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	Anses Plant Health Laboratory - Pests and Tropical Pathogens Unit Pôle de Protection des Plantes, 7 Chemin de l'IRAT, 97410 Saint Pierre, France
Short description of the test	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
Date, reference of the validation report	2020-07-10 - HLB_qPCR_EUPHRESCO-2016-A-232
Link to other validation data	- 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
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?	Euphresco
If yes, please specify	2016-A-232
Description of the test	
Organism(s)	'Candidatus Liberibacter asiaticus'(LIBEAS) 'Candidatus Liberibacter africanus'(LIBEAF)
Detection / identification	detection and identification
Matrix(ces) tested	Leaves pedoncule & midrib
Plant species tested	Citrus sp.
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic	

protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	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.
Other information	
Other details on the test	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).
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/121 ‘Candidatus Liberibacter africanus’, ‘Candidatus Liberibacter americanus’ and ‘Candidatus Liberibacter asiaticus’ (version 1)
Name of the test	Real-time PCR targeting hyvI/hyvII gene (according to Morgan et al. 2012)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes Use of SYBR green instead of Taqman probe
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**'Candidatus Liberibacter asiaticus'(LIBEAS)**
Analytical sensitivity	

What is smallest amount of target that can be detected reliably?	
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	0.626 (DSE=PA/N+, Fav. Hypothesis, considering CLas and CLaf samples)
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	
Specificity value	
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	0.980 (DSP=NA/N-, Fav. Hypothesis, considering CLas and CLaf samples)
Specify the test(s)	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	CO=0.952 (considering CLas and CLaf samples)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	DA=0.976 (considering CLas and CLaf samples)
Organism 2.:	**'Candidatus Liberibacter africanus'(LIBEAF)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	0.626 (DSE=PA/N+, Fav. Hypothesis, considering CLas and CLaf samples)
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	

Specificity value	
Cross reacts with	'Candidatus Liberibacter solanacearum'
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	0.980 (DSP=NA/N-, Fav. Hypothesis, considering CLas and CLaf samples)
Specify the test(s)	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	CO=0.952 (considering CLas and CLaf samples)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	DA=0.976 (considering CLas and CLaf samples)
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	Test performance study organized in the framework of a EUPHRESCO project involving 8 international laboratories.
Other information	
Any other information considered useful	Publication available at: https://link.springer.com/content/pdf/10.1007/s10658-020-02052-3.pdf 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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