

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	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	Detection of <i>Phyllosticta citricarpa</i> by PCR (Bonants et al. 2003)
Date, reference of the validation report	2014-09-15 -
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?	no
Description of the test	
Organism(s)	<i>Phyllosticta citricarpa</i> (GUIGCI)
Detection / identification	detection
Matrix(ces) tested	Fruits, Other Fungal mycelium, fruits
Plant species tested	Citrus x limon
Method(s)	Molecular Extraction DNA RNA Molecular Conventional PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Kit	
Is a kit used	yes
Manufacturer name	MACHEREY-NAGEL
Specify the kit used	Nucleospin plant II kit
Kit used following the manufacturer's instructions?	yes
Other information	
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes

EPPO Diagnostic Protocol name	PM 7/017 Guignardia citricarpa (version 2)
Name of the test	Conventional PCR (Bonants et al., 2003)
Other information	
Other details on the test	See details in report
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Phyllosticta citricarpa(GUIGCI)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	20 pg of DNA
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	4 target strains for PCR
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	3 non-target strains (see validation report)
Specificity value	100% No cross reaction
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	The robustness of the method was verified through a Test Performance Study among 7 laboratories. For each lab 6 positive samples (3 containing the target DNA slightly above the relative limit of detection and 3 containing the target DNA ten times the relative limit of detection) and 6 negative samples (3 containing no DNA and 3 containing DNA of non-target strains) were tested. The results showed: -100% relative sensitivity -100% relative specificity -100% repeatability -100% reproducibility
Other information	
Any other information considered useful	The verification of performance criteria did not give the same value as the limit of detection defined in the EPPO bulletin PM 7/17 (20 pg instead of 1pg), so a new validation was performed See full report for details or contact lab1

The following complementary files are available online:

- [Validation process of the conventional PCR for the identification of *Phyllosticta citricarpa* \(Bonants et al., 2003\)](#)

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