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	Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands
Short description of the test	MeloTuber Test: a real-time TaqMan® PCR-based test to detect the root-knot nematodes Meloidogyne chitwoodi and M. fallax directly in potato tubers,
Date, reference of the validation report	2008-01-01 - 2008 and 2012
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Meloidogyne chitwoodi (MELGCH) Meloidogyne fallax (MELGFA)
Detection / identification	detection
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/041 Meloidogyne chitwoodi and M. fallax (version 2)
Name of the test	TaqMan ITS based real-time PCR (Zijlsta & van Hoof, 2006)
Other information	
Reaction type	Probe
Other details on the test	The protocol is based on the article of Zijlstra and Van Hoof (2006) as described in appendix 6 of PM 7/41 and was developed by the Dutch General Inspection Service for agricultural seeds and seed potatoes (NAK). The article is published as: The MeloTuber Test: a real-time TaqMan® PCRbased assay to detect the root-knot nematodes Meloidogyne chitwoodi and M. fallax directly in potato tubers, E.G. de Haan, C.C.E.M. Dekker, W.I.L.

	Tameling, L.J.M.F. den Nijs, G.W. van den Bovenkamp and M. Kooman-Gersmann. EPPO Bulletin Volume 44, Issue 2, pages 166-175, August 2014
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Meloidogyne chitwoodi(MELGCH)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	1 female in a sample of 100 peelings
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Diagnostic sensitivity: 100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	M. minor, M. hapla
Specificity value	No cross reactions with other organisms
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Diagnostic specificity: 100%
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%
Organism 2.:	**Meloidogyne fallax(MELGFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	1 female in a sample of 100 peelings
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Diagnostic sensitivity: 100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	M. minor, M. hapla
Specificity value	No cross reactions with other organisms
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Diagnostic specificity: 100%

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
Other information	
Any other information considered useful	Stability testing: secondary peelings and/or homogenate can be stored at - 20°C without affecting the analytical sensitivity. The MeloTuber Test is insensitive to variation in sample material (potato varieties). The different performance criteria such as analytical sensitivity, repeatability, reproducibility, diagnostic sensitivity and diagnostic specificity seem not to be influenced by matrix effects caused by the different varieties and this confirms the selectivity and the robustness of the molecular test. Results of the validations have been described in the article “The MeloTuber Test: a real-time TaqMan® PCR-based assay to detect the root-knot nematodes Meloidogyne chitwoodi and M. fallax directly in potato tubers, E.G. de Haan, C.C.E.M. Dekker, W.I.L. Tameling, L.J.M.F. den Nijs, G.W. van den Bovenkamp and M. Kooman-Gersmann. EPPO Bulletin Volume 44, Issue 2, pages 166-175, August 2014”

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