

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> , <i>X. oryzae</i> pv. <i>oryzae</i> and <i>X. oryzae</i> pv <i>oryzicola</i> by Triplex Conventional PCR in Seeds
Date, reference of the validation report	2026-07-24 - Detection of <i>Xanthomonas oryzae</i> , <i>X. oryzae</i> pv. <i>oryzae</i> and <i>X. oryzae</i> pv <i>oryzicola</i> by triplex PCR 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 Conventional PCR
Method: Molecular Conventional 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 7/080 <i>Xanthomonas oryzae</i> (version 1)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes

Kit	
Is a kit used	no
Other information	
Reaction type	Triplex
Other details on the test	The test is suitable for the detection of <i>X. oryzae</i> , <i>X. oryzae</i> pv <i>oryzae</i> , <i>X. oryzae</i> pv. <i>oryzicola</i> in rice seeds and identification of bacterial cultures. The test is adapted by Vera Cruz et al., 2017. The target sequences are located in the gene coding a hypothetical protein of <i>X. oryzae</i> and <i>X. oryzae</i> pv. <i>oryzae</i> and a LPS O-antigen biosynthesis protein of <i>X. oryzae</i> pv. <i>oryzicola</i> .
Performance Criteria :	
Organism 1.:	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>(XANTOR)
<u>Analytical sensitivity</u>	
What is the smallest amount of target that can be detected reliably?	10 ³ cfu/mL for <i>X. oryzae</i> pv. <i>oryzae</i> ; 10 ⁴ cfu/mL for <i>X. oryzae</i> pv. <i>oryzicola</i> . Evaluated using three panel of rice spiked samples with bacterial suspensions of <i>X. oryzae</i> pv. <i>oryzae</i> strain LMG 26970 and of <i>X. oryzae</i> pv. <i>oryzicola</i> strain NCPPB 1585 from 106 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.
<u>Diagnostic sensitivity</u>	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	95,8%
Standard test(s)	Comparison of samples with known status
<u>Analytical specificity - inclusivity</u>	
Number of strains/populations of target organisms tested	<i>X. oryzae</i> pv. <i>oryzae</i> and <i>X. oryzae</i> pv. <i>oryzicola</i> : 11 strains of <i>X. oryzae</i> pv. <i>oryzae</i> and 12 strains of <i>X. oryzae</i> pv. <i>oryzicola</i> .
Specificity value	100%
<u>Analytical specificity - exclusivity</u>	
Number of non-target organisms tested	<i>X. oryzae</i> pv. <i>oryzae</i> : tested on 12 strain of <i>Xanthomonas oryzae</i> pv. <i>oryzicola</i> , 3 bacterial isolates from rice seeds, 7 strains of <i>Xanthomonas</i> spp., 3 strains of <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> , 2 strains of <i>X. maliensis</i> , one strain of <i>X. arboricola</i> pv. <i>pruni</i> , <i>X. axonopodis</i> pv. <i>poinsettiicola</i> , <i>X. citri</i> pv. <i>citri</i> , <i>X. citri</i> pv. <i>glycines</i> , <i>Burkholderia andropogonis</i> , <i>Pseudomonas putida</i> , <i>Pseudomonas syringae</i> pv. <i>theae</i> , <i>Pseudomonas viridiflava</i> , <i>Pantoea stewartii</i> subsp. <i>indologenes</i> . <i>Pantoea stewartii</i> subsp. <i>stewartii</i> . <i>X. oryzae</i> pv. <i>oryzicola</i> : 11 strain of <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> , 3 bacterial isolates from rice seeds, 7

	strains of <i>Xanthomonas</i> spp., 3 strains of <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> , 2 strains of <i>X. maliensis</i> , one strain of <i>X. arboricola</i> pv. <i>pruni</i> , <i>X. axonopodis</i> pv. <i>poinsettiicola</i> , <i>X. citri</i> pv. <i>citri</i> , <i>X. citri</i> pv. <i>glycines</i> , <i>Burkholderia andropogonis</i> , <i>Pseudomonas putida</i> , <i>Pseudomonas syringae</i> pv. <i>theae</i> , <i>Pseudomonas viridiflava</i> , <i>Pantoea stewartii</i> subsp. <i>indologenes</i> . <i>Pantoea stewartii</i> subsp. <i>stewartii</i> .
Specificity value	<i>X. oryzae</i> pv. <i>oryzae</i> : 95% <i>X. oryzae</i> pv. <i>oryzicola</i> : 100%
Cross-reacts with	
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Diagnostic specificity: 100%.
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—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% (evaluated on three technical replicates of spiked rice samples at the LOD 103 cfu/mL for <i>X. oryzae</i> pv. <i>oryzae</i> and 104 cfu/mL for <i>X. oryzae</i> pv. <i>oryzicola</i>) and ten-fold higher concentration: 100%)
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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