

**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	Aphelenchoides besseyi	
Short description	Diagnostic Real-time PCR assay for identification and detection of Aphelenchoides besseyi	
Laboratory contact details	ClearDetections P.O. Box 170, NL-6700 PD Wageningen, The Netherlands www.cleardetections.com	
Date and reference of the validation report	2011 - '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 PM 7/98:	Yes	
Reference of the test description	N/R Test considered for inclusion in a revision of PM 7/039(1)	
Is the test the same as described in the EPPO DP?		
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Not relevant	
Matrices tested (if relevant)	Nematode suspensions obtained from plant extracts	
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		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	< one individual nematode (~ 3 cells of target nematode)	
<u>Diagnostic sensitivity</u>		
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	
<u>Analytical specificity</u>		
Specificity value	100%	
Number of strains/populations of target organisms tested	A. besseyi (2160 and E9192) obtained from Dutch PPO (ref. Gerrit Karssen)	
Number of non-target organisms tested	Aphelenchoides subtenuis; A. fragariae; A. ritzemabosi; A. saprophilus; Ditylenchus dipsaci; D. destructor	
Cross reacts with (specify the species)	No cross reaction	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%	
Specify the standard test	Morphological identification	
<u>Reproducibility</u>		

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
<u>Test performance study</u>	
Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
<u>Other information</u>	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	<p>Accuracy: 100% Dynamic range: between 10-100 and 0.1 billion copies of target rDNA Selectivity: 100% Robustness: OK</p> <p>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).</p>
The following complementary files are available online:	<ul style="list-style-type: none"> • Validation report