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 of Pantoea stewartii subsp. stewartii by Molecular real time PCR in maize seeds according to Tambong et al., 2008.
Date, reference of the validation report	2024-03-01 - CREA-DC_PT2022-07-Ps_Tambong et al., 2008; Scala et al., 2023
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?	Other_project
If yes, please specify	Proteggo 1.4
Description of the test	
Organism(s)	Pantoea stewartii subsp. stewartii(ERWIST)
Detection / identification	detection
Method(s)	Molecular real time PCR
Method: Molecular real time 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?	no
EPPO Diagnostic Protocol name	PM 7/060 Pantoea stewartii (version 2)
Name of the test	Real -time PCR (Tambong et al., 2008)
Kit	
Is a kit used	no
Other information	
Reaction type	Probe
Other details on the test	The validation data related to the analytical sensitivity, analytical specificity and repeatability

	are pubblished 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 partecipation of 8 Italian OL. The extraction and amplification of DNA were performed by the different partecipants according to their routine method adopted to perform the official analysis of Pantoea stewartii subsp stewartii.
Performance Criteria :	
Organism 1.:	**Pantoea stewartii subsp. stewartii(ERWIST)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	10^2 cfu/ml evaluated on DNA extracted from bacterial cell suspension, from 10^8 to 10^1 CFU/ml. 10^4 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 10^8 to 10 CFU/ml to healthy seed extracts. Each sample was repeated in three biological replicates and each biological replcate was repeated in two technical replicates.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% calculated with 12 samples consisted of 6 artificially infected positive samples, 6 negative samples, prepared as described below (details of PT).
Standard test(s)	comparison of samples with known status
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	5 strains: CREA-DC 1775; CREA-DC 1869; CREA-DC 1870; CREA-DC 1899; CREA-DC 1900
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	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
Specificity value	56%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	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 10^6 cfu/ml of Pantoea stewartii subsp. indologenes and P. ananatis respectively.
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%

Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	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 with10^4 cfu/ml of bacterial suspension of P. stewartii subsp. stewartii, 3 samples of maize seed extract spiked with10^5 cfu/ml of bacterial suspension of P. stewartii subsp. stewartii, 1 sample of maize seed extract spiked with10^6 cfu/ml of bacterial suspension of P. stewartii subsp. stewartii, 1 sample of 10^6 cfu/ml of bacterial suspension of Pantoea stewartii subsp. indologenes, 1 samples of 10^6 cfu/ml of bacterial suspension of P. ananatis.
The following complementary files are available online:	• Scala • CREA-DC_PT

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