

**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 <i>Meloidogyne chitwoodi</i> and <i>M. fallax</i>
<b>Date, reference of the validation report</b>	2013-08-01 - ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of <i>Meloidogyne chitwoodi</i> and <i>M. fallax</i>
<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>Meloidogyne fallax</i> (MELGFA) <i>Meloidogyne chitwoodi</i> (MELGCH)
<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 7/041 <i>Meloidogyne chitwoodi</i> and <i>M. fallax</i> (version 2)
<b>Name of the test</b>	Real-time SYBR-green PCR (LSU rDNA based Test (ClearDetections))
<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>	

Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
Reaction type	Simplex
Other details on the test	Real-time PCR: based on detection of a fluorescent DNA-binding dye.
Are the performance characteristics included in the EPPO diagnostic protocol?	no
<b>Performance Criteria :</b>	
Organism 1.:	<b>Meloidogyne fallax(MELGFA)</b>
<b>Analytical sensitivity</b>	
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
<b>Diagnostic sensitivity</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>Analytical specificity - inclusivity</b>	
Number of strains/populations of target organisms tested	1 for each
Specificity value	100%
<b>Analytical specificity - exclusivity</b>	
Number of non-target organisms tested	Meloidogyne minor, Meloidogyne hapla, Meloidogyne naasi, Meloidogyne arenaria, Meloidogyne ichinohei, Pratylenchus penetrans
Specificity value	100% No cross reaction observed
<b>Diagnostic Specificity</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>Reproducibility</b>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
<b>Repeatability</b>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Organism 2.:	<b>Meloidogyne chitwoodi(MELGCH)</b>
<b>Analytical sensitivity</b>	
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
<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>	1 for each
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	Meloidogyne minor, Meloidogyne hapla, Meloidogyne naasi, Meloidogyne arenaria, Meloidogyne ichinohei, Pratylenchus penetrans
<b>Specificity value</b>	100% No cross reaction observed
<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 given level of the pest (see PM 7/98)</b>	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><u>Test performance study</u></b>	
<b>Test performance study?</b>	no
<b><u>Other information</u></b>	
<b>Any other information considered useful</b>	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 ?Ct values remain < 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.clear-detections.com">www.clear-detections.com</a> ).
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Validation report</a></li> </ul>

