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	Aphelench	hoides 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, Netherlands	
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 cons	idered 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	e 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	х	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

profiling, fatty acid profiling & DNA

Morphological and morphometrical methods intended for identification				
Biochemical methods: e.g. enzyme electrophoresis, protein profiling				
Other				
Analytical sensitivity (= limit of detection)				
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%			
Specify the standard test	Morpholog	gical identification		
Analytical specificity				
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			
Diagnostic Specificity				
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%			
Specify the standard test	Morpholog	gical 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			
Include brief details of the test performance study and its output.It available, provide a link to published article/report				
Other information				
Any other information considered	Accuracy:	100%		

useful e.g. robustness, ease of performing the test, etc.	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 A. besseyi 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.cleardetections.com).	
The following complementary files are available online:	Validation report	