EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE 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.

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 diagnòstics 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 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. http://dx.doi.org/10.1094/PHYTO-07-13-0182-R	
Is the test the same as described in the EPPO DP?	No 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,		

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		
<u>Analytical sensitivity (= limit of detection)</u>		
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.	
<u>Diagnostic sensitivity</u>		
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)	
<u>Analytical specificity</u>		
Specificity value		
Number of strains/populations of target organisms tested		
Number of non-target organisms tested		
Cross reacts with (specify the species)		
<u>Diagnostic Specificity</u>		
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)	
<u>Reproducibility</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	83% (465/530)	
<u>Repeatability</u>		
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
Include brief details of the test performance study and its output.It available, provide a link to published article/report	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 Fitopatoloxica -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;
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	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 maxium accuracy a CTAB or other types of DNA extraction is adviced.