

**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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|---|--|--|
| 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, | | |

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| 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> | | |
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|---|---|
| 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 |