

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	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Detection and identification of <i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> by molecular real time PCR in herbaceous cuttings and in pure culture
Date, reference of the validation report	2026-07-22 - 2025.molbio.006
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 and identification
Matrix(ces) tested	Herbaceous cuttings, Pure culture Pieces of the stems and petioles of common bean grown in the greenhouse. And pure cultures in water.
Plant species tested	<i>Phaseolus vulgaris</i>
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
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	Simplex
Other details on the test	DNA isolation from plant extract was done using the Quickpick Plant DNA kit (Bio-Nobile). Four assays were selected: 1. Naktuinbouw 1: Real-time PCR (Barnhoorn, R., unpublished) primer/probe set #1 2. Naktuinbouw 2: Real-time PCR (Barnhoorn, R., unpublished) primer/probe set #2 3. Woodhall: Real-time PCR (Woodhall et al., unpublished) 4. Tarakanov: Real time PCR (Tarakanov et al., 2023) based on the conventional PCR by Tegli et al., 2002. An 18S rRNA internal control was used. PerfeCTa qPCR ToughMix (Quantabio) was used. Machine: BioRad CFX Opus Real-Time PCR system
Performance Criteria :	
Organism 1.:	Curtobacterium flaccumfaciens pv. flaccumfaciens(CORBFL)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Assay Naktuinbouw 1 = 7.9 cfu per reaction Assay Naktuinbouw 2 = 3.6 cfu per reaction
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	25 isolates of C. flaccumfaciens pv. flaccumfaciens (isolated from various host plants in various countries and from different years), 3 isolates of C. poinsettiae, and 1 C. aurantiacum. All isolates contain the virulence plasmid.
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	32 isolates of bacterial genera associated with the Fabaceae family.
Specificity value	100%
Cross-reacts with	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%. Three different technicians tested 3 sample sets of a high (3.5x10 ⁵ CFU/mL) and a low (3.5x10 ² CFU/mL) concentration, each technician on a different day and on different Bio-Rad CFX Opus real-time PCR machines.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Three different technicians tested 3 sample sets of a high (3.5x10 ⁵ CFU/mL) and a low (3.5x10 ² CFU/mL) concentration, each technician on a different day and on different Bio-Rad CFX Opus real-time PCR machines.
Test performance study	
Test performance study?	no

