

**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>	Anses Plant Health Laboratory - Nematology Unit Domaine de la Motte au Viconte BP 35327, 35653 Le Rheu, France
<b>Short description of the test</b>	Method for the Identification of <i>Nacobbus aberrans</i> from Isolated Nematodes by Real-Time PCR
<b>Date, reference of the validation report</b>	2024-12-01 - Method for the Identification of <i>Nacobbus aberrans</i> from Isolated Nematodes by Real-Time PCR (ANSES/LSV/MA079)
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	EURL
<b>If yes, please specify</b>	EU-funded project EURLs-EURCs 2025-2027. Grant number: 101202127
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Nacobbus aberrans</i> sensu lato (NACOBAs)
<b>Detection / identification</b>	identification
<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>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/005 <i>Nacobbus aberrans</i> sensu lato (version 2)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes The method was adapted from the conventional PCR developed by Anthoine & Mugniéry (2005) to Real-Time PCR
<b>Kit</b>	
<b>Is a kit used</b>	no

<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	DNA extraction was performed with the use of lysis buffer (see details in the report). Amplicon size: 295 bp
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Nacobbus aberrans sensu lato(NACOBA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	1 J2, 1 male (both 100%) and also 1 female, but only when the female is in good condition.
<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>	100%
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	4 populations of N. aberrans sensu lato originating from Bolivia, Peru and Argentina
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	34 populations from the genera Meloidogyne (11 species); Globodera (4 species); Heterodera (4 species); Pratylenchus (1) and Punctodera (1)
<b>Specificity value</b>	100%
<b>Cross reacts with</b>	
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%. The operators, the days on which the tests were carried out and two different thermocyclers: one uses a xenon lamp and the other LED technology. Different reaction consumables (Roche 96-well plate and Roche 8-well strips on an adapter) were also used.
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% positive results for the 8 technical replicates of the DNA analysis of 1J2 and 2J2.
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	The report is not publicly available, but can be provided on request (eurl.nematodes@anses.fr). It is restricted to the NRLs registered to the EURL website (see link below): <a href="https://sitesv2.anses.fr/en/minisite/plant-parasitic-nematodes/method-and-test-validation-reports">https://sitesv2.anses.fr/en/minisite/plant-parasitic-nematodes/method-and-test-validation-reports</a> The report has been published to Zenodo with restricted access with the following citation: European Union Reference

	Laboratory for Plant Parasitic Nematodes. (2024). Method for the identification of <i>Nacobbus aberrans</i> by Real-Time PCR (Version 1). Zenodo. <a href="https://doi.org/10.5281/zenodo.14653989">https://doi.org/10.5281/zenodo.14653989</a>
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