

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	Bacteriology. Instituto Valenciano de Investigaciones Agrarias CV-315, km. 10.7, 46113 Moncada, Spain
Short description of the test	Detection of 'Candidatus Liberibacter solanacearum' by real time PCR in different types of plant material using Plant Print diagnostics kit
Date, reference of the validation report	2016-04-28 - Report 2016-04-28; Validation assay October 2012 - Performance study n°1
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	'Candidatus Liberibacter solanacearum' (LIBEPS)
Detection / identification	detection
Matrix(ces) tested	Leaves leaf extracts of Daucus carota, Solanum tuberosum, Apium graveolens, Nicotiana tabacum, Vinca pervinca
Plant species tested	Apium graveolens, Daucus carota, Nicotiana tabacum, Solanum tuberosum, Vinca sp.
Method(s)	Extraction Molecular real time PCR
Method: Extraction	
Reference of the test description	
Other information	
Other details on the test	Direct sample preparation without DNA purification (spot procedure)
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/143 ' <i>Candidatus</i> Liberibacter solanacearum' (version 1)

Name of the test	Real-time PCR based on 16S rRNA gene (Teresani et al., 2014)
Is the test modified compared to the reference test	yes Use of a kit
Kit	
Is a kit used	yes
Manufacturer name	PLANT PRINT
Specify the kit used	'Candidatus Liberibacter solanacearum' Complete real-time PCR kit for direct screening (Ref: CaLsol/100)
Kit used following the manufacturer's instructions?	
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	'Candidatus Liberibacter solanacearum'(LIBEPS)
Analytical sensitivity	
What is the 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.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	87% 232 samples agreement / 265 (including replications performed in some labs)
Standard test(s)	Standard test was real time PCR according to Teresani et al. after CTAB extract
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	99% 263 samples agreement / 265 (including replications performed in some labs.)
Specify the test(s)	Standard test was real time PCR according to Teresani et al. after CTAB extract
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	83% (465/530)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	95% 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
Test performance study	
Test performance study?	yes

Brief details of the test performance study and its output. It available, link to published article/report	<p>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 Fitopatologica -Areiro, Spain; Sanidad Vegetal-Huelva, Spain; Lab Regional-Logroño, Spain; IVIA / Bacteriologia, 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;</p>
Other information	
Any other information considered useful	<p>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.</p>

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