Globodera rostochiensis(HETDRO)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	1 viable juveniles or egg
<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>	G. rostochiensis: 100 %
<b>Standard test(s)</b>	Bulman & Marshall (1997), Van de Vossenberg (2014)
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	6 strains of G. rostochiensis (Ro1 SCRI, Ro1 Mierenbos, Ro2,3 C262, Ro2,3 PRI 2013, Ro4 F515, Ro5 G1518)

<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	6 non-target organisms were tested (Heterodera betae, Heterodera glycines, Heterodera schachtii, Heterodera trifolii, Globodera tabacum, Cactodera cacti)
<b>Specificity value</b>	100% - No cross reactions with other organisms
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	G. rostochiensis: 100%
<b>Specify the test(s)</b>	Visual determination
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Reproducibility: 100%, using 20 samples including a sample with low level (12) of viable eggs
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Repeatability: 100%, using 20 samples including a sample with low level (12) of viable eggs
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	<p>Various KingFisher apparatus and ABI 7500 apparatus did not influence the outcome of the tests. The above described method will be applied in a qualitative way at the NAK. The new test has been applied to 184 field samples that were analysed by the old and the new method. No false-negative results were found. The new test found 7 positive results that were not detected with the old method. Overview of comparison between a previous used non-published DNA PCR and the RT-PCR of Beniers et al.(2014) for a set of 184 routine samples. Results are for G. pallida and G. rostochiensis,. Green markings are added to indicate the positive and negative agreement. G. pallida G. rostochiensis Beniers RT-PCR Beniers RT-PCR + - <math>\Sigma</math> + - <math>\Sigma</math> Old DNA PCR + 134 2 136 + 40 2 42 - 7 41 48 - 0 142 142 <math>\Sigma</math> 141 43 184 <math>\Sigma</math> 40 144 184</p>
The following complementary files are available online:	
<ul style="list-style-type: none"> <li>• <a href="#">Globodera NAK presentation april EPPO site</a></li> </ul>	

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