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’ using real-time PCR test	
Laboratory contact details	Finnish Food Authority / Plant Pest Section Mustialankatu 3, 00790 Helsinki, Finland	
Date and reference of the validation report	2018-04-03 - Validation_report_Evira7629, ID DOC-1280-6573-fi	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0	
Is the test the same as described in the EPPO DP?	Modified The primers and probes adopted from Li et al. (2009) Journal of Microbiological Methods 78:59–65 (universal HLBr primer and HLBp probe, CLso specific LsoF primer) or Teresani et al. (2014) Phytopathology 104: 804–811 (universal CaLsppF and CaLsppR primers, CLso specific CaLsolP probe) were used in conjunction with in-house developed DNA extraction method and modified real-time PCR (qPCR). Deviations from the references: - Sample preparation - DNA extraction - PCR reagents and reaction volume - Primer and probe concentrations - BHQ1-quencher is used instead of TAMRA for CaLsolP probe - PCR program (Teresani et al. 2014 is also used for Li et al. 2009 primers)	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Solanum tuberosum	
Matrices tested (if relevant)	Tuber	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Potato tuber sample can be taken from a single tuber, or from the heel end core sample for ring rots (LAB4001) directly or after the ring rots have been extracted. Heel end cores are homogenized in Bioreba extraction bag without the buffer, 25 ml PBST is added and the bacteria are extracted by rubbing the buffer into the tuber pulp inside the bag. The bacteria are concentrated from the extract by 2-step centrifugation. In the first step (clarification) the coarse plant material is removed from the sample by 100×g 5 min centrifugation at

		10 °C, the bacteria are then pelleted from the supernatant (~8.5 ml) by 7000×g 5 min centrifugation at 10 °C. Bacterial pellet is suspended in 500 µl PBST buffer, 200 µl of the suspension is used for DNA extraction and the rest can be frozen in case the analysis needs to be repeated. A single tuber sample is taken from the heel end or bud site, homogenized with Homex 6 and extracted 1:2 to PBST buffer. It is important to get as much vascular tissue as possible into the sample. Symptomatic sample does not need concentration. Carrot petioles and potato stolons are homogenized in in Bioreba extraction bag with Homex 6 and extracted 1:2 in PBST. 1 cm pieces from the petioles are cut circa 3 cm above the root. 200 µl of the unconcentrated extract is used for DNA extraction. Carrot seeds are washed before grinding to remove the seed dressing, in addition the washing softens the seeds and thus aids the grinding. One gram of seeds is weighed into 50 ml centrifuge tube and 30–40 ml 0.5 % Triton X-100 is added. Larger volume reduces the flow of liquid in the mixing and results in poor washing result. The tube is shaken 30 minutes on a platform rocker. The liquid is carefully poured out, the tube is filled with UP-water, shaken vigorously and the water is carefully decanted. The rinsing is performed three times total, on the third time the seeds are poured with the water into Bioreba extraction bag and the water is subsequently decanted from the bag. The seeds are ground to fine powder with Homex 6, 10 ml PBST is added and the bacteria are extracted by rubbing the buffer into the seed powder inside the bag. 200 µl of the extract is used for DNA extraction. Color from seed dressing may remain in the sample, it does not interfere with the extraction. DNA extraction with KingFisher Flex and QuickPick™ Plant DNA-kit (BioNobile) 1. Add 300 µl Lysis Buffer and 20 µl proteinase K to 200 µl sample 2. Incubate 30 minutes in thermal shaker 700 rpm at 65°C 3. Pipet KingFisher Deepwell plates (Cat. 95040450) during incubation Sample: 500 µl Binding Buffer + 20 µl Magnetic Particles Wash 1: 900 µl Wash Buffer Wash 2_1: 800 µl Wash Buffer Wash 2_2: 800 µl Wash Buffer Elution: 50 µl Elution Buffer 4. Centrifuge the samples 5 minutes 18000×g at room temperature. Pipet 450 µl of the supernatant carefully to the Sample plate wells avoiding the sediment. 5. Start KingFisher Flex and run PURE_DNAPlant_Flex96 program

		6. Pipet the DNA from Elution plate to eppendorf tube and use for real-time PCR analysis. The DNA can be kept short time (1–2 weeks) in the fridge or longer times in the freezer for further use.
Molecular methods, e.g. hybridization, PCR and real time PCR	X	PCR reaction for one well Reagent Conc. Volume (µl) Final conc. Nuclease free water 4.0 Maxima Probe qPCR Master Mix 2× 12.5 1× CaLsppF OR LsoF OR COX-F 5 µM 1.5 0.3 µM CaLsppR OR HLBr OR COX-RW 5 µM 1.5 0.3 µM CaLsolP OR HLBp OR COX-P 5 µM 0.5 0.1 µM Total 20.0 DNA 5.0 Total 25.0 PCR An initial step at 95ºC for 10 min followed by 45 cycles (95ºC for 15 s, and 60ºC for 1 min; Teresani 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		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	0.01% contamination rate or 10 target copies per PCR reaction	
<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	N.A.	
Specify the standard test	N.A.	
<u>Analytical specificity</u>		
Specificity value	100%	
Number of strains/populations of target organisms tested	4: CLso-positive Daucus carota subsp. sativus midrib (83-2013, haplotype C, Minna Haapalainen, University of	

	Helsinki) and seeds [cv. Maestro (11-2016, haplotype D, Mikko Lehtonen, Evira)], Solanum tuberosum tuber frozen (2-2011, haplotype B, Anne Nissinen, LUKE) and fresh material [cv. Atlantic (1–7-2017, haplotype B, Rodney Cooper, USDA)]
Number of non-target organisms tested	4: Ralstonia solanacearum NCPPB 4156, Clavibacter michiganensis var. sepedonicus NCPPB 4053, and Dickeya solani S0432-1 and Pectobacterium carotovorum SCC1 (Minna Pirhonen, UH).
Cross reacts with (specify the species)	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	N.A.
Specify the standard test	N.A.
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for HLBp, 66% for CaLsolP
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
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 **e.g. robustness, ease of performing the test, etc.**	
The following complementary files are available online:	• SOP english • Validation report