EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

Target Organism	Phyllosticta citricarpa	
Short description	Detection of Phyllosticta citricarpa by PCR (Bonants et al. 2003)	
Laboratory contact details	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy	
Date and reference of the validation report	2014-09-15 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 Bonants P.J.M., Caroll G.C., de Weerdt M., van Brouwershaven I.R.,Baayen R.P., 2003. Development and validation of a fast PCR-based method for pathogenic isolates of the citrus black spot fungus Guignardia citricarpa. European Journal of Plant Pathology, 109, 503–513. van Gent-Pelzer M.P.E,. van Brouwershaven I.R., Kox L.F.F., Bonants P.J.M., 2007. A Taqman PCR method for routine diagnosis of the quarantine fungus Guignardia citricarpa. Journal of Phytopathology, 155, 357–363. EPPO PM 7/17(2), 2009. Guignardia citricarpa. EPPO Bulletin, 39, 318–327.	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Citrus lemon	
Matrices tested (if relevant)	Fungal mycelium, fruits	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	PCR method
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		

Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	20 pg of DNA	
<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		
Specify the standard test		
<u>Analytical specificity</u>		
Specificity value		
Number of strains/populations of target organisms tested	4 target strains for PCR	
Number of non-target organisms tested	3 non-target strains (see validation report)	
Cross reacts with (specify the species)	No cross reaction	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test		
Specify the standard test		
<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?	Yes
Include brief details of the test performance study and its output.It available, provide a 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 **e.g. robustness, ease of performing the test, etc.**	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)