

**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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| <b>Laboratory contact details</b>                                      | Anses Plant Health Laboratory - Pests and Tropical Pathogens Unit<br>Pôle de Protection des Plantes, 7 Chemin de l'IRAT,<br>97410 Saint Pierre, France                                                    |
| <b>Short description of the test</b>                                   | Detection and identification of 'Candidatus Liberibacter asiaticus' and 'Candidatus Liberibacter africanus' by Molecular real time PCR (PLANT PRINT) in Citrus sp. leaves                                 |
| <b>Date, reference of the validation report</b>                        | 2020-07-10 - HLB_qPCR_EUPHRESCO-2016-A-232                                                                                                                                                                |
| <b>Link to other validation data</b>                                   | - 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 |
| <b>Validation process according to EPPO Standard PM7/98?</b>           | yes                                                                                                                                                                                                       |
| <b>Is the lab accredited for this test?</b>                            | no                                                                                                                                                                                                        |
| <b>Was the validated data generated in the framework of a project?</b> | Euphresco                                                                                                                                                                                                 |
| <b>If yes, please specify</b>                                          | 2016-A-232                                                                                                                                                                                                |
| <b>Description of the test</b>                                         |                                                                                                                                                                                                           |
| <b>Organism(s)</b>                                                     | 'Candidatus Liberibacter africanus' (LIBEAF)<br>'Candidatus Liberibacter asiaticus' (LIBEAS)                                                                                                              |
| <b>Detection / identification</b>                                      | detection and identification                                                                                                                                                                              |
| <b>Method(s)</b>                                                       | Molecular Extraction DNA RNA<br>Molecular real time PCR                                                                                                                                                   |
| <b>Method: Molecular Extraction DNA RNA</b>                            |                                                                                                                                                                                                           |
| <b>Reference of the test description</b>                               |                                                                                                                                                                                                           |
| <b>Kit</b>                                                             |                                                                                                                                                                                                           |
| <b>Is a kit used</b>                                                   | yes                                                                                                                                                                                                       |
| <b>Manufacturer name</b>                                               | QIAGEN                                                                                                                                                                                                    |
| <b>Specify the kit used</b>                                            | DNeasy Plant Mini Kit                                                                                                                                                                                     |
| <b>Kit used following the manufacturer's instructions?</b>             | yes DNA extraction was performed on ground citrus leaves using the DNeasy Plant Mini Kit (Qiagen, 1 / 4 Germantown, MD, USA) following the                                                                |

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|                                                                                                                                      | manufacturer's recommendations.                                                                                                                                                                                                           |
| <b>Other information</b>                                                                                                             |                                                                                                                                                                                                                                           |
| <b>Other details on the test</b>                                                                                                     | 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) |
| <b>Method: Molecular real time PCR</b>                                                                                               |                                                                                                                                                                                                                                           |
| <b>Reference of the test description</b>                                                                                             |                                                                                                                                                                                                                                           |
| <b>As or adapted from an EPPO diagnostic protocol</b>                                                                                | no                                                                                                                                                                                                                                        |
| <b>As or adapted from an IPPC diagnostic protocol</b>                                                                                | no                                                                                                                                                                                                                                        |
| <b>Is the test modified compared to the reference test</b>                                                                           | no                                                                                                                                                                                                                                        |
| <b>Kit</b>                                                                                                                           |                                                                                                                                                                                                                                           |
| <b>Is a kit used</b>                                                                                                                 | yes                                                                                                                                                                                                                                       |
| <b>Manufacturer name</b>                                                                                                             | PLANT PRINT                                                                                                                                                                                                                               |
| <b>Specify the kit used</b>                                                                                                          | Liberibacter' spp. (Huanglongbing-HLB citrus disease). Rapid screening test                                                                                                                                                               |
| Kit used following the manufacturer's instructions?                                                                                  | yes                                                                                                                                                                                                                                       |
| <b>Other information</b>                                                                                                             |                                                                                                                                                                                                                                           |
| <b>Reaction type</b>                                                                                                                 | Simplex - Probe                                                                                                                                                                                                                           |
| <b>Other details on the test</b>                                                                                                     | Real-time PCR targeting 16S rRNA gene (according to Bertolini et al. (2010, 2014))                                                                                                                                                        |
| <b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>                                                 | <b>no</b>                                                                                                                                                                                                                                 |
| <b>Performance Criteria :</b>                                                                                                        |                                                                                                                                                                                                                                           |
| <b>Organism 1.:</b>                                                                                                                  | <b>'Candidatus Liberibacter africanus'(LIBEAF)</b>                                                                                                                                                                                        |
| <b>Analytical sensitivity</b>                                                                                                        |                                                                                                                                                                                                                                           |
| <b>What is smallest amount of target that can be detected reliably?</b>                                                              |                                                                                                                                                                                                                                           |
| <b>Diagnostic sensitivity</b>                                                                                                        |                                                                                                                                                                                                                                           |
| <b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b> | 1 (DSE=PA/N+, Fav. Hypothesis, considering CLas and CLaf samples)                                                                                                                                                                         |
| <b>Diagnostic Specificity</b>                                                                                                        |                                                                                                                                                                                                                                           |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | 0.679 (DSP=NA/N-, Fav. Hypothesis, considering CLas and CLaf samples)                                                                                                                                                                     |
| <b>Reproducibility</b>                                                                                                               |                                                                                                                                                                                                                                           |
| <b>Provide the calculated % of agreement for a</b>                                                                                   | CO=0.889 (considering CLas and CLaf samples)                                                                                                                                                                                              |

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| <b>given level of the pest (see PM 7/98)</b>                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b><u>Repeatability</u></b>                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                             | DA=1 (considering CLas and CLaf samples)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Organism 2.:</b>                                                                                                                  | <b>'Candidatus Liberibacter asiaticus'(LIBEAS)</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b><u>Analytical sensitivity</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>What is smallest amount of target that can be detected reliably?</b>                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b><u>Diagnostic sensitivity</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b> | 1 (DSE=PA/N+, Fav. Hypothesis, considering CLas and CLaf samples)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b><u>Analytical specificity - exclusivity</u></b>                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Number of non-target organisms tested</b>                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Specificity value</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b><u>Diagnostic Specificity</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | 0.679 (DSP=NA/N-, Fav. Hypothesis, considering CLas and CLaf samples)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b><u>Reproducibility</u></b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                             | CO=0.889 (considering CLas and CLaf samples)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b><u>Repeatability</u></b>                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                             | DA=1 (considering CLas and CLaf samples)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b><u>Test performance study</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Test performance study?</b>                                                                                                       | yes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Brief details of the test performance study and its output.It available, link to published article/report</b>                     | Test performance study organized in the framework of a EUPHRESKO project involving 8 international laboratories.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b><u>Other information</u></b>                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Any other information considered useful</b>                                                                                       | Publication available at: <a href="https://link.springer.com/content/pdf/10.1007/s10658-020-02052-3.pdf">https://link.springer.com/content/pdf/10.1007/s10658-020-02052-3.pdf</a> 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. |