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	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Detection and identification of Pantoea stewartii subsp. stewartii with real-time PCR
Date, reference of the validation report	2022-04-28 - 2022.molbio.003
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	Other_project
If yes, please specify	Dutch phytosanitary project
Description of the test	
Organism(s)	Pantoea stewartii subsp. stewartii(ERWIST)
Detection / identification	detection and identification
Method(s)	Molecular real time RT PCR
Method: Molecular real time RT 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)
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 Pal et al. real-time PCR was run in 20 µL

	including 4 µL of sample using 10 µL of GoTaq qPCR mastermix by Promega with the following PCR protocol: 10 minutes at 95˚C followed by 40 cycles of 95˚C for 15 seconds and 66˚C for 1 minute. Subsequently, a melt curve was produced: An increase from 65˚C to 95˚C in increments of 0.5˚C per 10 seconds. Primers were used in accordance with the original protocol (Table 1). The reaction mix was finished off with DNAse/RNAse free water. Primers were obtained from Eurogentec. The real-time protocol was run on the CFX96 Touch Real-Time PCR Detection System. Threshold values were automatically calculated for each run. Table 1: Primers used during this validation Oligo name Sequence (5' - 3') Final concentration cpsAB2313F AGA AAA CGC TGA TGC CAG AC 400 nM cpsR ACT ATC CTG ACT CAG GCA CT 400 n
Performance Criteria :	
Organism 1.:	**Pantoea stewartii subsp. stewartii(ERWIST)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	12.14 CFU / reaction or 3035 CFU / mL
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% The host matrix did not influence the performance of the test.
Standard test(s)	Two different sweet corn varieties were used (Tasty Sweet F1 and Jubilee). The status of the samples was known.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	13 isolates of Pss
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Clavibacter nebraskensis (1x) Dickeya zea (2x) P. agglomerans pv. milletae (1x) P. alli (1x) P. ananatis (6x) P. ananatis pv. anantis (1x) P. ananatis pv. uredovora (1x) P. stewartii subsp. indologenes (6x) Paraburkholderia andropogonis (1x) Pseudomonas syringae pv. syringae (2x) Pseudomonas syringae pv. lapsa (1xc) Xanthomonas campestris pv. vasculorum (1x)
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	Phosphate buffer was used as a negative control.

Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Three samples were isolated and tested by all three technicians.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Three samples were isolated and tested in duplicate by all three technicians.
Test performance study	
Test performance study?	no
The following complementary files are available online:	• Real-time PCR Pss

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