

**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.

<b>Laboratory contact details</b>	ILVO Institute for Agricultural and Fisheries Research Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium
<b>Short description of the test</b>	Detection of <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> by TaqMan real-time PCR in <i>Phaseolus vulgaris</i> seeds (method EURL-BAC-2024-CORBFL-TM-01)
<b>Date, reference of the validation report</b>	2024-12-23 - Validation report for the detection of Cff in common bean seeds by TaqMan real-time PCR
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	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.
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> (CORBFL)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no

<b>Reference of the test</b>	EURL-BAC-2024-CFF-TPS-01; EURL-BAC-2024-CORBFL-TM-01
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	BIONOBILE
<b>Specify the kit used</b>	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)
<b>Other information</b>	
<b>Other details on the test</b>	Validated with the KingFisher Flex System
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	EURL-BAC-2024-CORBFL-TM-01 (Real-time TaqMan PCR by Naktuinbouw, from Naktuinbouw protocol SPN-B005)
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Triplex - Probe
<b>Other details on the test</b>	Reagent: PerfeCTa qPCR ToughMix
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Curtobacterium flaccumfaciens pv. flaccumfaciens(CORBFL)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	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 $9 \times 10^5$ , $9 \times 10^4$ , $9 \times 10^3$ , $4.5 \times 10^3$ , $9 \times 10^2$ and $4.5 \times 10^2$ 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 $9.0 \times 10^2$ CFU per mL of concentrated seed extract (equivalent to 18 CFU per mL of crude unconcentrated extract).
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	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.
<b>Standard test(s)</b>	Conventional PCR by Tegli et al. (2002)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	51 Cff strains with confirmed pathogenicity (see attached report for complete list)
<b>Specificity value</b>	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.
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	44 non-target strains were tested: 9 strains from other pathovars within <i>C. flaccumfaciens</i> (Cf), 31 other Cf strains that are not pathogenic to bean, and 4 <i>Frigoribacterium</i> isolates from Phaseolus bean and soybean (see attached report for complete list)
<b>Specificity value</b>	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).
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% evaluated with 3 biological replicates at $9 \times 10^5$ , $9 \times 10^4$ , $9 \times 10^3$ , $4.5 \times 10^3$ and $9 \times 10^2$ 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 $4.5 \times 10^2$ CFU/mL was still detected by operator 1 but not by operator 2.
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% evaluated on 3 biological replicates at $9 \times 10^5$ , $9 \times 10^4$ , $9 \times 10^3$ , $4.5 \times 10^3$ , $9 \times 10^2$ and $4.5 \times 10^2$ CFU/mL.

<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	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.
<b>Other information</b>	
<b>Any other information considered useful</b>	Validation data generated in support of method EURL-BAC-2024-CORBFL-TM-01
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">EURL-BAC-2024-CORBFL-TR-01</a></li> </ul>

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