

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

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

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Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Reproducibility: 100%, using 20 samples including a sample with low level (12) of viable eggs																																				
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Test performance study																																					
Test performance study?	no																																				
Other information																																					
Any other information considered useful	<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.</p> <table border="1"> <tr> <td>G. pallida</td> <td>G. rostochiensis</td> <td>Beniers RT-PCR</td> <td>Beniers RT-PCR</td> </tr> <tr> <td>+</td> <td>-</td> <td>+</td> <td>-</td> </tr> <tr> <td>Σ</td> <td>+</td> <td>Σ</td> <td>Old DNA PCR</td> </tr> <tr> <td>+</td> <td>134</td> <td>2</td> <td>136</td> </tr> <tr> <td>+</td> <td>40</td> <td>2</td> <td>42</td> </tr> <tr> <td>-</td> <td>7</td> <td>41</td> <td>48</td> </tr> <tr> <td>-</td> <td>0</td> <td>142</td> <td>142</td> </tr> <tr> <td>Σ</td> <td>141</td> <td>43</td> <td>184</td> </tr> <tr> <td>Σ</td> <td>40</td> <td>144</td> <td>184</td> </tr> </table>	G. pallida	G. rostochiensis	Beniers RT-PCR	Beniers RT-PCR	+	-	+	-	Σ	+	Σ	Old DNA PCR	+	134	2	136	+	40	2	42	-	7	41	48	-	0	142	142	Σ	141	43	184	Σ	40	144	184
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The following complementary files are available online:																																					
<ul style="list-style-type: none"> • Globodera NAK presentation april EPPO site 																																					

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