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 Monilinia fructicola by multiplex PCR
Date, reference of the validation report	2013-04-06 -
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?	
Description of the test	
Organism(s)	Monilinia fructicola (MONIFC)
Detection / identification	detection
Method(s)	Molecular Conventional PCR
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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
Other information	
Reaction type	Multiplex (>3)
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Monilinia fructicola(MONIFC)**
Analytical sensitivity	

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)
Standard test(s)	End point PCR (Ioos and Frey, 2000)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	10 for Multiplex PCR 6 for Standard method
Specificity value	
Analytical specificity - exclusivity	
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
Specificity value	Not occured
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
Specify the test(s)	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
Brief details of the test performance study and its output.It available, link to published article/report	see Annex 2
Other information	
Any other information considered useful	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. 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.
The following complementary files are available online:	• Annex 1 - list of strains used • Annex 2 - performance test

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