

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 carrot seeds 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 2016/05/02; Validation assay June 2015 - PNT-18/2015	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 E. Bertolini et al. Transmission of 'Candidatus Liberibacter solanacearum' in carrot seeds. Plant Pathology 2014. Doi:10.1111/ppa.12245	
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 Bertolini 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	
Matrices tested (if relevant)	Seeds	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Direct sample preparation without DNA purification (spot procedure) (Bertolini et al. 2014a, Teresani et al. 2014)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Real time PCR using Plant Print diagnostic kit, based on Bertolini 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		
Analytical sensitivity (= limit of detection)		
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.	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100% (Standard test was real time PCR according to Bertolini et al. after CTAB extraction)	
Specify the standard test	75 samples agreement / 75 (including replications performed in some labs)	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested		
Number of non-target organisms tested		
Cross reacts with (specify the species)		
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% (Standard test was real time PCR according to Bertolini et al. after CTAB extraction)	
Specify the standard test	75 samples agreement / 75 (including replications performed in some labs)	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (150/150)	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% The repeatability was calculated in 5 laboratories that performed 2 replications	
Test performance study		
Test performance study?	Yes	
Include brief details of the test	Ring test during accreditation process.	

<p>performance study and its output. It available, provide a link to published article/report</p>	<p>10 official Laboratories of Diagnostic of Spain tested this method: Laboratorio de Producción y Sanidad Vegetal, Huelva; Laboratorio de Producción y Sanidad Vegetal, Sevilla; Laboratorio de Sanidad Vegetal-ICIA, Tenerife; Centro Regional de Diagnóstico, Salamanca; Laboratorio de Diagnóstico Fitopatológico (Bacteriología), Valencia; Laboratorio de Bacteriología- IVIA, Valencia; Laboratorio Nacional de Referencia de Bacteriología (MAGRAMA), Valencia; Laboratorio Regional de la CC. AA. de La Rioja, Logroño; Laboratorio de Bacteriología-INIA, Madrid; Sanidad Vegetal-INIA, Madrid. The test performance study was organized by IVIA.</p>
<p>Other information</p>	
<p>Any other information considered useful e.g. robustness, ease of performing the test, etc.</p>	<p>The diagnostic kit evaluated is simple to use, rapid and accurate. It showed a high robustness in 10 laboratories, and can be applied for rapid testing of carrot seeds. For maximum accuracy a previous CTAB extraction or other types of DNA extraction is advised.</p>
<p>The following complementary files are available online:</p>	<ul style="list-style-type: none"> • Ejercicio colaborativo CaLsol