

**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>	Anses Plant Health Laboratory - Pests and Tropical Pathogens Unit Pôle de Protection des Plantes, 7 Chemin de l'IRAT, 97410 Saint Pierre, France
<b>Short description of the test</b>	Detection and identification of 'Candidatus Liberibacter asiaticus' and 'Candidatus Liberibacter africanus' by Molecular real time PCR (according to Li et al., 2006) in Citrus sp. Leaves
<b>Date, reference of the validation report</b>	2020-07-10 - HLB_qPCR_EUPHRESCO-2016-A-232
<b>Link to other validation data</b>	- HLB_qPCR_EUPHRESCO-2016-A-232 Detection and identification of 'Candidatus Liberibacter asiaticus' and 'Candidatus Liberibacter africanus' by Molecular real time PCR (according to Li et al., 2006) in Citrus sp. leaves
<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>	Euphresco
<b>If yes, please specify</b>	2016-A-232
<b>Description of the test</b>	
<b>Organism(s)</b>	'Candidatus Liberibacter africanus' (LIBEAF) 'Candidatus Liberibacter asiaticus' (LIBEAS)
<b>Detection / identification</b>	detection and identification
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	DNeasy Plant Mini Kit
<b>Kit used following the manufacturer's instructions?</b>	yes DNA extraction was performed on ground citrus leaves using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) following the

	manufacturer's recommendations.
<b>Other information</b>	
<b>Other details on the test</b>	Ground using a HOMEX 6 homogenizer (Bioreba AG, Reinach, Switzerland) with 5 mL of extraction buffer (pH = 8): 50 mM Sigma 7-9® TRIS (Merck KGaA, Darmstadt, Germany); 5 mM EDTA (Merck KGaA); and 1% sodium dodecyl sulfate (Merck KGaA).
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/121 ' <i>Candidatus</i> Liberibacter africanus', ' <i>Candidatus</i> Liberibacter americanus' and ' <i>Candidatus</i> Liberibacter asiaticus' (version 1)
<b>Name of the test</b>	Real-time PCR targeting 16S rRNA gene (according to Li et al., 2006)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes using a final volume of 13 µL per reaction instead of 25 µL / primers were used at the original concentration (0.25 µM), but the Taqman probe was added at 0.13 µM instead of 0.15 µM
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	no
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	' <i>Candidatus</i> Liberibacter africanus'(LIBEAF)
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	5.5E-03 (DL 100) DL100 is defined as the smallest number of target analytes detected in the samples 100% of the time by a given method. This value is relative and is only relevant for comparison with the data produced for the other tests in the framework of the 2016-A-232 Euphresco project.
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	5 strains (see details in paper)
<b>Specificity value</b>	100.0%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	See paper: samples infected by Clso and Xcc +

	several non-target DNA samples corresponding to different non infected matrices
<b>Specificity value</b>	100.00%
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	DL 100: 91.7%
<b>Organism 2.:</b>	<b>'Candidatus Liberibacter asiaticus'(LIBEAS)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	4.40E-04 (DL 100) DL100 is defined as the smallest number of target analytes detected in the samples 100% of the time by a given method. This value is relative and is only relevant for comparison with the data produced for the other tests in the framework of the 2016-A-232 Euphresco project.
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	12 strains (see details in paper)
<b>Specificity value</b>	94.40%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	See paper: samples infected by Clso and Xcc + several non-target DNA samples corresponding to different non infected matrices
<b>Specificity value</b>	100.00%
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	DL 100: 92.2%
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	no
<b><u>Other information</u></b>	
<b>Any other information considered useful</b>	Publication available at: <a href="https://link.springer.com/content/pdf/10.1007/s10658-020-02052-3.pdf">https://link.springer.com/content/pdf/10.1007/s10658-020-02052-3.pdf</a> Cellier, G., C. Redondo, J. Cubero, M. Roselló, E. de Andrade, L. Cruz, E. Ince, H. N. Yildiz, P. G. Güler, A. M. D'Onghia, T. Yaseen, K. Djelouah, E. Metz-Verschure, F. Gaffuri, R. A. Gottsberger, and B. Giovani. 2020. "Comparison of the performance of the main real-time and conventional PCR detection tests for 'Candidatus Liberibacter' spp., plant pathogenic bacteria causing the Huanglongbing disease in Citrus spp." European Journal of Plant Pathology. doi: 10.1007/s10658-020-02052-3.

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