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 | Meloidogyne chitwoodi  
| Meloidogyne fallax |
| Short description | Diagnostic Real-time PCR assays for identification and detection of Meloidogyne chitwoodi and M. fallax |
| 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 Meloidogyne chitwoodi and M. fallax |
| Validation process according to EPPO Standard PM 7/98 | Yes |
| Reference of the test description | PM 7/041(2) Appendix 7 |
| 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 specimens  
| Nematodes suspensions isolated from 100 ml soil samples |

**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  
<p>| 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 |<br />
| Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA |<br />
| Real-time PCR: based on detection of a fluorescent DNA-binding dye. |</p>
<table>
<thead>
<tr>
<th>profiling</th>
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<tbody>
<tr>
<td>Morphological and morphometrical methods intended for identification</td>
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<tr>
<td>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</td>
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<tr>
<td>Other</td>
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**Analytical sensitivity (= limit of detection)**

| What is smallest amount of target that can be detected reliably? | One individual target nematode (M. chitwoodi or M. fallax) against a DNA background of thousands of non-target nematodes |

**Diagnostic sensitivity**

| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | 100% |
| Specify the standard test | Morphological identification |

**Analytical specificity**

| Specificity value | 100% |
| Number of strains/populations of target organisms tested | 1 for each |
| Number of non-target organisms tested | Meloidogyne minor, Meloidogyne hapla, Meloidogyne naasi, Meloidogyne arenaria, Meloidogyne ichinohei, Pratylenchus penetrans |
| Cross reacts with (specify the species) | No cross reaction observed |

**Diagnostic Specificity**

| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | 100% |
| Specify the standard test | Morphological identification |

**Reproducibility**

| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 100% |

**Repeatability**

| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | 100% |

**Test performance study**

<p>| Test performance study? | No |
| Include brief details of the test performance study and its output. If available, provide a link to published article/report |</p>
<table>
<thead>
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<th><strong>Other information</strong></th>
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<td>Any other information considered useful e.g. robustness, ease of performing the test, etc.</td>
<td>No test 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 real-time PCR tests for the detection of M. chitwoodi and M. fallax are robust. The two qPCR assays for identification and detection of M. chitwoodi and M. fallax 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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| The following complementary files are available online: | • **Validation report** |