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.

Laboratory contact details	ClearDetections P.O. Box 170, NL-6700 PD Wageningen, Netherlands
Short description of the test	Diagnostic Real-time PCR assays for identification and detection of Meloidogyne chitwoodi and M. fallax
Date, reference of the validation report	2013-08-01 - ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of Meloidogyne chitwoodi and M. fallax
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Meloidogyne fallax (MELGFA) Meloidogyne chitwoodi (MELGCH)
Detection / identification	detection and identification
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/041 Meloidogyne chitwoodi and M. fallax (version 2)
Name of the test	Real-time SYBR-green PCR (LSU rDNA based Test (ClearDectections))
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	
Specify the kit used	

Kit used following the manufacturer's instructions?	yes
Other information	
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**
Performance Criteria :	
Organism 1.:	**Meloidogyne fallax(MELGFA)**
Analytical sensitivity	
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%
Standard test(s)	Morphological identification
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	1 for each
Specificity value	100%
Analytical specificity - exclusivity	
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
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	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%
Organism 2.:	**Meloidogyne chitwoodi(MELGCH)**
Analytical sensitivity	
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%
Standard test(s)	Morphological identification
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	1 for each
Specificity value	100%
Analytical specificity - exclusivity	
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
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	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
Other information	
Any other information considered useful	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 www.cleardetections.com).
The following complementary files are available online:	• Validation report

Creation date: 2014-11-12 00:00:00 - Last update: 2021-03-12 08:51:02