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 duplex conventional PCR 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 duplex conventional PCR 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?	EURL
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 Conventional PCR
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no

Reference of the test	Conventional PCR targeting 16S rRNA gene (according to Teixeira et al., 2005a,b) duplexed with the conventional PCR targeting rplKAJL – rpoBC operon gene (according to Hocquellet et al., 1999)
Is the test modified compared to the reference test	yes Two conventional PCR were duplexed
Kit	
Is a kit used	no
Other information	
Reaction type	Duplex
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-02 (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	80.0%
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: 94.4%
Organism 2.:	**'Candidatus Liberibacter asiaticus'(LIBEAS)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	2.60E-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	12 strains (see details in paper)
Specificity value	80.60%
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: 96.7%
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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