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.

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Short description of the test	Detection of Phytophthora ramorum by Molecular Conventional PCR in Leaves, Pure culture
Date, reference of the validation report	2020-06-04 - Validierung/Verifizierung – Plan und Bericht: A51_02_05 (konv PCR mit Phyto1/Phyto4) von pfl. Materialien und in vitro-Kulturen auf Phytophthora ramorum
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?	no
Description of the test	
Organism(s)	Phytophthora ramorum (PHYTRA)
Detection / identification	detection
Matrix(ces) tested	Leaves, Pure culture
Plant species tested	Rhododendron ponticum
Method(s)	Molecular Conventional PCR
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/066 Phytophthora ramorum (version 1)
Name of the test	Conventional PCR (Wagner & Werres, 2003)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes Reduced primer concentration: 0,1 µM: Use of HOT FIREPol® Blend Master Mix Initial denaturation step extendet to 13 min due to hot-start activation
Kit	

Is a kit used	no
Other information	
Reaction type	Simplex
Performance Criteria :	
Organism 1.:	**Phytophthora ramorum(PHYTRA)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	1.2 pg Phytophthora ramorum BBA 9/95 genomic DNA
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	n.a.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	- P. ramorum BBA 9/95 (=CBS 101553, genotype EU1, mating type A1, isolated from Rhododendron) - P. ramorum BBA 26/02 (genotype EU1, mating type A2, isolated from Viburnum bodnantense) - P. ramorum JKI-055-13-8-00-0-0 (genotype NA1, mating type A2, isolated from Rhododendron campylocarpum) - P. ramorum JKI-057-13-8-00-0-0 (genotype NA2, mating type A2, isolated from Gaultheria shallon)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	P. plurivora JKI-017-09-09-01-02-00 P. cambivora BBA 21/95 KII P. lateralis BBA CBS 168.42 P. foliorum JKI CBS 121655
Specificity value	No cross reactions were detected or P. plurivora JKI-017-09-09-01-02-00 and P. cambivora BBA 21/95 KII even at high DNA concentrations. For P. lateralis BBA CBS 168.42 and P. foliorum JKI CBS 121655 sporadic very weak bands at high concentrations (> 2.1 ng and > 3.3 ng)
Cross-reacts with	Phytophthora lateralis Phytophthora foliorum
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	n.a.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Reproducibility was 100% for the detection of P. ramorum BBA 9/95 genomic DNA at concentrations ≥ 1.2 pg. Concentration evaluated using 4 replicates of 10-fold dilution series of a genomic DNA extract in 6 different runs performed by two operators over a period of one week. Reproducibility of the test using leaf material was

	assessed using healthy leaf material supplemented with 2% and 10% symptomatic leaf material. DNA extraction and conventional PCR were performed in 4 replicates by two operators over a period of one week. A third test was performed by a third operator 16 months later.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability was 100% for the detection of P. ramorum BBA 9/95 genomic DNA at concentrations ≥ 1.2 pg. Concentration evaluated using 4 replicates of 10-fold dilution series of a genomic DNA extract under standardised conditions in 6 different runs performed by two operators over a period of one week. Repeatability of the test using leaf material was assessed using healthy leaf material supplemented with 2% and 10% symptomatic leaf material. DNA extraction and conventional PCR were performed in 4 replicates by two operators over a period of one week. A third test was performed by a third operator 16 months later.
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	Prior to DNA extraction using the DNeasy Plant Mini Kit (Qiagen), mycelium or leaf pieces were homogenized with two sterile stainless steel beads (Ø 5 mm) and 400 µl of AP1 buffer using a mixer mill (Retsch MM 400, Retsch GmbH) for 5 min at a frequency of 27 Hz and room temperature.
The following complementary files are available online:	• Summary - Data Validation Sheet - Phytophthora ramorum - conventional PCR

Creation date: 2026-08-26 13:57:02 - Last update: 2026-08-26 14:16:31