

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	Qualitative detection of viability and identification of Globodera spp.	
Laboratory contact details	Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands	
Date and reference of the validation report	July 2014 - Qualitative detection of Globodera spp. viability version 2	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/040(2) FES research: Validation of the determination of viability of Globodera cysts/eggs based on RNA detection version 3. Theo v.d. Lee. Quantification of viable eggs of the potato cyst nematodes (Globodera spp.) using either trehalose or RNA-specific Real-Time PCR Beniers et al. 2014 The test is based on the article of Beniers et al. 2014 but some changes have been made (another internal control is used and the extraction method is automated).	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)		
Matrices tested (if relevant)	Potato 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	Qualitative real-time RT-PCR
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	
Analytical sensitivity (= limit of detection)	
What is smallest amount of target that can be detected reliably?	1 -10 viable juveniles or eggs
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	G. pallida: 100 % G. rostochiensis: 100 %
Specify the standard test	-Dead or alive visual assessment using the table according to Beniers, 2008 -Soortbepaling Globodera pallida en Globodera rostochiensis m.b.v. realtime RT-PCR, PRI (niet gepubliceerd) -Validatierapport Nematoden-onderzoek Soortbepaling Globodera pallida en G. rostochiensis m.b.v. real-time PCR, versie 2, NAK, T. Dekker, mei 2012 -EPPO protocol PM7/40 (3), appendix 4 Visual determination
Analytical specificity	
Specificity value	
Number of strains/populations of target organisms tested	8 strains of G. pallida (Pa2 WUR 248, Pa2 Averis 2013, Pa3 Ecnavornay, Pa3 Rookmaker 2009, Pa3 Rookmaker 2013, Pa3 Averis 2013) 6 strains of G. rostochiensis (Ro1 SCRI, Ro1 Mierenbos, Ro2,3 C262, Ro2,3 PRI 2013, Ro4 F515, Ro5 G1518)
Number of non-target organisms tested	6 non-target organisms were tested (Heterodera betae, Heterodera glycines, Heterodera schachtii, Heterodera trifolii, Globodera tabacum, Cactodera cacti)
Cross reacts with (specify the species)	No cross reactions with other organisms
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	G. pallida: 72.2% G. rostochiensis: 86.7%

Specify the standard test	Dead or alive assessment according to the table in Beniers, 2008. Adapted and inserted in EPPO protocol PM7/40 (3), appendix 4 Visual determination. Species identification of <i>Globodera pallida</i> and <i>Globodera rostochiensis</i> by real-time RT-PCR, PRI (intern report FES)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Reproducibility: 100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability: 100%
Test performance study	
Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	Various KingFisher apparatus and ABI 7500 apparatus did not influence the outcome of the tests. The above described method will be applied in a qualitative way at the NAK. The new test has been applied to 184 field samples that were analysed by the old and the new method. No false-negative results were found. The new test found 6 positive results that were not detected with the old method.