**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** | 'Candidatus Liberibacter solanacearum' | |
| **Short description** | Analytical sensitivity of the 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 2016-05-06; Validation assay December 2015 - PNT 14 Validacion Xcc y CaLSol_REV 3 / 2015-2 | |
| **Validation process according to EPPO Standard PM 7/98:** | Yes | |
| **Reference of the test description** | 0<br>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 | |
| **Is the test the same as described in the EPPO DP?** | No<br>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 | |
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| ***List of methods used*** | | |
| **Method for extraction / isolation / baiting of target organism from matrix** | X | Direct sample preparation without DNA purification (spot procedure)<br>according to Bertolini et al. 2014, Teresani et al. 2014 |
| **Molecular methods, e.g. hybridization, PCR and real time PCR** | X | Real time PCR using Plant Print diagnòstics kit, based on Bertolini et al. 2014, Teresani et al 2014 |
| **Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay** | | |
| **Plating methods: selective isolation** | | |

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| **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?** | 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.)<br><br>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)<br>(Approx. 3-100 cells by direct sample preparation according calculation of Bertolini et al. 2014.) | |
| **Diagnostic sensitivity** | | |
| **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<br>76% for the standard test (standard test was the same real-time PCR performed with a previous extraction of DNA by CTAB protocol) | |
| **Specify the standard test** | -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<br>-36 samples positive /60 using the spot method<br>-46 samples positive /60 using the standard test (performed with a previous extraction of DNA by CTAB protocol) | |
| **Analytical specificity** | | |
| **Specificity value** | Not evaluated because there were not negative samples included | |
| **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** | | |

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| **standard test** | |
| **Specify the standard test** | |
| **Reproducibility** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 100%<br>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%<br>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 |
| **Include brief details of the test performance study and its output.It available, provide a link to published article/report** | |
| **Other information** | |
| **Any other information considered useful**<br>**e.g. robustness, ease of performing the test, etc.** | 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.<br>For maximum accuracy a previous CTAB extraction or other types of DNA extraction is adviced. |
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| The following complementary files are available online: | • Ejercicio control interno CaLsol y Xcc 2015_2 |