

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**  
**ORGANISATION EUROPEENNE ET MEDITERRANEEENNE 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.

<b>Target Organism</b>	Ralstonia solanacearum	
<b>Short description</b>	Detection of Ralstonia solanacearum by egl LAMP in plant material	
<b>Laboratory contact details</b>	National Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia	
<b>Date and reference of the validation report</b>	2017-02-09 - Dreo, T., 2017. Summary of validation data on egl LAMP for Ralstonia solanacearum (No. D0004/17), Report on Suitability Testing. National Institute of Biology, Ljubljana.	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	N/R	
<b>Is the test the same as described in the EPPO DP?</b>		
<b>Is the lab accredited for this test?</b>	No	
<b>Plant species tested (if relevant)</b>	Solanum tuberosum, Solanum lycopersicon	
<b>Matrices tested (if relevant)</b>	latent (tuber cores) and symptomatic potato tubers (tuber vascular tissue), symptomatic tomato plants (stems)	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>		
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	X	LAMP egl
<b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>		
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b>		
<b>Pathogenicity test</b>		
<b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA</b>		

<b>profiling</b>		
<b>Morphological and morphometrical methods intended for identification</b>		
<b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>		
<b>Other</b>		
<b><u>Analytical sensitivity (= limit of detection)</u></b>		
<b>What is smallest amount of target that can be detected reliably?</b>	10E4 cells/mL (25 cells per LAMP reaction) when tested on strains belonging to phylotypes I and III, and a sensitivity limit of 10E5-10E6 cells/mL for strains from phylotypes IIA, IIB and IV; 10E5 cells/mL in potato tubers (cores of 200) as tested on three standard curves.	
<b><u>Diagnostic sensitivity</u></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 %	
<b>Specify the standard test</b>	Potato cores. The official testing scheme (EC 98/57) including screening tests, isolation on media, identification tests and pathogenicity testing with re-isolation and identification for positive samples.	
<b><u>Analytical specificity</u></b>		
<b>Specificity value</b>	99 % (1 false negative, no false positives)	
<b>Number of strains/populations of target organisms tested</b>	88 strains of RSSC	
<b>Number of non-target organisms tested</b>	26	
<b>Cross reacts with (specify the species)</b>	no cross-reactions observed	
<b><u>Diagnostic Specificity</u></b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100 %	
<b>Specify the standard test</b>	Immunofluorescence and real-time PCR (Weller et al., 2000) for negative samples.	
<b><u>Reproducibility</u></b>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100 % detection for samples with at least 10E4 copies of Rs DNA or more.	
<b><u>Repeatability</u></b>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100 % using different machines (SmartCycler, Roche Light Cycler, Genie II).	
<b><u>Test performance study</u></b>		
<b>Test performance study?</b>	No	

<p><b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b></p>	
<p><b><u>Other information</u></b></p>	
<p><b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b></p>	<p>The test is proposed as identification test for pure cultures. While the validation data indicates that it may well detect concentrations of <i>R. solanacearum</i> usually seen in latently infected samples there is not sufficient data on the R.s. concentrations encountered in routine testing.</p>
<p>The following complementary files are available online:</p>	<ul style="list-style-type: none"> <li>• <a href="#">Summary of Rs LAMP validation data</a></li> </ul>