

**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 <i>Aphelenchoides besseyi</i>
Date, reference of the validation report	2011-01-01 - 86 ; 'Validatie van moleculaire identificatie- en detectiemethoden van <i>Aphelenchoides fragariae</i> , <i>A. ritzemabosi</i> , <i>A. subtenuis</i> en <i>A. besseyi</i> '. 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
If yes, please specify	
Description of the test	
Organism(s)	<i>Aphelenchoides besseyi</i> (APLOBE)
Detection / identification	detection and identification
Matrix(ces) tested	Specimen Nematode suspensions obtained from plant extracts
Plant species tested	
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
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/039 <i>Aphelenchoides besseyi</i> (version 2)
Name of the test	Real-time PCR test based on SSU rDNA (Clear detection)
As or adapted from an IPPC diagnostic protocol	

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
Cross reacts with	
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
Brief details of the test performance study and its output. It available, link to published article/report	
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 <i>A. besseyi</i> 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.clear-detections.com).
The following complementary files are available online:	<ul style="list-style-type: none"> • Validation report

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