**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.

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| **Laboratory contact details** | ClearDetections<br>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?** | |
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| **Description of the test** | |
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| **Organism(s)** | Ditylenchus dipsaci (DITYDI)<br>Ditylenchus destructor (DITYDE) |
| **Detection / identification** | detection and identification |
| **Method(s)** | Molecular real time PCR<br>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? | |

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| ***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. |

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| **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** | |

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| **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). |
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| 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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