

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**  
**ORGANISATION EUROPEENNE ET MEDITERRANEEENNE POUR LA PROTECTION DES PLANTES**  
(11-17239)

**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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| Target Organism  | 'Candidatus Liberibacter solanacearum'   |   |
| Short description  | Detection of 'Candidatus Liberibacter solanacearum' by real time PCR in different types of plant material using Plant Print diagnostics kit  |   |
| Laboratory contact details   | Bacteriology. Instituto Valenciano de Investigaciones Agrarias CV-315, km. 10.7, 46113 Moncada, Spain  |   |
| Date and reference of the validation report                                | Report 2016-04-28; Validation assay October 2012 - Performance study nº1   |   |
| Validation process according to EPPO Standard PM 7/98:                     | Yes  |   |
| Reference of the test description  | 0<br>G.R. Teresani, E. Bertolini, A. Alfaro-Fernández et al. 2014. Association of ‘Candidatus Liberibacter solanacearum’ with a Vegetative Disorder of Celery in Spain and Development of a Real-Time PCR Method for Its Detection.<br><a href="http://dx.doi.org/10.1094/PHYTO-07-13-0182-R">http://dx.doi.org/10.1094/PHYTO-07-13-0182-R</a> |   |
| Is the test the same as described in the EPPO DP?                          | No<br>There is not yet a protocol published by the EPPO or IPPC. The test was performed following Teresani et al. 2014 , following a method included in the draft of the EPPO protocol in preparation.   |   |
| Is the lab accredited for this test?                                       | Yes  |   |
| Plant species tested (if relevant)   | Daucus carota, Solanum tuberosum, Apium graveolens, Nicotiana tabacum, Vinca pervinca  |   |
| Matrices tested (if relevant)  | leaf extracts  |   |
|  |  |   |
| List of methods used   |  |   |
| Method for extraction / isolation / baiting of target organism from matrix | X  | Direct sample preparation without DNA purification (spot procedure)             |
| Molecular methods, e.g. hybridization, PCR and real time PCR               | X  | Real time PCR using Plant Print diagnostic kit, based on Teresani et al. (2014) |
| Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay            |  |   |
| Plating methods: selective isolation                                       |  |   |
| Bioassay methods: selective enrichment in host plants, baiting,            |  |   |

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| plant test and grafting.   |   |  |
| Pathogenicity test   |   |  |
| Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling   |   |  |
| Morphological and morphometrical methods intended for identification   |   |  |
| Biochemical methods: e.g. enzyme electrophoresis, protein profiling  |   |  |
| Other  |   |  |
| <b>Analytical sensitivity (= limit of detection)</b>   |   |  |
| What is smallest amount of target that can be detected reliably?   | Not calculated for a non-culturable bacterium. The performance study was oriented to receive qualitative results.   |  |
| <b>Diagnostic sensitivity</b>  |   |  |
| Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98 | 87% (standard test was real time PCR according to Teresani et al. after CTAB extract)   |  |
| Specify the standard test  | 232 samples agreement / 265 (including replications performed in some labs)   |  |
| <b>Analytical specificity</b>  |   |  |
| Specificity value  |   |  |
| Number of strains/populations of target organisms tested   |   |  |
| Number of non-target organisms tested  |   |  |
| Cross reacts with (specify the species)  |   |  |
| <b>Diagnostic Specificity</b>  |   |  |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test         | 263 samples agreement / 265 (including replications performed in some labs.)  |  |
| Specify the standard test  | 99% (standard test was real time PCR according to Teresani et al. after CTAB extract)   |  |
| <b>Reproducibility</b>   |   |  |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98)  | 83% (465/530)   |  |
| <b>Repeatability</b>   |   |  |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98)  | 95%<br>The repeatability was calculated in 7 laboratories that performed 3 replications each one and at least in one replication 100% true positives and true negatives according to the standard test (20/21), were detected |  |

| <b>Test performance study</b>   |  |
|---|--|
| <b>Test performance study?</b>  | Yes  |
| <b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b> | There were involved 28 laboratories from 15 countries: AGES, Austria; INTA-EEA, Argentina; PROINPA, Bolivia; Agronômica, Brazil; ANSES, France (2 Labs.); FN3PT/RD3PT-UMT INNOPLANT/ INRA Paris, France; BPI, Greece; Genlogs Ltd. Hungary; Ministry Agricult. and Rural Develop., Israel; University of Catania Italy; NPPO/NRC, The Netherlands; Ministry for Primary Industries, New Zealand; Plant Prot. Central Research Institute, Turkey; SASA, United Kingdom; IFAPA-Sevilla, Spain; INIA-Madrid, Spain; Sanidad Vegetal-Sevilla, Spain; Centro Regional de Diagnostico-Salamanca, Spain; Estación Fitopatológica -Areiro, Spain; Sanidad Vegetal-Huelva, Spain; Lab Regional-Logroño, Spain; IVIA / Bacteriología, Spain; IVIA / Virología e Inmunología, Spain; IVIA / Reference Laboratory MAGRAMA, Spain; USDA/ARS Prosser, WA, USA; USDA-ARS, USA; Experiment Station Rd, Bushland, Texas, USA; |
| <b>Other information</b>  |  |
| <b>Any other information considered useful<br/>e.g. robustness, ease of performing the test, etc.</b>                               | The diagnostic kit evaluated is simple to use, rapid and accurate. It showed a high robustness in 28 laboratories from 15 countries, and can be applied for rapid testing of plant material of at least the five plant species evaluated. For maximum accuracy a CTAB or other types of DNA extraction is advised.   |