

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>oryzae</i> by real time PCR of Lu et al (2014) in Seeds
Date, reference of the validation report	2026-07-24 - Detection of <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> by real-time PCR (Lu et al., 2014) 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>oryzae</i> (XANTOR)
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	
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	Lu et al (2014)
Kit	
Is a kit used	no

Other information	
Other details on the test	The test targets the putative glycosyltransferase gene (Genbank accession number AF169030). This gene is
Performance Criteria :	
Organism 1.:	Xanthomonas oryzae pv. oryzae(XANTOR)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	10 ² cfu/mL - Evaluated using three panel of rice spiked samples with bacterial suspensions of X. oryzae pv. oryzae strain LMG 26970 from 10 ⁶ cfu/mL to 10 cfu/mL. 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	85.4% - This value is the result of false positive obtained on the sample spiked with the Xoo type strain by the 8 laboratories involved in the EURL-BAC-2024-XANTOZ-TPS-FR Test Performance Study (TPS).
Standard test(s)	Comparison of samples with known status
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Evaluated on 11 strains X. oryzae pv. oryzae. False positive result occurred on the Xoo type strain ICMP 3125 (NCPB 3002).
Specificity value	91%
Analytical specificity - exclusivity	
Number of non-target organisms tested	tested on 7 strains of Xanthomonas spp., 11 strains of X. oryzae pv. oryzicola, 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. glycines, 1 strain of Pantoea stewartii subsp. stewartii, 1 strain of Pantoea stewartii subsp. indologenes, 1 strain of Xanthomonas citri pv. citri, 1 strain of Burkholderia andropogonis, 1 strain of Xanthomonas axonopodis pv. poinsettiicola, 1 strain of Pseudomonas syringae pv. theae, 1 strain of Pseudomonas viridiflava, 1 strain of Pseudomonas putida, 1 strain of Xanthomonas arboricola pv. pruni.
Specificity value	97.1% - X. oryzae pv. oryzicola LMG27109 showed a peak amplification signal with high Cq value (Cq>33)
Cross-reacts with	Xanthomonas oryzae pv. oryzicola
Diagnostic Specificity	
Proportion of uninfected/uninfested samples	100%

(true negatives) testing negative compared to results from a standard test	
Specify the test(s)	Comparison of samples with known status
<u>Reproducibility</u>	
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 within the TPS—of spiked rice samples at the LOD (102 cfu/mL for <i>X. oryzae</i> pv. <i>oryzae</i> and at a ten fold higher concentration)
<u>Repeatability</u>	
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 and evaluated on three technical replicates of spiked rice samples at the LOD 102 cfu/mL for <i>X. oryzae</i> pv. <i>oryzae</i> and ten-fold higher concentration).
Test performance study	
Test performance study?	yes
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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