

**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.

<b>Target Organism</b>	Aphelenchoides besseyi	
<b>Short description</b>	Diagnostic Real-time PCR assay for identification and detection of Aphelenchoides besseyi	
<b>Laboratory contact details</b>	ClearDetections P.O. Box 170, NL-6700 PD Wageningen, The Netherlands www.cleardetections.com	
<b>Date and reference of the validation report</b>	2011 - 'Validatie van moleculaire identificatie- en detectiemethoden van Aphelenchoides fragariae, A. ritzemabosi, A. subtenuis en A. besseyi'. Validation report (in Dutch) of FES study.	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	N/R Test considered for inclusion in a revision of PM 7/039(1)	
<b>Is the test the same as described in the EPPO DP?</b>		
<b>Is the lab accredited for this test?</b>	No	
<b>Plant species tested (if relevant)</b>	Not relevant	
<b>Matrices tested (if relevant)</b>	Nematode suspensions obtained from plant extracts	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>		
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	X	Real-time PCR; based on detection of a fluorescent DNA-binding dye
<b>Serological methods: IF, ELISA,</b>		

<b>Direct Tissue Blot Immuno Assay</b>		
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b>		
<b>Pathogenicity test</b>		
<b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA profiling</b>		
<b>Morphological and morphometrical methods intended for identification</b>		
<b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>		
<b>Other</b>		
<b><u>Analytical sensitivity (= limit of detection)</u></b>		
<b>What is smallest amount of target that can be detected reliably?</b>	< one individual nematode (~ 3 cells of target nematode)	
<b><u>Diagnostic sensitivity</u></b>		
<b>Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98</b>	100%	
<b>Specify the standard test</b>	Morphological identification	
<b><u>Analytical specificity</u></b>		
<b>Specificity value</b>	100%	
<b>Number of strains/populations of target organisms tested</b>	A. besseyi (2160 and E9192) obtained from Dutch PPO (ref. Gerrit Karssen)	
<b>Number of non-target organisms tested</b>	Aphelenchoides subtenuis; A. fragariae; A. ritzemabosi; A. saprophilus; Ditylenchus dipsaci; D. destructor	
<b>Cross reacts with (specify the species)</b>	No cross reaction	
<b><u>Diagnostic Specificity</u></b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100%	
<b>Specify the standard test</b>	Morphological identification	
<b><u>Reproducibility</u></b>		

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	No
<b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b>	
<b><u>Other information</u></b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	<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 <a href="http://www.clear-detections.com">www.clear-detections.com</a>).</p>
<b>The following complementary files are available online:</b>	<ul style="list-style-type: none"> <li>• <a href="#">Validation report</a></li> </ul>