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.

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Target Organism	'Candidati	us Liberibacter solanacearum'
Short description	Detection of 'Candidatus Liberibacter solanacearum' by real time PCR in carrot seeds 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 2016/05/02; Validation assay June 2015 - PNT-18/2015	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 E. Bertolir solanacea Doi:10.11	ni et al. Transmision of 'Candidatus Liberibacter nrum' in carrot seeds. Plant Pathology 2014. 1/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 ca	arota
Matrices tested (if relevant)	Seeds	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	Х	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	Х	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 sample in some la	es agreement / 75 (including replications performed abs)		
Reproducibility				
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (15	0/150)		
<u>Repeatability</u>				
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% The performed	e repeatibility was calculated in 5 laboratories that d 2 replications		
Test performance study				
Test performance study?	Yes			
Include brief details of the test	Ring test	during acreditation process.		

performance study and its output.It available, provide a link to published article/report	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 Bacteriologia- IVIA, Valencia; Laboratorio Nacional de Referencia de Bacteriología (MAGRAMA), Valencia; Laboratorio Regional de la CC. AA. de La Rioja, Logroño; Laboratorio de Bacteriologia-INIA, Madrid; Sanidad Vegetal-INIA, Madrid. The test performance study was organized by IVIA.
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 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 adviced.
The following complementary files are available online:	Ejercicio colaborativo CaLsol