

**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.

<b>Laboratory contact details</b>	Council for Agricultural Research and Economics- Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
<b>Short description of the test</b>	Detection of <i>Monilinia fructicola</i> by multiplex PCR
<b>Date, reference of the validation report</b>	2013-04-06 -
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Monilinia fructicola</i> (MONIFC)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Conventional PCR
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Multiplex PCR: Côté MJ, Tardif MC, Meldrum AJ: Identification of <i>Monilinia fructigena</i> , <i>M. fructicola</i> , <i>M. laxa</i> , and <i>Monilia polystroma</i> on inoculated and naturally infected fruit using multiplex PCR. Plant Dis 2004. 88:1219-1225
<b>Other information</b>	
<b>Reaction type</b>	Multiplex (>3)
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	no
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b><i>Monilinia fructicola</i>(MONIFC)</b>
<b>Analytical sensitivity</b>	

<b>What is smallest amount of target that can be detected reliably?</b>	25 pg for Multiplex PCR 0.5 pg for Standard method
<b><u>Diagnostic sensitivity</u></b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	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)
<b>Standard test(s)</b>	End point PCR (Ioos and Frey, 2000)
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	10 for Multiplex PCR 6 for Standard method
<b>Specificity value</b>	
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	22 for Multiplex PCR (M. laxa, M. fructigena, Monilia polistroma, fruit) 19 for Standard method (M. laxa, M. fructigena, Monilia polistroma) See Annex 1
<b>Specificity value</b>	Not occurred
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	1
<b>Specify the test(s)</b>	End point PCR (Ioos and Frey, 2000)
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not requested when comparing with a standard method (appendix 3 PM 7/98) 100% for Standard method during performance verification
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not requested when comparing with a standard method (appendix 3 PM 7/98) 100% for Standard method during performance verification
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	see Annex 2
<b><u>Other information</u></b>	
<b>Any other information considered useful</b>	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 culture and from infected tissue. Positive

	results could be confirmed by the end point PCR that uses specific primers. loos 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:	<ul style="list-style-type: none"><li>• <a href="#">Annex 1 - list of strains used</a></li><li>• <a href="#">Annex 2 - performance test</a></li></ul>

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