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	Anses Plant Health Laboratory - Nematology Unit Domaine de la Motte au Viconte BP 35327, 35653 Le Rheu, France
Short description of the test	Method for the Identification of Nacobbus aberrans from Isolated Nematodes by Real-Time PCR
Date, reference of the validation report	2024-12-01 - Method for the Identification of Nacobbus aberrans from Isolated Nematodes by Real-Time PCR (ANSES/LSV/MA079)
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?	EURL
If yes, please specify	EU-funded project EURLs-EURCs 2025-2027. Grant number: 101202127
Description of the test	
Organism(s)	Nacobbus aberrans sensu lato (NACOBA)
Detection / identification	identification
Matrix(ces) tested	Specimen Isolated nematodes
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
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
EPPO Diagnostic Protocol name	PM 7/005 Nacobbus aberrans sensu lato (version 2)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes The method was adapted from convention PCR to Real-Time PCR
Kit	
Is a kit used	no

Other information	
Reaction type	Simplex - Probe
Other details on the test	Based on the use of lysis buffer (see details in the report). Final volume 100 microliter evaluated.
Performance Criteria :	
Organism 1.:	**Nacobbus aberrans sensu lato(NACOBA)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	1 J2, 1 male (both 100%) and also 1 female, but only when the female is in good condition.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	4 populations of N. aberrans sensu lato originating from Bolivia, Peru and Argentina
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	34 populations from the genera Meloidogyne (11 species); Globodera (4 species); Heterodera (4 species); Pratylenchus (1) and Punctodera (1)
Specificity value	100%
Cross-reacts with	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	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.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% positive results for the 8 technical replicates of the DNA analysis of 1J2 and 2J2.
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	The report is not publicly available, but can be provided on request (eurl.nematodes@anses.fr). It is resticted to those registered to the EURL website (see link below): https://sitesv2.anses.fr/en/minisite/plant-parasitic-nematodes/method-and-test-validation-reports . 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 Nacobbus aberrans by Real-Time PCR (Version 1). Zenodo. https://doi.org/10.5281/zenodo.14653989

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