

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.

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| 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) 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) 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 |
| 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, Germantown, MD, USA) following the |

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| | 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 |
| EPPO Diagnostic Protocol name | PM 7/121 ' <i>Candidatus</i> Liberibacter africanus', ' <i>Candidatus</i> Liberibacter americanus' and ' <i>Candidatus</i> 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 | no |
| Kit | |
| Is a kit used | no |
| Other information | |
| Reaction type | Simplex |
| 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? | 7.80E-05 (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 | 88.90% |
| 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 |

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| Specificity value | 100.00% |
| Repeatability | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | DL 100: 92.2% |
| Organism 2.: | 'Candidatus Liberibacter africanus'(LIBEAF) |
| Analytical sensitivity | |
| What is smallest amount of target that can be detected reliably? | NPC |
| Analytical specificity - inclusivity | |
| Number of strains/populations of target organisms tested | 5 strains (see details in paper) |
| Specificity value | 60.0% |
| 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% |
| Repeatability | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | DL 100: 100% |
| Test performance study | |
| Test performance study? | no |
| 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. Giovanni. 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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