

**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.

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| <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<br>P.O. Box 170, NL-6700 PD Wageningen,<br>The Netherlands<br>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<br>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><i>List of methods used</i></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>  |  |  |

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| <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>   |  |  |
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| <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<br/>e.g. robustness, ease of performing the test, etc.</b>                               | <p>Accuracy: 100%<br/> Dynamic range: between 10-100 and 0.1 billion copies of target rDNA<br/> Selectivity: 100%<br/> 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>   |