

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	Identification of <i>Curtobacterium flaccumfaciens</i> pv <i>flaccumfaciens</i> by conventional PCR
Date, reference of the validation report	2011-06-24 - 2011 Validation report PCR for identification of <i>Curtobacterium flaccumfaciens</i> pv <i>flaccumfaciens</i>
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> (CORBFL)
Detection / identification	identification
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Conventional PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Kit	
Is a kit used	yes
Manufacturer name	BIONOBILE
Specify the kit used	QuickPick Plant DNA kit
Kit used following the manufacturer's instructions?	
Other information	
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
Kit	
Is a kit used	yes

Manufacturer name	ROCHE
Specify the kit used	High Pure PCR Template Preparation Kit
Kit used following the manufacturer's instructions?	
Other information	
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPP0 diagnostic protocol	yes
EPP0 Diagnostic Protocol name	PM 7/102 Curtobacterium flaccumfaciens pv. flaccumfaciens (version 1)
Name of the test	Conventional PCR (Tegli et al., 2002)
Is the test modified compared to the reference test	no
Other information	
Are the performance characteristics included in the EPP0 diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Curtobacterium flaccumfaciens pv. flaccumfaciens(CORBFL)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	2,3 x 10 ⁷ 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)	IF in combination with Fatty Acid Analysis
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	11 strains of Curtobacterium flaccumfaciens pv flaccumfaciens
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	20 strains of non-target organisms
Specificity value	100% - no cross reaction
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	IF in combination with Fatty Acid Analysis
Reproducibility	
Provide the calculated % of agreement for a	100%

given level of the pest (see PM 7/98)	
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	For the robustness: the DNA isolation methodology was tested in order to investigate whether this could have any influence on the outcome of the test. Both the QuickPick Plant DNA Kit (Bio-Nobile) on the Kingfisher and the High Pure PCR template preparation kit (Roche) performed equally well.
The following complementary files are available online:	
	<ul style="list-style-type: none"> • 2011 Validation report PCR for identification of Cff

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