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	Real Time PCR for the identification of Phyllosticta citricarpa (van Gent-Pelzer et al., 2007)	
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 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	N/R Modification from EPPO PM 7/17(2), 2009. Guignardia citricarpa. EPPO Bulletin, 39, 318–327.	
Is the test the same as described in the EPPO DP?		
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Citrus lemon	
Matrices tested (if relevant)	Fungal pure culture and lemon fruit	
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	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.
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		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	10 fg of DNA	
Diagnostic sensitivity		
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		
Analytical specificity		
Specificity value	100%	
Number of strains/populations of target organisms tested	3 target strains	
Number of non-target organisms tested	3 non-target strains (see validation report)	
Cross reacts with (specify the species)	no cross reaction	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test		
Specify the standard test		
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	
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 6 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.**	When verifying the performance criteria cross reactions wih the non-target organism Phyllosticta citriasiana was noted so the protocol was slightly changed and a new validation was performed. It is suggested to use the amplification commercial kit in RealTime-PCR to avoid no specific amplification with P. citriasiana.
The following complementary files are available online:	• Validation process of the Real Time PCR for the identification of Phyllosticta citricarpa (van Gent-Pelzer et al., 2007)