

**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.

<b>Target Organism</b>	Erwinia amylovora	
<b>Short description</b>	Detection of Erwinia amylovora in plant extract by Real-time PCR, targeting chromosomal DNA	
<b>Laboratory contact details</b>	Federal State Organization "All-Russian Plant Quarantine Center" Pogranichnaya str.32, Ramensky region, Moscow obl., 140150 Bykovo, Russian Federation	
<b>Date and reference of the validation report</b>	2014 - none	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	PM 7/020(1) Gottsberger RA (2010) Development and evaluation of a real-time PCR assay targeting chromosomal DNA of Erwinia amylovora. Letters in Applied Microbiology 51, 285–292.	
<b>Is the test the same as described in the EPPO DP?</b>	Yes	
<b>Is the lab accredited for this test?</b>	Yes	
<b>Plant species tested (if relevant)</b>	Several plant species from the Rosaceae family, see full report (in Russian)	
<b>Matrices tested (if relevant)</b>	Shoots and leaves	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>	X	Commercial DNA extraction kit - "Proba-GS" produced by OOO "AgroDiagnostika"
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	X	Real-time PCR (Gottsberger, 2010)
<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</b>		

<b>profiling, fatty acid profiling &amp; DNA 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>Analytical sensitivity (= limit of detection)</b>		
<b>What is smallest amount of target that can be detected reliably?</b>	24 cfu/μl // 1,2*10 <sup>3</sup> /ml	
<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>		
<b>Specify the standard test</b>	FLASH-PCR for Erwinia amylovora	
<b>Analytical specificity</b>		
<b>Specificity value</b>		
<b>Number of strains/populations of target organisms tested</b>	126 strains: 1 strain of Erwinia amylovora CFBP 1430 + 99 strains isolated in different regions of Russian Federation + 26 strains isolated in Kazakhstan, Kyrgyzstan, Poland and Moldova	
<b>Number of non-target organisms tested</b>	92 strains including other Erwinia species	
<b>Cross reacts with (specify the species)</b>	No cross reaction observed	
<b>Diagnostic Specificity</b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>		
<b>Specify the standard test</b>		
<b>Reproducibility</b>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	for samples with level of infection 10 <sup>3</sup> and 10 <sup>4</sup> - 100 %, when tested with different operators	
<b>Repeatability</b>		
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	for samples with level of infection 10 <sup>3</sup> and 10 <sup>4</sup> - 100 %	
<b>Test performance study</b>		
<b>Test performance study?</b>	No	
<b>Include brief details of the test performance study and its output.It available, provide a link to</b>		

<b>published article/report</b>	
<b><u>Other information</u></b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	Primers, probe, MasterMix and Internal control were made by Russian companies.  You can find attached full validation report in Russian
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">2014. Validation of Real-time PCR (according to Gottsberger, 2010) for detection of E.a. in plant extract</a></li> </ul>