

**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	National Reference Centre, National Plant Protection Organization 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?	
If yes, please specify	
Description of the test	
Organism(s)	<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> (CORBFL)
Detection / identification	identification
Matrix(ces) tested	
Plant species tested	
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Conventional PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
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	
Other details on the test	
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
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	
Other details on the test	
Method: Molecular Conventional 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?	
EPPO Diagnostic Protocol name	PM 7/102 Curtobacterium flaccumfaciens pv. flaccumfaciens (version 1)
Name of the test	Conventional PCR (Tegli et al., 2002)
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	no
Kit	
Is a kit used	
Other information	
Reaction type	

Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Curtobacterium flaccumfaciens pv. flaccumfaciens(CORBFL)
<u>Analytical sensitivity</u>	
What is smallest amount of target that can be detected reliably?	2,3 x 10 ⁷ cfu/ml.
<u>Diagnostic sensitivity</u>	
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
<u>Analytical specificity - inclusivity</u>	
Number of strains/populations of target organisms tested	11 strains of Curtobacterium flaccumfaciens pv flaccumfaciens
Specificity value	100%
<u>Analytical specificity - exclusivity</u>	
Number of non-target organisms tested	20 strains of non-target organisms
Specificity value	100% - no cross reaction
Cross reacts with	
<u>Diagnostic Specificity</u>	
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
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
<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
Brief details of the test performance study and its output.It available, link to published article/report	
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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