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 (PLANT PRINT) 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 (PLANT PRINT) 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
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
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, 1 / 4 Germantown, MD, USA) following the

Other details on the test Ground using a HOMEX 6 homogenizer (Bioreba AG, Reinach, Switzerland) with 5 m. d extraction buffer (pH = 8): 50 mM Sigma 7-9® TRIS (Merck KGaA); and 1%sodium dodecyl sulfate (Merck KGA); and 1%sodium dodecyl sulfate (Merck KGA); and 1%sodium dodecyl sulfate Merch MGA, and the sprotocol? Nate test test description no Stitu used following the manufacturer's instruction? yes Other information Reaction type Reaction type Simplex - Probe Other details on the test Nealttime PCR targeting 16S rRNA gene (according to Bertolini		manufacturer's recommendations.	
AG, Reinach, Switzerland) with 5 m.u of sym Argen 7490 TRIS (Merck KGaA, Darmstadt, Germany): 5 mM EDTA (Merck KGaA,) and 1%sodium dodecyl sulfate (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? New test being considered for inclusion in the next version of the EPPO diagnostic protocol? As or adapted from an IPPC 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 PLANT PRINT Specify the kit used Liberibacter' spp. (Huanglongbing-HLB citrus disease). Rapid screening test Kit used following the manufacturer's instructions? yes Other information Simplex - Probe Other deails on the test Real-time PCR targeting 165 rRNA gene (according to Bertolini et al. (2010, 2014)) Are the performance characteristics included in the EPPO diagnostic protocol? no Performance Criteria : 'Candidatus Liberibacter africanus'(LIBEAF) Analytical sensitivity Number of target that can be detected in the samplest in the gramework of the 2016-A-232 Euphresco project. Analytical specificity - inclusivity S strains (see details in paper)	Other information		
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Number of strains/populations of target 5 strains (see details in paper) organisms tested 93.3%	What is smallest amount of target that can be detected reliably?	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	
organisms tested 93.3%	Analytical specificity - inclusivity		
	Number of strains/populations of target organisms tested	5 strains (see details in paper)	
Analytical specificity - exclusivity	Specificity value	93.3%	

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	91.70%	
Cross reacts with	'Candidatus Liberibacter solanacearum'	
<u>Repeatability</u>		
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?	6.20E-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.	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	12 strains (see details in paper)	
Specificity value	83.30%	
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	91.70%	
Cross reacts with	'Candidatus Liberibacter solanacearum'	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	DL 100: 91.1%	
Test performance study		
Test performance study?	no	
Other information		
Any other information considered useful	Publication available at: https://link.springer.com/co ntent/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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