

**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>	ClearDetections P.O. Box 170, NL-6700 PD Wageningen, Netherlands
<b>Short description of the test</b>	Diagnostic Real-time PCR assays for identification and detection of Globodera rostochiensis, G. pallida and G. tabacum
<b>Date, reference of the validation report</b>	2013-08-01 - ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of Globodera rostochiensis & G. pallida & G. tabacum
<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>	Globodera pallida(HETDPA) Globodera rostochiensis(HETDRO)
<b>Detection / identification</b>	detection and identification
<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>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/040 Globodera rostochiensis and Globodera pallida (version 2)
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	
<b>Specify the kit used</b>	
<b>Kit used following the manufacturer's instructions?</b>	yes

<b>Other information</b>	
<b>Other details on the test</b>	Real-time PCR; based on detection of a fluorescent DNA-binding dye
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>yes</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Globodera pallida(HETDPA)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	The analytical sensitivity is one single PCN juvenile or egg, against a background of 1000 juveniles or eggs of non-target cyst nematodes.
<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>	100%
<b>Standard test(s)</b>	Morphological identification
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	3 Globodera pallida populations, 4 G. rostochiensis populations and 2 G. tabacum populations
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	Globodera achilleae, Globodera artemisiae, Gobodera mexicana, Heterodera goettingiana, Heterodera schachtii, Heterodera betae, Punctodera stonei
<b>Specificity value</b>	Several target and non-target species (from different origins) were tested and no cross reactions were noted for the G. tabacum qPCR test. The G. pallida qPCR tests is specific for the G. pallida populations tested, including one from South America. In addition, it picks up its close relative G. mexicana. The qPCR test for G. rostochiensis is specific for G. rostochiensis populations, including South American populations, and G. tabacum. These results demonstrate that in all cases where G. rostochiensis and G. tabacum cysts may be jointly found in samples and positive qPCR signals are found for G. rostochiensis, the qPCR test for G. tabacum must be used to verify possible false positive results.
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100%
<b>Specify the test(s)</b>	Morphological identification
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a</b>	100%

given level of the pest (see PM 7/98)	
<b><u>Repeatability</u></b>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Organism 2.:	<b>Globodera rostochiensis(HETDRO)</b>
<b><u>Analytical sensitivity</u></b>	
What is smallest amount of target that can be detected reliably?	The analytical sensitivity is one single PCN juvenile or egg, against a background of 1000 juveniles or eggs of non-target cyst nematodes.
<b><u>Diagnostic sensitivity</u></b>	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Standard test(s)	Morphological identification
<b><u>Analytical specificity - inclusivity</u></b>	
Number of strains/populations of target organisms tested	3 Globodera pallida populations, 4 G. rostochiensis populations and 2 G. tabacum populations
Specificity value	100%
<b><u>Analytical specificity - exclusivity</u></b>	
Number of non-target organisms tested	Globodera achilleae, Globodera artemisiae, Gobodera mexicana, Heterodera goettingiana, Heterodera schachtii, Heterodera betae, Punctodera stonei
Specificity value	Several target and non-target species (from different origins) were tested and no cross reactions were noted for the G. tabacum qPCR test. The G. pallida qPCR tests is specific for the G. pallida populations tested, including one from South America. In addition, it picks up its close relative G. mexicana. The qPCR test for G. rostochiensis is specific for G. rostochiensis populations, including South American populations, and G. tabacum. These results demonstrate that in all cases where G. rostochiensis and G. tabacum cysts may be jointly found in samples and positive qPCR signals are found for G. rostochiensis, the qPCR test for G. tabacum must be used to verify possible false positive results.
<b><u>Diagnostic Specificity</u></b>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	Morphological identification
<b><u>Reproducibility</u></b>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
<b><u>Repeatability</u></b>	

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	Robustness: No qPCR failure is observed when the primer combinations are exposed to a temperature gradient. With a deviation in Ta of (plus or minus) 1.0 oC from the normal Ta (63 oC), all ?Ct values remain < 1. The qPCR tests for the detection of G. pallida, G. rostochiensis and G. tabacum are therefore robust. The three qPCR assays for identification and detection of G. rostochiensis, G. pallida and G. tabacum are available as all-inclusive molecular kit, including primer sets, positive control DNA, PCR enhancer and PCR mix and a bench-side protocol describing the laboratory procedure (for information visit <a href="http://www.cleardetections.com">www.cleardetections.com</a> ).
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Validation report</a></li> </ul>

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