EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
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.

Laboratory contact details	ClearDetections P.O. Box 170, NL-6700 PD Wageningen, Netherlands
Short description of the test	Diagnostic Real-time PCR assays for identification and detection of Ditylenchus dipsaci and D. destructor
Date, reference of the validation report	2013-08-01 - ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of Ditylenchus dipsaci and D. destructor
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Ditylenchus dipsaci (DITYDI) Ditylenchus destructor (DITYDE)
Detection / identification	detection and identification
Method(s)	Molecular real time PCR Molecular real time PCR (2)
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/087 Ditylenchus destructor and Ditylenchus dipsaci (version 2)
Name of the test	Real-time PCR tests based on SSU rDNA (provided by ClearDetections)
Kit	
Is a kit used	yes
Manufacturer name	CLEAR DETECTIONS
Specify the kit used	RT-N-D-0704 ClearDetections Real-Time PCR Diagnostic kit: Ditylenchus dipsaci
Kit used following the manufacturer's instructions?	

Other information	
Other details on the test	Real-time PCR: based on detection of a fluorescent DNA-binding dye
Method: Molecular real time PCR (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/087 Ditylenchus destructor and Ditylenchus dipsaci (version 2)
Name of the test	Real-time PCR tests based on SSU rDNA (provided by ClearDetections)
Kit	
Is a kit used	yes
Manufacturer name	CLEAR DETECTIONS
Specify the kit used	RT-N-D-0701 ClearDetections Real-Time PCR Diagnostic kit: Ditylenchus destructor
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	Real-time PCR: based on detection of a fluorescent DNA-binding dye
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Ditylenchus dipsaci(DITYDI)**
Analytical sensitivity	
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)
Standard test(s)	Morphological identification
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	5 targets D. dipsaci or D. destructor (different origin) see Table 6 of the validation report.
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.
Analytical specificity - exclusivity	
Number of non-target organisms tested	17 non target species see Table 6 of the validation report.

Specificity value	No cross reaction observed
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
Specify the test(s)	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)
Organism 2.:	**Ditylenchus destructor(DITYDE)**
Analytical sensitivity	
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)
Standard test(s)	Morphological identification
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	5 targets D. dipsaci or D. destructor (different origin) see Table 6 of the validation report.
Specificity value	100% Details are provided in Table 6 of the validation report.
Analytical specificity - exclusivity	
Number of non-target organisms tested	17 non target species see Table 6 of the validation report.
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.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	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
Other information	
Any other information considered useful	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 ?Ct 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:	• ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of Ditylenchus dipsaci and D. destructor

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