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	Monilinia fructicola	
Short description	Detection of Monilinia fructicola by multiplex PCR	
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	2013-04-06 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 Multiplex PCR: Côté MJ, Tardif MC, Meldrum AJ: Identification of Monilinia fructigena, M. fructicola, M. laxa, and Monilia polystroma on inoculated and naturally infected fruit using multiplex PCR. Plant Dis 2004. 88:1219-1225. Standard method:PM 7/18 (2) and Ioos R, Frey P: Genomic variation within Monilinia laxa, M. fructigena and M. fructicola, and application to species identification by PCR. Eur J Plant Pathol 2000, 106: 373–378.	
Is the test the same as described in the EPPO DP?	No the multiplex PCR is not included in the EPPO DP	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Peach, pear, plum, apple	
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	Multiplex PCR
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?	25 pg for Multiplex PCR 0.5 pg for Standard method	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	96,0 % (after a Ring test with four laboratory and the lower DNA concentration in the samples was 25 pg); 54,5 % (during the test of validation carry out in the lab were the lower DNA concentration was that of the standard method, 0.5 pg (false negatives obtained by the multiplex PCR method were caused by the DNA concentration lower than the LOD)	
Specify the standard test	End point PCR (Ioos and Frey, 2000)	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested	10 for Multiplex PCR 6 for Standard method	
Number of non-target organisms tested	22 for Multiplex PCR (M. laxa, M. fructigena, Monilia polistroma, fruit) 19 for Standard method (M. laxa, M. fructigena, Monilia polistroma) See Annex 1	
Cross reacts with (specify the species)	Not occured	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%	
Specify the standard test	End point PCR (Ioos and Frey, 2000)	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not requested when comparing with a standard method (appendix 3 PM 7/98) 100% for Standard method during performance verification	

Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not requested when comparing with a standard method (appendix 3 PM 7/98) 100% for Standard method during performance verification
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	see Annex 2
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	The Multiplex PCR (Cotè et al., 2004) is just less sensitive than the end point PCR (Ioos and Frey, 2000) used here as standard method, but the LOD is enough for quarantine purpose and allow to identify the different species in one test, both from pure colture and from infected tessue. Positive results could be confirmed by the end point PCR that uses specific primers.
The following complementary files are available online:	• Annex 1 - list of strains used • Annex 2 - performance test