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 assay for identification and detection of Aphelenchoides besseyi
Date, reference of the validation report	2011-01-01 - 86 ; 'Validatie van moleculaire identificatie- en detectiemethoden van Aphelenchoides fragariae, A. ritzemabosi, A. subtenuis en A. besseyi'. Validation report (in Dutch) of FES study.
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)	Aphelenchoides besseyi (APLOBE)
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 7/039 Aphelenchoides besseyi (version 2)
Name of the test	Real-time PCR test based on SSU rDNA (Clear detection)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	CLEAR DETECTIONS
Specify the kit used	RT-N-D-0302 ClearDetections Real-Time PCR Diagnostic kit: Aphelenchoides besseyi

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?	**yes**
Performance Criteria :	
Organism 1.:	**Aphelenchoides besseyi(APLOBE)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	< one individual nematode (~ 3 cells of target nematode)
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	A. besseyi (2160 and E9192) obtained from Dutch PPO (ref. Gerrit Karssen)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Aphelenchoides subtenuis; A. fragariae; A. ritzemabosi; A. saprophilus; Ditylenchus dipsaci; D. destructor
Specificity value	100% No cross reaction
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	Accuracy: 100% Dynamic range: between 10-100 and 0.1 billion copies of target rDNA Selectivity:

	100% Robustness: OK This qPCR assay for identification and detection of A. besseyi is 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

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