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	Analytical sensitivity of the detection of 'Candidatus Liberibacter solanacearum' by real time PCR in carrot seeds using Plant Print diagnòstics kit
Date, reference of the validation report	2016-05-06 - Validation assay December 2015 - PNT 14 Validacion Xcc y CaLSol_REV 3 / 2015-2
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
Method(s)	Extraction Molecular real time PCR
Method: Extraction	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Teresani, GR, Bertolini, E., Alfaro-Fernandez, A. 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 E. Bertolini et al. Transmision of 'Candidatus Liberibacter solanacearum' in carrot seeds. Plant Pathology 2014. Doi:10.111/ppa.12245
Other information	
Other details on the test	Direct sample preparation without DNA purification

	(spot procedure) according to Bertolini et al. 2014, Teresani et al. 2014
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 ‘Candidatus 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	
Other details on the test	Real time PCR using Plant Print diagnòstics kit, based on Bertolini et al. 2014, Teresani et al 2014
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**'Candidatus Liberibacter solanacearum'(LIBEPS)**
<u>Analytical sensitivity</u>	
What is smallest amount of target that can be detected reliably?	Detection of 100% (12/12) dilution 1/1000 (-3) of a spiked sample extract of carrot seeds spiked with an approximate concentration of 1,7 nanograms/microlitre of purified DNA. (Approx. 10-100 cells by direct sample preparation according calculation of Bertolini et al. 2014.) Detection of 75% (9/12) dilution 1/10000 (-4) of the same spiked samples by the standard test (standard test was the same real-time PCR performed with a previous extraction of DNA by CTAB protocol) (Approx. 3-100 cells by direct sample preparation according calculation of Bertolini et al. 2014.)
<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	60% for the spot test 76% for the standard test (standard test was the same real-time PCR performed with a previous extraction of DNA by CTAB protocol)

Standard test(s)	-60 spiked samples of -1, -2, -3, -4 and -5 dilutions of a spiked sample extract of seeds spiked with purified DNA from naturally contaminated seeds -36 samples positive /60 using the spot method -46 samples positive /60 using the standard test (performed with a previous extraction of DNA by CTAB protocol)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% The reproducibility was calculated performing three series of dilutions of spiked samples (-1 to -5 dilution) analyzed in different days.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% The repeatability was calculated performing the analysis of three series of spiked samples (-1 to -5 dilution) and three replicates/serie.
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	The method of analysis of carrot seeds with the diagnostic kit evaluated is simple to use, rapid and accurate. It can be applied for rapid testing of large number of samples 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 control interno CaLsol y Xcc 2015_2

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