

**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.

<b>Laboratory contact details</b>	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
<b>Short description of the test</b>	detection of <i>Pantoea stewartii</i> subsp. <i>stewartii</i> by Molecular real time PCR in maize seeds according to Tambong et al., 2008.
<b>Date, reference of the validation report</b>	2024-03-01 - CREA-DC_PT2022-07-Ps_Tambong et al., 2008; Scala et al., 2023
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	Proteggio 1.4
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Pantoea stewartii</i> subsp. <i>stewartii</i> (ERWIST)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular real time PCR
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/060 <i>Pantoea stewartii</i> (version 2)
<b>Name of the test</b>	Real -time PCR (Tambong et al., 2008)
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Probe
<b>Other details on the test</b>	The validation data related to the analytical sensitivity, analytical specificity and repeatability

	are published in Scala et al 2023. The validation data related to diagnostic sensitivity, diagnostic specificity and reproducibility are the results of PT organized by CREA-DC with the participation of 8 Italian OL. The extraction and amplification of DNA were performed by the different participants according to their routine method adopted to perform the official analysis of <i>Pantoea stewartii</i> subsp <i>stewartii</i> .
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b><i>Pantoea stewartii</i> subsp. <i>stewartii</i>(ERWIST)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	10 <sup>2</sup> cfu/ml evaluated on DNA extracted from bacterial cell suspension, from 10 <sup>8</sup> to 10 <sup>1</sup> CFU/ml. 10 <sup>4</sup> cfu/ml evaluated on DNA extracted from maize seeds extract spiked with bacterial cell suspension at known concentration. The spiked samples have been prepared by adding bacterial suspensions from 10 <sup>8</sup> to 10 CFU/ml to healthy seed extracts. Each sample was repeated in three biological replicates and each biological replicate was repeated in two technical replicates.
<b><u>Diagnostic sensitivity</u></b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	100% calculated with 12 samples consisted of 6 artificially infected positive samples, 6 negative samples, prepared as described below (details of PT).
<b>Standard test(s)</b>	comparison of samples with known status
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	5 strains: CREA-DC 1775; CREA-DC 1869; CREA-DC 1870; CREA-DC 1899; CREA-DC 1900
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	40 strains belonging to different genera or species of bacterial pathogens present in CREA-DC bacterial collection. The list of the 40 strains were reported in Scala et al. 2023
<b>Specificity value</b>	56%
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	83.3 % calculated with 12 samples consisted of 6 artificially infected positive samples, 6 negative samples. In the samples panel were included two samples of bacterial suspension at 10 <sup>6</sup> cfu/ml of <i>Pantoea stewartii</i> subsp. <i>indologenes</i> and <i>P. ananatis</i> respectively.
<b>Specify the test(s)</b>	comparison of samples with known status
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%

<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	The PT was performed with 8 Italian OL. Each participant received a panel of 12 samples consisted of: 4 samples of healthy maize seed extract, 2 samples of maize seed extract spiked with $10^4$ cfu/ml of bacterial suspension of <i>P. stewartii</i> subsp. <i>stewartii</i> , 3 samples of maize seed extract spiked with $10^5$ cfu/ml of bacterial suspension of <i>P. stewartii</i> subsp. <i>stewartii</i> , 1 sample of maize seed extract spiked with $10^6$ cfu/ml of bacterial suspension of <i>P. stewartii</i> subsp. <i>stewartii</i> , 1 sample of $10^6$ cfu/ml of bacterial suspension of <i>Pantoea stewartii</i> subsp. <i>indologenes</i> , 1 samples of $10^6$ cfu/ml of bacterial suspension of <i>P. ananatis</i> .
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Scala</a></li> <li>• <a href="#">CREA-DC_PT</a></li> </ul>

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