

**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>	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
<b>Short description of the test</b>	Addition of a direct seed extract TaqMan based PCR to pre-screen bean seed samples of 1000 seed for presence and absence of DNA of <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> .
<b>Date, reference of the validation report</b>	2025-09-05 - Validation report Cff SPN-B005 v5.0
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	no
<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>Matrix(ces) tested</b>	Seeds
<b>Plant species tested</b>	<i>Glycine max</i> , <i>Phaseolus coccineus</i> , <i>Phaseolus lunatus</i> , <i>Phaseolus vulgaris</i> , <i>Pisum sativum</i> , <i>Vigna angularis</i> , <i>Vigna mungo</i> , <i>Vigna radiata</i>
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular other
<b>Method: Extraction</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>Is the test modified compared to the reference test</b>	yes introduction of a SE-PCR prescreen detection method to the existing protocol.
<b>Other information</b>	

<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>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>Other details on the test</b>	extraction of DNA from seed extract of the seed subsamples is carried out first by concentration via centrifugation, followed by DNA isolation with a magnetic particle approach ending with the use of a multiplex Cff specific TaqMan PCR .
<b>Method: Molecular other</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>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>	Multiplex (>3)
<b>Other details on the test</b>	Two Cff specific assays designed on the Cff plasmid were used together with a internal isolation control assay for Cmtes spiked to each test sample prior to concentration.
<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>	the LOD of the test lays somewhere between 17,4 and 1,74 CFU/mL
<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%
<b>Standard test(s)</b>	the standard test used is dilution plating on two semi-selective media mMSCFF+b and D2ANX.
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	since the same Cff specific assays are used as for the earlier validated protocol version SPN-B005 vers2, this was not again demonstrated
<b>Specificity value</b>	100%
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	>95%
<b>Specify the test(s)</b>	the standard test used is dilution plating on two semi-selective media mMSCFF+b and D2ANX.
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% (evaluated on 3 replicates at 20CFU/mL)
<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 replicates at 20CFU/mL)
<b>Test performance study</b>	
<b>Test performance study?</b>	no
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">Validation report Cff SPN-B005 v5.0 EPPO</a></li> </ul>

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