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	Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	detection and identification of Phytophthora cinnamomi by Molecular real time PCR in Roots, Wood, Soil of walnut and chestnut
Date, reference of the validation report	2024-07-05 - Haegi et al. 2024, https://doi.org/10.3390/agriculture14070999
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?	no
Description of the test	
Organism(s)	Phytophthora cinnamomi(PHYTCN)
Detection / identification	detection and identification
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Haegi et al. 2024
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Probe

Other details on the test	GoTaq G2 Hot Start DNA Polymerase (Promega, Madison WI, USA)
Performance Criteria :	
Organism 1.:	**Phytophthora cinnamomi(PHYTCN)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	50 fg of P.cinnamomi genomic DNA
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	15 isolates of P. cinnamomi from CREADC collection: Om69 (Quercus rubra France); Om70 (Juglans regia Italy); Om74 (Juglans regia Italy); Om76 (Juglans regia Italy); Om119 (Juglans regia Italy); Om194 (Juglans regia Italy); Om202 (Juglans regia Italy); Om274 (Juglans regia Italy); Om281 (Juglans regia Italy); Om283 (Juglans regia Italy); Om139 (Castanea sativa Spain); Om141 (Castanea sativa Spain); Om142 (Castanea sativa Spain); Om144 (Quercus sp. Italy); Om145 (Quercus sp. Italy).
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Specificity was tested on the genomic DNA from 50 isolates belonging to eight different Phytophthora clades, and all the results obtained are summarized in Table 1 of the Reference test. Detection in qPCR occurred only for the expected sequences of P. cinnamomi. None of the other Phytophthora species showed cross amplifications, nor did the closely related ones belonging to clade 7. P. alni subsp. alni, P. alni subsp. multiformis, P. alni subsp. uniformis, P.cambivora (subclade 7a), P. niederhauserii (subclade 7b), and P. parvispora (subclade 7c as P. cinnamomi) did not amplify with our method at high DNA concentrations (10 ng).
Specificity value	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability was 100% for quantity of P.cinnamomi genomic DNA higher than 200 fg (defined as limit of detection LOD in the reference test). Concentrations evaluated with 3 replicates are 5, 2, and 1 ng/µL, 500, 200, 100, 50, 20, 10, 5, 2, and 1 pg/µL, and 500, 200, 100, and 50 fg/µL.
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	The reference test attached describes the development of a qPCR method for the specific identification and quantification of P. cinnamomi in natural soil samples. Primers and probe were

	developed targeting the nuclear single-copy ras-related protein gene Ypt1, suitable for Phytophthora-specific PCR. Additionally, this study established a systematic and repeatable soil sampling method and developed an efficient soil DNA extraction technique to apply the developed qPCR in naturally infested soils of walnut orchards.
The following complementary files are available online:	• Haegi et al., 2024

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