

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	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
Short description of the test	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> .
Date, reference of the validation report	2025-09-05 - Validation report Cff SPN-B005 v5.0
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> (CORBFL)
Detection / identification	detection
Method(s)	Extraction Molecular Extraction DNA RNA Molecular other
Method: Extraction	
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
Is the test modified compared to the reference test	yes introduction of a SE-PCR prescreen detection method to the existing protocol.
Other information	
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic	no

protocol	
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
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Other details on the test	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 .
Method: Molecular other	
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
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Multiplex (>3)
Other details on the test	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.
Performance Criteria :	
Organism 1.:	Curtobacterium flaccumfaciens pv. flaccumfaciens(CORBFL)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	the LOD of the test lays somewhere between 17,4 and 1,74 CFU/mL
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Standard test(s)	the standard test used is dilution plating on two

	semi-selective media mMSCFF+b and D2ANX.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	since the same Cff specific assays are used as for the earlier validated protocol version SPN-B005 vers2, this was not again demonstrated
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	>95%
Specify the test(s)	the standard test used is dilution plating on two semi-selective media mMSCFF+b and D2ANX.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (evaluated on 3 replicates at 20CFU/mL
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (evaluated on 3 replicates at 20CFU/mL
Test performance study	
Test performance study?	no
The following complementary files are available online:	• Validation report Cff SPN-B005 v5.0 EPPO

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