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	Ditylenchus dipsaci Ditylenchus destructor	
Short description	Diagnostic Real-time PCR assays for identification and detection of Ditylenchus dipsaci and D. destructor	
Laboratory contact details	ClearDetections P.O. Box 170, NL-6700 PD Wageningen, Netherlands	
Date and reference of the validation report	2013-08 - ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of Ditylenchus dipsaci and D. destructor	
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 7/087	
Is the test the same as described in the EPPO DP?	No	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)		
Matrices tested (if relevant)	individual specimens nematode suspensions isolated from 100 ml soil samples	
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		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	One individual target nematode (D. dipsaci or D. destructor) against a DNA background of thousands of non-target nematodes.	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100 % (both for D. dipsaci and D. destructor)	
Specify the standard test	Morphological identification	
Analytical specificity		
Specificity value	100% Several target and non target species were tested an no cross reactions were noted for D. dipsaci or D. destructor. Details are provided in Table 6 of the validation report.	
Number of strains/populations of target organisms tested	5 targets D. dipsaci or D. destructor (different origin) see Table 6 of the validation report.	
Number of non-target organisms tested	17 non target species see Table 6 of the validation report.	
Cross reacts with (specify the species)	No cross reaction observed	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100 %	
Specify the standard test	Morphological identification	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for both primer combinations (detecting D. dipsaci and D. destructor).	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for both primer combinations (detecting D. dipsaci and D. destructor)	
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 useful **e.g. robustness, ease of performing the test, etc.**	Robustness: No test failure was observed when the primer combinations were exposed to a temperature gradient. With a deviation in Ta of (plus or minus) 1.0 oC from the normal Ta (63 oC), all ΔCt values remain < 1. The tests for the detection of D. dipsaci and D. destructor are therefore robust. The two qPCR assays for identification and detection of D. dipsaci and D. destructor are 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