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	
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 next version of the EPPO diagnostic protocol?	yes	
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 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 , 9×10^4 , 9×10^3 , 4.5×10^3 , 9×10^2 and 4.5×10^2 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).	
<u>Diagnostic sensitivity</u>		
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 nontargets, 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	
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The following complementary files are available online:	• EURL-BAC-2024-CORBFL-TR-01	

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