

**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>	Intralaboratory validation of <i>Ceratocystis platani</i> _SYBR Green Real-Time PCR in wood of <i>Platanus acerifolia</i>
<b>Date, reference of the validation report</b>	2025-05-04 - Intralaboratory validation of <i>Ceratocystis platani</i> _Real-Time PCR_SYBR Green (corresponding M. Pilotti)
<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>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Ceratocystis platani</i> (CERAFP)
<b>Detection / identification</b>	detection and identification
<b>Matrix(ces) tested</b>	Wood Necrotic wood of artificially and naturally <i>C. platani</i> -infected trees
<b>Plant species tested</b>	<i>Platanus x hispanica</i>
<b>Method(s)</b>	Molecular real time PCR
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Lumia et al., 2018
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	

<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	SsoAdvanced Universal SYBR Green Supermix (BIO-RAD)
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Ceratocystis platani(CERAFP)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	3 fg of C. platani gDNA per PCR reaction (the detection limit was also confirmed by spiking with 6 ul of an extract from C. platani-uninfected and Fomitiporia mediterranea-infected necrotic wood)
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	100%
<b>Standard test(s)</b>	Comparison with samples of known status
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% - evaluated by two different operators; each operator performed three different experiment testing 8 replicates at the LOD (3 fg)
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% - evaluated on 8 replicates at the LOD (3 fg). The result was also confirmed by testing the gDNA of five additional C. platani strains, each in a single experiment performed by one operator, at the limit of detection and with eight replications.
<b>Test performance study</b>	
<b>Test performance study?</b>	no
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">Lumia et al (2018)</a></li> </ul>

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