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.

Target Organism	Globodera pallida Globodera rostochiensis	
Short description	Identification of potato cyst nematodes using a real-time PCR test	
Laboratory contact details	Finnish Food Authority / Plant Pest Section Mustialankatu 3, 00790 Helsinki, Finland	
Date and reference of the validation report	2013-08-16 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 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 the same as described in the EPPO DP?	No Based on Nakhla et al. 2010. TaqMan real-time PCR with modified primer concentrations and PCR program, including modified DNA extraction	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)		
Matrices tested (if relevant)	Isolated nematodes (larvae, cysts)	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	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.
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		

Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	Validation samples were prepared from larvae of two cysts of either G. pallida or G. rostochiensis. G. pallida could be detected with certainty at a 10-3 dilution and G. rostochiensis 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.	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98		
Specify the standard test		
<u>Analytical specificity</u>		
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)	
Number of strains/populations of target organisms tested	G. rostochiensis 100 + populations (see validation report) G. pallida 1 population	
Number of non-target organisms tested	G. tabacum 1 population G. artemisiae 1 population	
Cross reacts with (specify the species)	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.	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test		
Specify the standard test		
<u>Reproducibility</u>		
Provide the calculated % of	On the positive/negative scale:	

agreement for a given level of the pest (see PM 7/98)	100 % for G. pallida 100% for G. rostochiensis
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for G. pallida 100% for G. rostochiensis
Test performance study	
Test performance study?	No
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	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