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 Curtobacterium flaccumfaciens pv. flaccumfaciens in seeds, by Isolation, Molecular conventional PCR and Molecular real time PCR.
Date, reference of the validation report	2025-09-01 - EURL-BAC-2024-CORBFL-TPS-FR
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	TPS EURL-BAC-2024-CORBFL-TPS-FR: EURL TPS on a method to detect Curtobacterium flaccumfaciens pv. flaccumfaciens in seeds of common bean (Phaseolus vulgaris).
Description of the test	
Organism(s)	Curtobacterium flaccumfaciens pv. flaccumfaciens (CORBFL)
Detection / identification	detection and identification
Matrix(ces) tested	Seeds The contaminated bean seed lot that was used in the TPS originated from a seed company in the USA and was imported into Belgium, where it was subjected to routine testing for the presence of C. flaccumfaciens pv. flaccumfaciens. Since the seed lot showed adequate homogeneity of contamination in replicate testing this seed lot was considered appropriate for the TPS.
Plant species tested	Phaseolus vulgaris
Method(s)	Molecular Conventional PCR Molecular real time PCR Isolation
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?	no
EPPO Diagnostic Protocol name	PM 7/102 Curtobacterium flaccumfaciens pv. flaccumfaciens (version 1)
Name of the test	Conventional PCR (Tegli et al., 2002)
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 labs that participated in the TPS used the reagents of their own choice.
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	Naktuinbouw Taqman PCR
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Probe
Other details on the test	The labs that participated in the TPS used reagents of their own choice. They could also choose which PCR they performed.
Method: Isolation	
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 7/102 Curtobacterium flaccumfaciens pv. flaccumfaciens (version 1)
As or adapted from an IPPC diagnostic	no

protocol	
Is the test modified compared to the reference test	yes A different semi-selective agar medium was used.
Other information	
Other details on the test	A modified King's medium B named CFFKB was used for isolation.
Performance Criteria :	
Organism 1.:	**Curtobacterium flaccumfaciens pv. flaccumfaciens(CORBFL)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Not determined; not included in the TPS.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	- 19 labs plated crude seed extract on CFFKB. Of the 95 subsamples, 85.3% tested positive. - 18 labs performed the Naktuinbouw Taqman PCR. 90 subsamples were tested. 72.2% tested positive with a Ct value at or below 30. 92.2% tested positive with a Ct value at or below 32. - 6 labs performed the conventional PCR (Tegli). 30 subsamples were tested of which 63.3% tested positive.
Standard test(s)	Details can be found in the attached report.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Not determined; not included in the TPS.
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	Not determined; not included in the TPS.
Specificity value	
Cross-reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Not determined; not included in the TPS.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not determined; not included in the TPS.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not determined; not included in the TPS.
Test performance study	
Test performance study?	yes

Brief details of the test performance study and its output.It available, link to published article/report	The conclusion of the TPS is as follows: The endorsement of the Tegli conventional PCR test for testing Cff in seed samples is debatable. The report of the TPS was published here: https://eurlplanthealth.nl/groups/view/4f9f5037-a16d-4ea9-aa81-4c3fe915a884/bacteriology/wiki/view/f1048a55-ec15-4887-9de6-c43a08bc0e5d/proficiency-tests-and-test-performance-studies
The following complementary files are available online:	• TPS report C. flaccumfaciens (real time) PCR

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