

**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.

<b>Target Organism</b>	Ditylenchus dipsaci Ditylenchus destructor	
<b>Short description</b>	Diagnostic Real-time PCR assays for identification and detection of Ditylenchus dipsaci and D. destructor	
<b>Laboratory contact details</b>	ClearDetections P.O. Box 170, NL-6700 PD Wageningen, The Netherlands www.cleardetections.com	
<b>Date and reference of the validation report</b>	2013-08 - ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of Ditylenchus dipsaci and D. destructor	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	N/R Test considered for inclusion in a revision of PM 7/087	
<b>Is the test the same as described in the EPPO DP?</b>	No	
<b>Is the lab accredited for this test?</b>	No	
<b>Plant species tested (if relevant)</b>		
<b>Matrices tested (if relevant)</b>	individual specimens nematode suspensions isolated from 100 ml soil samples	
<b>List of methods used</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, 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</b>		

<b>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 target nematode (D. dipsaci or D. destructor) against a DNA background of thousands of non-target nematodes.	
<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 % (both for D. dipsaci and D. destructor)	
<b>Specify the standard test</b>	Morphological identification	
<b><u>Analytical specificity</u></b>		
<b>Specificity value</b>	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.	
<b>Number of strains/populations of target organisms tested</b>	5 targets D. dipsaci or D. destructor (different origin) see Table 6 of the validation report.	
<b>Number of non-target organisms tested</b>	17 non target species see Table 6 of the validation report.	
<b>Cross reacts with (specify the species)</b>	No cross reaction observed	
<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>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% for both primer combinations (detecting D. dipsaci and D. destructor).	
<b><u>Repeatability</u></b>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% for both primer combinations (detecting D. dipsaci and D. destructor)	
<b><u>Test performance study</u></b>		
<b>Test performance study?</b>	No	

<p><b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b></p>	
<p><b>Other information</b></p>	
<p><b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b></p>	<p>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 <math>\Delta C_t</math> values remain &lt; 1. The tests for the detection of D. dipsaci and D. destructor are therefore robust.</p> <p>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 <a href="http://www.clear-detections.com">www.clear-detections.com</a>).</p>
<p>The following complementary files are available online:</p>	<ul style="list-style-type: none"> <li>• <a href="#">Validation report</a></li> </ul>