

**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.

<b>Laboratory contact details</b>	Bacteriology. Instituto Valenciano de Investigaciones Agrarias CV-315, km. 10.7, 46113 Moncada, Spain
<b>Short description of the test</b>	Detection of 'Candidatus Liberibacter solanacearum' by real time PCR in carrot seeds using Plant Print diagnostics kit
<b>Date, reference of the validation report</b>	2016-05-02 - Report 2016/05/02; Validation assay June 2015 - PNT-18/2015
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	'Candidatus Liberibacter solanacearum'(LIBEPS)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Molecular real time PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Bertolini et al. 2014a, Teresani et al. 2014
<b>Other information</b>	
<b>Other details on the test</b>	Direct sample preparation without DNA purification (spot procedure) ( Bertolini et al. 2014a, Teresani et al. 2014)
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes

<b>EPPO Diagnostic Protocol name</b>	PM 7/143 ' <i>Candidatus Liberibacter solanacearum</i> ' (version 1)
<b>Name of the test</b>	Real-time PCR based on 16S rRNA gene (Teresani et al., 2014)
<b>Is the test modified compared to the reference test</b>	yes Use of a kit
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	PLANT PRINT
<b>Specify the kit used</b>	'Candidatus Liberibacter solanacearum' Complete real-time PCR kit for direct screening (Ref: CaLsol/100)
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	Real time PCR using Plant Print diagnostic kit, based on Bertolini et al. 2014
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>'Candidatus Liberibacter solanacearum'(LIBEPS)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Not calculated for a non-culturable bacterium. The performance study was oriented to receive qualitative results.
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	100% (Standard test was real time PCR according to Bertolini et al. after CTAB extraction)
<b>Standard test(s)</b>	75 samples agreement / 75 (including replications performed in some labs)
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100% (Standard test was real time PCR according to Bertolini et al. after CTAB extraction)
<b>Specify the test(s)</b>	75 samples agreement / 75 (including replications performed in some labs)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% (150/150)
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% The repeatability was calculated in 5 laboratories that performed 2 replications

<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Ring test during accreditation process. 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.
<b>Other information</b>	
<b>Any other information considered useful</b>	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.
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Ejercicio colaborativo CaLsol</a></li> </ul>

Creation date: 2016-05-02 00:00:00 - Last update: 2020-10-27 17:41:30