EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

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	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 profiling, fatty acid profiling & DNA		

profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
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		
Test performance study?	No	
Include brief details of the test performance study and its output.It available, provide a link to published article/report		

Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	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 < 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 www.cleardetections.com).
The following complementary files are available online:	• Validation report