

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 <i>Xanthomonas oryzae</i> pv. <i>oryzicola</i> by real time PCR Xoc3866 in Seeds
Date, reference of the validation report	2026-07-24 - Detection of <i>Xanthomonas oryzae</i> pv. <i>oryzicola</i> by real-time PCR Xoc 3866 from rice seeds
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?	EURL
If yes, please specify	EURL for Pest of Plants on Bacteria (2023-2024)
Description of the test	
Organism(s)	<i>Xanthomonas oryzae</i> pv. <i>oryzicola</i> (XANTTO)
Detection / identification	detection and identification
Matrix(ces) tested	Seeds
Plant species tested	<i>Oryza sativa</i>
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
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
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex

Other details on the test	The test is adapted from the tetraplex conventional PCR (cPCR) assay developed by Lang et al. (2010) and targets the lipopolysaccharide (LPS) O-antigen biosynthesis protein gene. The assay uses the primer pair Xoc3866F and Xoc3866R.
Performance Criteria :	
Organism 1.:	Xanthomonas oryzae pv. oryzicola(XANTTO)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	10 ⁴ cfu/mL - Evaluated using three series of rice spiked samples with bacterial suspensions of X. oryzae pv. oryzicola strain NCPPB 1585 from 10 ⁶ cfu/mL to 10 cfu/mL. Three panel of samples tested. Spiked samples were prepared by soaking rice seeds (5 gr) of cv. "Carnaroli" with the inoculum for 24 hours at 4°C and pelleting to obtain a bacterial concentrate which was used for DNA extraction.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Standard test(s)	Comparison of samples with known status
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	12 strains of X. oryzae pv. oryzicola.
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	97%
Specificity value	Evaluated on 7 strains of Xanthomonas spp., 11 strains of X. oryzae pv. oryzae, 2 strains of Xanthomonas maliensis, 2 strains of Xanthomonas oryzae pv. leersiae, 3 strains of Curtobacterium flaccumfaciens pv. flaccumfaciens, 1 strain of Xanthomonas citri pv. citri, 1 strain of Burkholderia andropogonis, 1 strain of Xanthomonas axonopodis pv. poinsetticola, 1 strain of Xanthomonas arboricola pv. pruni.
Cross-reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	Comparison of samples with known status
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (evaluated using two technical replicates—analyzed across eight different laboratories—of spiked rice samples at the LOD (104 cfu/mL for X. oryzae pv. oryzicola and at a ten

	fold higher concentration).
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (obtained within an intra-laboratory activity performed at CREA on three technical replicates of rice seeds spiked with 104 cfu/ml and ten-fold higher concentration).
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output. It available, link to published article/report	Test performance study organized within the EURL Bac activity (EURL-BAC-2024-XANTOZ-TPS-FR). See the report attached.
The following complementary files are available online:	• TEST PERFORMANCE STUDY ON THE MOLECULAR DETECTION OF XANTHOMONAS ORYZAE PV. ORYZAE AND XANTHOMONAS ORYZAE PV. ORYZICOLA IN RICE SEEDS

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