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.

| Target Organism          | Globodera rostochiensis  
|                         | Globodera pallida  
|                         | Globodera tabacum  
| Short description       | Diagnostic Real-time PCR assays for identification and detection of Globodera rostochiensis, G. pallida and G. tabacum  
| Laboratory contact details | ClearDetections  
|                         | P.O. Box 170, NL-6700 PD Wageningen, The Netherlands  
|                         | www.cleardetections.com  
| Date and reference of the validation report | 2013-08 - ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of Globodera rostochiensis & G. pallida & G. tabacum  
| Validation process according to EPPO Standard PM 7/98: | Yes  
| Reference of the test description | PM 7/040(2) Appendix 3 D  
| Is the test the same as described in the EPPO DP? | Yes  
| Is the lab accredited for this test? | No  
| Plant species tested (if relevant) | not relevant  
| Matrices tested (if relevant) | individual cyst or larvae cyst mixtures  

**List of methods used**

| Method for extraction / isolation / baiting of target organism from matrix |  
| Molecular methods, e.g. hybridization, PCR and real time PCR | X Real-time PCR; based on detection of a fluorescent DNA-binding dye  
| Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay |  
| Plating methods: selective isolation |  
| Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting. |  
| Pathogenicity test |  
| Fingerprint methods: protein |  

<table>
<thead>
<tr>
<th>Analytical sensitivity (= limit of detection)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What is smallest amount of target that can be detected reliably?</strong></td>
<td>The analytical sensitivity is one single PCN juvenile or egg, against a background of 1000 juveniles or eggs of non-target cyst nematodes.</td>
</tr>
<tr>
<td><strong>Diagnostic sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</td>
<td>100%</td>
</tr>
<tr>
<td>Specify the standard test</td>
<td>Morphological identification</td>
</tr>
<tr>
<td><strong>Analytical specificity</strong></td>
<td></td>
</tr>
<tr>
<td>Specificity value</td>
<td>100%</td>
</tr>
<tr>
<td>Number of strains/populations of target organisms tested</td>
<td>3 Globodera pallida populations, 4 G. rostochiensis populations and 2 G. tabacum populations</td>
</tr>
<tr>
<td>Number of non-target organisms tested</td>
<td>Globodera achilleae, Globodera artemisiae, Gobodera mexicana, Heterodera goettingiana, Heterodera schachtii, Heterodera betae, Punctodera stonei</td>
</tr>
<tr>
<td>Cross reacts with (specify the species)</td>
<td>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.</td>
</tr>
<tr>
<td><strong>Diagnostic Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</td>
<td>100%</td>
</tr>
<tr>
<td>Specify the standard test</td>
<td>Morphological identification</td>
</tr>
<tr>
<td><strong>Reproducibility</strong></td>
<td></td>
</tr>
<tr>
<td>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</td>
<td>100%</td>
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<tr>
<td><strong>Repeatability</strong></td>
<td></td>
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<th>100%</th>
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<tbody>
<tr>
<td><strong>Test performance study</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Test performance study?</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Include brief details of the test performance study and its output. If available, provide a link to published article/report</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Other information</strong></td>
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</tr>
<tr>
<td><strong>Any other information considered useful e.g. robustness, ease of performing the test, etc.</strong></td>
<td>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 ΔCt values remain &lt; 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>).</td>
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<tr>
<td><strong>The following complementary files are available online:</strong></td>
<td>• Validation report</td>
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