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 Globodera rostochiensis, G. pallida and G. tabacum
Date, reference of the validation report	2013-08-01 - ClearDetections Validation Report: Diagnostic qPCR assays for identification and detection of Globodera rostochiensis & G. pallida & G. tabacum
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?	no
Description of the test	
Organism(s)	Globodera pallida (HETDPA) Globodera rostochiensis (HETDRO)
Detection / identification	detection and identification
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/040 Globodera rostochiensis and Globodera pallida (version 2)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	
Specify the kit used	
Kit used following the manufacturer's instructions?	yes

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?	**yes**
Performance Criteria :	
Organism 1.:	**Globodera pallida(HETDPA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	The analytical sensitivity is one single PCN juvenile or egg, against a background of 1000 juveniles or eggs of non-target cyst 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%
Standard test(s)	Morphological identification
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	3 Globodera pallida populations, 4 G. rostochiensis populations and 2 G. tabacum populations
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Globodera achilleae, Globodera artemisiae, Gobodera mexicana, Heterodera goettingiana, Heterodera schachtii, Heterodera betae, Punctodera stonei
Specificity value	Several target and non-target species (from different origins) were tested and no cross reactions were noted for the G. tabacum qPCR test. The G. pallida qPCR tests is specific for the G. pallida populations tested, including one from South America. In addition, it picks up its close relative G. mexicana. The qPCR test for G. rostochiensis is specific for G. rostochiensis populations, including South American populations, and G. tabacum. These results demonstrate that in all cases where G. rostochiensis and G. tabacum cysts may be jointly found in samples and positive qPCR signals are found for G. rostochiensis, the qPCR test for G. tabacum must be used to verify possible false positive results.
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	100%

given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Organism 2.:	**Globodera rostochiensis(HETDRO)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	The analytical sensitivity is one single PCN juvenile or egg, against a background of 1000 juveniles or eggs of non-target cyst 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%
Standard test(s)	Morphological identification
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	3 Globodera pallida populations, 4 G. rostochiensis populations and 2 G. tabacum populations
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Globodera achilleae, Globodera artemisiae, Gobodera mexicana, Heterodera goettingiana, Heterodera schachtii, Heterodera betae, Punctodera stonei
Specificity value	Several target and non-target species (from different origins) were tested and no cross reactions were noted for the G. tabacum qPCR test. The G. pallida qPCR tests is specific for the G. pallida populations tested, including one from South America. In addition, it picks up its close relative G. mexicana. The qPCR test for G. rostochiensis is specific for G. rostochiensis populations, including South American populations, and G. tabacum. These results demonstrate that in all cases where G. rostochiensis and G. tabacum cysts may be jointly found in samples and positive qPCR signals are found for G. rostochiensis, the qPCR test for G. tabacum must be used to verify possible false positive results.
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%
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
Other information	
Any other information considered useful	Robustness: No qPCR failure is observed when the primer combinations are 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 qPCR tests for the detection of G. pallida, G. rostochiensis and G. tabacum are therefore robust. The three qPCR assays for identification and detection of G. rostochiensis, G. pallida and G. tabacum 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

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