

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	Meloïdogyne chitwoodi Meloïdogyne fallax	
Short description	MeloTuber Test: a real-time TaqMan® PCR-based test to detect the root-knot nematodes <i>Meloïdogyne chitwoodi</i> and <i>M. fallax</i> directly in potato tubers,	
Laboratory contact details	Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands	
Date and reference of the validation report	2008 and 2012 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/041(2) 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® PCR-based assay to detect the root-knot nematodes <i>Meloïdogyne chitwoodi</i> and <i>M. fallax</i> 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	
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)	<i>Solanum tuberosum</i>	
Matrices tested (if relevant)	tubers	
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	Real-time 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		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	1 female in a sample of 100 peelings	
<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	Diagnostic sensitivity: 100%	
Specify the standard test		
<u>Analytical specificity</u>		
Specificity value		
Number of strains/populations of target organisms tested		
Number of non-target organisms tested	M. minor, M. hapla	
Cross reacts with (specify the species)	No cross reactions with other organisms	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Diagnostic specificity: 100%	
Specify the standard test		
<u>Reproducibility</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Reproducibility: 100%	
<u>Repeatability</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability: 100%	
<u>Test performance study</u>		

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.	<p>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).</p> <p>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.</p> <p>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 <i>Meloidogyne chitwoodi</i> and <i>M. fallax</i> 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"</p>