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 Li et al., 2006) 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 Li et al., 2006) 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 africanus'(LIBEAF) 'Candidatus Liberibacter asiaticus'(LIBEAS)
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 16S rRNA gene (according to Li et al., 2006)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	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
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Probe
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	

Organism 1.:	**'Candidatus Liberibacter africanus'(LIBEAF)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	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.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	5 strains (see details in paper)
Specificity value	100.0%
Analytical specificity - exclusivity	
Number of non-target organisms tested	See paper: samples infected by Clso and Xcc + several non-target DNA samples corresponding to different non infected matrices
Specificity value	100.00%
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	DL 100: 91.7%
Organism 2.:	**'Candidatus Liberibacter asiaticus'(LIBEAS)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	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.
Diagnostic sensitivity	
Proportion of infected/infested samples	

tested positive compared to results from the standard test, see appendix 2 of PM 7/98	
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	12 strains (see details in paper)
Specificity value	94.40%
Analytical specificity - exclusivity	
Number of non-target organisms tested	See paper: samples infected by Clso and Xcc + several non-target DNA samples corresponding to different non infected matrices
Specificity value	100.00%
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	DL 100: 92.2%
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	
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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