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	ILVO Institute for Agricultural and Fisheries Research Burg. Van Gansberghelaan 96, 9820 Merelbeke - Melle, Belgium
Short description of the test	Detection of Curtobacterium flaccumfaciens pv. flaccumfaciens by TaqMan real-time PCR in Phaseolus vulgaris seeds (method EURL-BAC-2024-CORBFL-TM-01)
Date, reference of the validation report	2024-12-23 - Validation report for the detection of Cff in common bean seeds by TaqMan real-time PCR
Validation process according to EPPO Standard PM7/98?	no
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	The research that yielded these results, was funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment through contract RF 23/08 CurtoALERT. It was also supported by EU-funded project 101143941 - EURL BACfyto 2023-2024.
Description of the test	
Organism(s)	Curtobacterium flaccumfaciens pv. flaccumfaciens (CORBFL)
Detection / identification	detection
Matrix(ces) tested	Seeds Commercially available seeds, tested in subsamples of 1000 seeds
Plant species tested	Phaseolus vulgaris
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the	yes

next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	EURL-BAC-2024-CFF-TPS-01; EURL-BAC-2024-CORBFL-TM-01
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	BIONOBILE
Specify the kit used	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	no See Appendix 1 in technical report EURL-BAC-2024-CORBFL-TR-01 (attached as complementary file)
Other information	
Other details on the test	Validated with the KingFisher Flex System
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	EURL-BAC-2024-CORBFL-TM-01 (Real-time TaqMan PCR by Naktuinbouw, from Naktuinbouw protocol SPN-B005)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Triplex - Probe
Other details on the test	Reagent: PerfeCTa qPCR ToughMix
Performance Criteria :	
Organism 1.:	**Curtobacterium flaccumfaciens pv. flaccumfaciens(CORBFL)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Evaluated on DNA extracted from 50x concentrated seed extracts that were spiked with different amounts of Curtobacterium flaccumfaciens pv. flaccumfaciens (Cff) to the final concentrations of 9 x 10^5, 9 x 10^4, 9 x 10^3, 4.5 x 10^3, 9 x 10^2

	and 4.5 x 10^2 CFU per mL of concentrated extract. Three biological replicates were tested per Cff level and two technical replicates were included in real-time PCR. The lowest amount of target that could be detected reliably by two different operators was 9.0 x 10^2 CFU per mL of concentrated seed extract (equivalent to 18 CFU per mL of crude unconcentrated extract).
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 standard detection tests included in the current version of EPPO PM 7/102 are conventional PCRs (e.g., Tegli et al., 2002), which are less sensitive than the newly validated TaqMan real-time PCR approach. All samples positive in conventional PCR were also detected in real-time PCR (100% diagnostic sensitivity), but due to the difference in sensitivity, several Tegli-negative samples also tested positive in TaqMan.
Standard test(s)	Conventional PCR by Tegli et al. (2002)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	51 Cff strains with confirmed pathogenicity (see attached report for complete list)
Specificity value	100%, all target strains were detected. Note that two Cff strains were not detected in the different PCR tests. Although these strains were originally reported as pathogenic on bean, they did not develop symptoms in the pathogenicity assays at ILVO. It was concluded that they had lost their virulence, underpinning the negative results in the PCR tests based on pathogenicity genes. Therefore, the two strains were excluded from the results.
Analytical specificity - exclusivity	
Number of non-target organisms tested	44 non-target strains were tested: 9 strains from other pathovars within C. flaccumfaciens (Cf), 31 other Cf strains that are not pathogenic to bean, and 4 Frigoribacterium isolates from Phaseolus bean and soybean (see attached report for complete list)
Specificity value	100%; Remark: There were no true cross reactions, but late signals were observed for several non-targets, probably due to the high concentration of bacterial DNA used in the PCR reactions (>10 ng).
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% evaluated with 3 biological replicates at 9 x 10^5, 9 x 10^4, 9 x 10^3, 4.5 x 10^3 and 9 x 10^2 CFU per mL of concentrated seed extract by 2 operators using the same PCR equipment on different days. Taking into account the in-house applied cut-off at Ct 35 for a positive result, the lowest concentration of 4.5 x 10^2 CFU/mL was still detected by operator 1 but not by operator 2.
Repeatability	

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% evaluated on 3 biological replicates at 9 x 10^5, 9 x 10^4, 9 x 10^3, 4.5 x 10^3, 9 x 10^2 and 4.5 x 10^2 CFU/mL.
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	A TPS organized in the framework of EURL is ongoing (EURL-BAC-2024-CFF-TPS-01), involving 19 laboratories from 18 countries. The report of this TPS will be made available in 2025.
Other information	
Any other information considered useful	Validation data generated in support of method EURL-BAC-2024-CORBFL-TM-01
The following complementary files are available online:	• EURL-BAC-2024-CORBFL-TR-01

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