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	Fera Sand Hutton, YO41 1LZ York, United Kingdom
Short description of the test	Diagnosis of Globodera pallida and Globodera rostochiensis (potato cyst nematodes) using Taqman® real-time PCR
Date, reference of the validation report	2013-09-01 - Potato Council Project Report 2009/15: Validation of quantitative DNA detection systems for PCN. Ref: R287
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?	Other_project
If yes, please specify	Potato Council Project R287 'Validation of quantitative DNA detection systems for PCN'
Description of the test	
Organism(s)	Globodera pallida (HETDPA) Globodera rostochiensis (HETDRO)
Detection / identification	detection and identification
Method(s)	Extraction Molecular Extraction DNA RNA Molecular real time PCR
Method: Extraction	
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 3)
Name of the test	Extraction Wye washer
Is the test modified compared to the reference test	no
Other information	
Other details on the test	Conventional flotation method to isolate cysts (Wye washer, following EPPO diagnostic protocol PM 7/40 (3))

Method: Molecular Extraction DNA RNA	
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 4)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	DNeasy Blood & Tissue Kits
Kit used following the manufacturer's instructions?	no
Other information	
Other details on the test	Spin column based DNA extraction
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 4)
Name of the test	Taqman Real-time PCR (Fera)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Other details on the test	Real-time PCR Applied BiosystemsTaqMan 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)
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?	DNA from single cyst detectable at 1000 fold dilution

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)	The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	20+ strains of G. pallida (see validation report)
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	Strains of G. tabacum (see validation report) Strains of G. achillae/millefolii (see validation report)
Specificity value	
Cross reacts with	Globodera tabacum tabacum
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	G. pallida 87.1% The testing gave no false negatives for either species.
Specify the test(s)	The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997 and morphological identification.
Reproducibility	
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
Repeatability	
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.
Organism 2.:	**Globodera rostochiensis(HETDRO)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	DNA from single cyst detectable at 1000 fold dilution
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)	The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997.
Analytical specificity - inclusivity	
Number of strains/populations of target	20+ strains of G. rostochiensis (see validation

organisms tested	report)
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	Strains of G. tabacum (see validation report) Strains of G. achillae/millefolii (see validation report)
Specificity value	
Cross reacts with	Globodera tabacum tabacum
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	G. rostochiensis 93.75% The testing gave no false negatives for either species.
Specify the test(s)	The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997 and morphological identification.
Reproducibility	
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
Repeatability	
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
Brief details of the test performance study and its output.It available, link to published article/report	100% correct identification results over recent proficiency tests.
Other information	
Any other information considered useful	The Globodera pallida probe is known to cross-react slightly with Globodera rostochiensis DNA. The cross reaction will show as a slight increase in delta Rn in the FAM channel (G. pallida) as the delta Rn increases exponentially in the TET channel (G. rostochiensis). This cross reaction is only observed when a sample is positive for G. rostochiensis.

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