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	Finnish Food Authority / Plant Pest Section Mustialankatu 3, 00790 Helsinki, Finland
Short description of the test	Identification of potato cyst nematodes using a real-time PCR test
Date, reference of the validation report	2013-08-16 -
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Globodera pallida (HETDPA) Globodera rostochiensis (HETDRO)
Detection / identification	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	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Nakhla, M. K., Owens, K. J., Li, W. & Wei, G. 2010. Multiplex real-time PCR assays for the identification of the potato cyst and tobacco cyst nematodes. Plant Disease 94 : 959 – 965.
Is the test modified compared to the reference test	yes Based on Nakhla et al. 2010. TaqMan real-time PCR with modified primer concentrations and PCR program, including modified DNA extraction
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Globodera pallida(HETDPA)**

Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Validation samples were prepared from larvae of two cysts of either G. pallida. G. pallida could be detected with certainty at a 10-3 dilution from these samples. The normal samples always contain at least 1 larva, which in validation process was easily detected in pure and mixed nematode populations.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	G. rostochiensis 100 + populations (see validation report) G. pallida 1 population
Specificity value	The specificity was 100 % for G. pallida and G. rostochiensis when specificity was tested using mixed populations of larvae of both species and pure populations of G. tabacum and G. artemisiae. However, slight cross-reactions of the probe of G. pallida was observed in repeatability testing (see this summary sheet 'Cross reacts with' and the validation report)
Analytical specificity - exclusivity	
Number of non-target organisms tested	G. tabacum 1 population G. artemisiae 1 population
Specificity value	G. pallida probe cross-reacted slightly with G. rostochiensis in some duplex reactions even though the fluorescence remained weak and the curve low. The result could be verified by running simplex reactions for both species. The simplex reactions did not show any cross reactions.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	On the positive/negative scale: 100 % for G. pallida
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for G. pallida
Organism 2.:	**Globodera rostochiensis(HETDRO)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Validation samples were prepared from larvae of two cysts G. rostochiensis. G. rostochiensis could be detected with certainty at a 10-2 dilution from these samples. The normal samples always contain at least 1 larva, which in validation process was easily detected in pure and mixed nematode populations.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	G. rostochiensis 100 + populations (see validation report) G. pallida 1 population
Specificity value	The specificity was 100 % for G. pallida and G. rostochiensis when specificity was tested using mixed populations of larvae of both species and pure populations of G. tabacum and G. artemisiae.

	However, slight cross-reactions of the probe of G. pallida was observed in repeatability testing (see this summary sheet 'Cross reacts with' and the validation report)
Analytical specificity - exclusivity	
Number of non-target organisms tested	G. tabacum 1 population G. artemisiae 1 population
Specificity value	G. pallida probe cross-reacted slightly with G. rostochiensis in some duplex reactions even though the fluorescence remained weak and the curve low. The result could be verified by running simplex reactions for both species. The simplex reactions did not show any cross reactions.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	On the positive/negative scale: 100% for G. rostochiensis
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for G. rostochiensis
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	The test was established and validated because the method of Bulman & Marshall (1997), which has been used for a long time in our laboratory has caused continuous problems with sensitivity and performance. In particular, when the sample material has consisted of old cysts of G. rostochiensis, it has sometimes been impossible to get any PCR amplicons. When the method of Nakhla et al. (2010, modified) was compared to the method of Bulman & Marshall (1997) with normal cyst samples in the validation process, the detection rates were 89.2 % and 52.3 %, respectively.
The following complementary files are available online:	• Validation report: Identification of potato cyst nematodes using a real-time PCR test

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