

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	Diagnosis of Globodera pallida and Globodera rostochiensis (potato cyst nematodes) using Taqman® real-time PCR	
Laboratory contact details	Fera Sand Hutton, YO41 1LZ York, United Kingdom	
Date and reference of the validation report	2013-09 - Potato Coucil Project Report 2009/15: Validation of quantitative DNA detection systems for PCN. Ref: R287	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/040 Based on: Bulman S. R. & Marshall J. W. (1997) Differentiation of Australasian potato cyst nematode (PCN) populations using the polymerase chain reaction (PCR). New Zealand Journal of Crop and Horticultural Science 25: 123-129.	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)		
Matrices tested (if relevant)	Dissected cyst and eggs. Half is retained if morphological examination is required	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Conventional flotation method to isolate cysts (Wye washer, following EPPO diagnostic protocol PM 7/40 (3))
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Spin column based DNA extraction and Real-time PCR PCR developed from Bulman S. R. & Marshall J. W. (1997) Applied Biosystems TaqMan Universal master mix II, no UNG (4440043) 50°C for 2 min; 95°C for 10 min; 95°C 15 sec, 60°C 1 min (40 repeats)
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?	DNA from single cyst detectable at 1000 fold dilution	
<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	G. pallida 100% G. rostochiensis 100%	
Specify the standard test	The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997.	
<u>Analytical specificity</u>		
Specificity value		
Number of strains/populations of target organisms tested	20+ strains of G. pallida (see validation report) 20+ strains of G. rostochiensis (see validation report)	
Number of non-target organisms tested	Strains of G. tabacum (see validation report) Strains of G. achillae/millefolii (see validation report)	
Cross reacts with (specify the species)	G. tabacum, however this gives a distinct profile and be differentiated for G.pallida and G.rostochiensis	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	G. pallida 87.1% G. rostochiensis 93.75% The testing gave no false negatives for either species.	
Specify the standard test	The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997 and morphological identification.	
<u>Reproducibility</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The testing has been successful carried out by multiple users on all equipment over several days	
<u>Repeatability</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Positive controls used on every run produce repeatable results.	

Test performance study	
Test performance study?	Yes
Include brief details of the test performance study and its output. If available, provide a link to published article/report	100% correct identification results over recent proficiency tests.
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	The <i>Globodera pallida</i> probe is known to cross-react slightly with <i>Globodera rostochiensis</i> DNA. The cross reaction will show as a slight increase in delta Rn in the FAM channel (<i>G. pallida</i>) as the delta Rn increases exponentially in the TET channel (<i>G. rostochiensis</i>). This cross reaction is only observed when a sample is positive for <i>G. rostochiensis</i> .
The following complementary files are available online:	<ul style="list-style-type: none"> • Validation of quantitative DNA detection systems for PCN Ref: R287 • Validation report: speciation of <i>Globodera pallida</i> and <i>G. rostochiensis</i> (potato cyst nematodes) using TaqMan® real-time PCR