

**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.

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| <b>Laboratory contact details</b>                                      | Netherlands Institute for Vectors, Invasive plants and Plant health<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands                                                                                                                                                                                                                                                                                |
| <b>Short description of the test</b>                                   | Detection and identification of <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> in seeds, by Isolation, Molecular conventional PCR and Molecular real time PCR.                                                                                                                                                                                                                       |
| <b>Date, reference of the validation report</b>                        | 2025-09-01 - EURL-BAC-2024-CORBFL-TPS-FR                                                                                                                                                                                                                                                                                                                                                             |
| <b>Validation process according to EPPO Standard PM7/98?</b>           | yes                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Is the lab accredited for this test?</b>                            | no                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Was the validated data generated in the framework of a project?</b> | EURL                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>If yes, please specify</b>                                          | TPS EURL-BAC-2024-CORBFL-TPS-FR: EURL TPS on a method to detect <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> in seeds of common bean ( <i>Phaseolus vulgaris</i> ).                                                                                                                                                                                                                |
| <b>Description of the test</b>                                         |                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Organism(s)</b>                                                     | <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> (CORBFL)                                                                                                                                                                                                                                                                                                                              |
| <b>Detection / identification</b>                                      | detection and identification                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Matrix(ces) tested</b>                                              | 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 <i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i> . Since the seed lot showed adequate homogeneity of contamination in replicate testing this seed lot was considered appropriate for the TPS. |
| <b>Plant species tested</b>                                            | <i>Phaseolus vulgaris</i>                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Method(s)</b>                                                       | Molecular Conventional PCR<br>Molecular real time PCR<br>Isolation                                                                                                                                                                                                                                                                                                                                   |
| <b>Method: Molecular Conventional PCR</b>                              |                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Reference of the test description</b>                               |                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>As or adapted from an EPPO diagnostic protocol</b>                  | yes                                                                                                                                                                                                                                                                                                                                                                                                  |

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| <b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b> | no                                                                                                                        |
| <b>EPPO Diagnostic Protocol name</b>                                                                | PM 7/102 Curtobacterium flaccumfaciens pv. flaccumfaciens (version 1)                                                     |
| <b>Name of the test</b>                                                                             | Conventional PCR (Tegli et al., 2002)                                                                                     |
| <b>As or adapted from an IPPC diagnostic protocol</b>                                               | no                                                                                                                        |
| <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                                                                                                                   |
| <b>Other details on the test</b>                                                                    | The labs that participated in the TPS used the reagents of their own choice.                                              |
| <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>                                                                        | Naktuinbouw Taqman PCR                                                                                                    |
| <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>                                                                                | Probe                                                                                                                     |
| <b>Other details on the test</b>                                                                    | The labs that participated in the TPS used reagents of their own choice. They could also choose which PCR they performed. |
| <b>Method: Isolation</b>                                                                            |                                                                                                                           |
| <b>Reference of the test description</b>                                                            |                                                                                                                           |
| <b>As or adapted from an EPPO diagnostic protocol</b>                                               | yes                                                                                                                       |
| <b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b> | yes                                                                                                                       |
| <b>EPPO Diagnostic Protocol name</b>                                                                | PM 7/102 Curtobacterium flaccumfaciens pv. flaccumfaciens (version 1)                                                     |
| <b>As or adapted from an IPPC diagnostic</b>                                                        | no                                                                                                                        |

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| <b>protocol</b>                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Is the test modified compared to the reference test</b>                                                                           | yes A different semi-selective agar medium was used.                                                                                                                                                                                                                                                                                                                                         |
| <b><i>Other information</i></b>                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Other details on the test</b>                                                                                                     | A modified King's medium B named CFFKB was used for isolation.                                                                                                                                                                                                                                                                                                                               |
| <b>Performance Criteria :</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Organism 1.:</b>                                                                                                                  | <b>Curtobacterium flaccumfaciens pv. flaccumfaciens(CORBFL)</b>                                                                                                                                                                                                                                                                                                                              |
| <b>Analytical sensitivity</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>What is the smallest amount of target that can be detected reliably?</b>                                                          | Not determined; not included in the TPS.                                                                                                                                                                                                                                                                                                                                                     |
| <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> | - 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. |
| <b>Standard test(s)</b>                                                                                                              | Details can be found in the attached report.                                                                                                                                                                                                                                                                                                                                                 |
| <b>Analytical specificity - inclusivity</b>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Number of strains/populations of target organisms tested</b>                                                                      | Not determined; not included in the TPS.                                                                                                                                                                                                                                                                                                                                                     |
| <b>Specificity value</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Analytical specificity - exclusivity</b>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Number of non-target organisms tested</b>                                                                                         | Not determined; not included in the TPS.                                                                                                                                                                                                                                                                                                                                                     |
| <b>Specificity value</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Cross-reacts with</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Diagnostic Specificity</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | Not determined; not included in the TPS.                                                                                                                                                                                                                                                                                                                                                     |
| <b>Reproducibility</b>                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                             | Not determined; not included in the TPS.                                                                                                                                                                                                                                                                                                                                                     |
| <b>Repeatability</b>                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                             | Not determined; not included in the TPS.                                                                                                                                                                                                                                                                                                                                                     |
| <b>Test performance study</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Test performance study?</b>                                                                                                       | yes                                                                                                                                                                                                                                                                                                                                                                                          |

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| <b>Brief details of the test performance study and its output. It is available, link to published article/report</b> | <p>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: <a href="https://eurlplanthealth.nl/groups/view/4f9f5037-a16d-4ea9-aa81-4c3fe915a884/bacteriology/wiki/view/f1048a55-ec15-4887-9de6-c43a08bc0e5d/proficiency-tests-and-test-performance-studies">https://eurlplanthealth.nl/groups/view/4f9f5037-a16d-4ea9-aa81-4c3fe915a884/bacteriology/wiki/view/f1048a55-ec15-4887-9de6-c43a08bc0e5d/proficiency-tests-and-test-performance-studies</a></p> |
|                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <p>The following complementary files are available online:</p>                                                       | <ul style="list-style-type: none"> <li>• <a href="#">TPS report C. flaccumfaciens (real time) PCR</a></li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

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