

**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 Morgan et al., 2012 using SYBR) 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 Morgan et al., 2012 using SYBR) 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 asiaticus' (LIBEAS) 'Candidatus Liberibacter africanus' (LIBEAF)
<b>Detection / identification</b>	detection and identification
<b>Matrix(ces) tested</b>	Leaves peduncule & midrib
<b>Plant species tested</b>	Citrus sp.
<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

Kit used following the manufacturer's instructions?	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 hyvI/hyvII gene (according to Morgan et al. 2012)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes Use of SYBR green instead of Taqman probe
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>'Candidatus Liberibacter asiaticus'(LIBEAS)</b>
<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>	0.626 (DSE=PA/N+, Fav. Hypothesis, considering CLas and CLaf samples)
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	0.980 (DSP=NA/N-, Fav. Hypothesis, considering CLas and CLaf samples)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	CO=0.952 (considering CLas and CLaf samples)
<b>Repeatability</b>	

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	DA=0.976 (considering CLas and CLaf samples)
<b>Organism 2.:</b>	<b>'Candidatus Liberibacter africanus'(LIBEAF)</b>
<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>	0.626 (DSE=PA/N+, Fav. Hypothesis, considering CLas and CLaf samples)
<b>Cross-reacts with</b>	'Candidatus Liberibacter solanacearum'
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	0.980 (DSP=NA/N-, Fav. Hypothesis, considering CLas and CLaf samples)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	CO=0.952 (considering CLas and CLaf samples)
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	DA=0.976 (considering CLas and CLaf samples)
<b>Test performance study</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>	Test performance study organized in the framework of a EUPHRESKO project involving 8 international laboratories.
<b>Other information</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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