## 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		
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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	

Creation date: 2014-11-12 00:00:00 - Last update: 2021-03-12 08:56:45