

**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.

<b>Laboratory contact details</b>	Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands
<b>Short description of the test</b>	MeloTuber Test: a real-time TaqMan® PCR-based test to detect the root-knot nematodes <i>Meloidogyne chitwoodi</i> and <i>M. fallax</i> directly in potato tubers,
<b>Date, reference of the validation report</b>	2008-01-01 - 2008 and 2012
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>If yes, please specify</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Meloidogyne chitwoodi</i> (MELGCH) <i>Meloidogyne fallax</i> (MELGFA)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Tubers tubers
<b>Plant species tested</b>	<i>Solanum tuberosum</i>
<b>Method(s)</b>	Molecular real time PCR
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	
<b>EPPO Diagnostic Protocol name</b>	PM 7/041 <i>Meloidogyne chitwoodi</i> and <i>M. fallax</i> (version 2)
<b>Name of the test</b>	TaqMan ITS based real-time PCR (Zijlstra & van Hoof, 2006)
<b>As or adapted from an IPPC diagnostic protocol</b>	
<b>Is the test modified compared to the reference test</b>	

<b>Kit</b>	
<b>Is a kit used</b>	
<b>Other information</b>	
<b>Reaction type</b>	Probe
<b>Other details on the test</b>	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 <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
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Meloidogyne chitwoodi(MELGCH)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	1 female in a sample of 100 peelings
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	Diagnostic sensitivity: 100%
<b>Standard test(s)</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	
<b>Specificity value</b>	
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	M. minor, M. hapla
<b>Specificity value</b>	No cross reactions with other organisms
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Diagnostic specificity: 100%
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a</b>	Reproducibility: 100%

<b>given level of the pest (see PM 7/98)</b>	
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Repeatability: 100%
<b>Organism 2.:</b>	<b>Meloidogyne fallax(MELGFA)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	1 female in a sample of 100 peelings
<b><u>Diagnostic sensitivity</u></b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	Diagnostic sensitivity: 100%
<b>Standard test(s)</b>	
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	
<b>Specificity value</b>	
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	M. minor, M. hapla
<b>Specificity value</b>	No cross reactions with other organisms
<b>Cross reacts with</b>	
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Diagnostic specificity: 100%
<b>Specify the test(s)</b>	
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Reproducibility: 100%
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Repeatability: 100%
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. If available, link to published article/report</b>	
<b><u>Other information</u></b>	
<b>Any other information considered useful</b>	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

<p>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 <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>
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