

**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 Erwinia amylovora from plant material by Real time PCR
<b>Date, reference of the validation report</b>	2012-03-01 - Not specified
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Erwinia amylovora (ERWIAM)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</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 7/020 Erwinia amylovora (version 2)
<b>Other information</b>	
<b>Other details on the test</b>	Ilop et al. 1999
<b>Method: Molecular Extraction DNA RNA (2)</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 7/020 Erwinia amylovora (version 2)
<b>Other information</b>	
<b>Other details on the test</b>	Taylor et al. 2001

<b>Method: Molecular Extraction DNA RNA (3)</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 7/020 Erwinia amylovora (version 2)
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	SIGMA-ALDRICH
<b>Specify the kit used</b>	RED-Extract N-Amp T Plant kit
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<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 7/020 Erwinia amylovora (version 2)
<b>Name of the test</b>	Real-time PCR (Pirc et al. 2009)
<b>Other information</b>	
<b>Other details on the test</b>	Pirc et al. (2009). Improved fire blight diagnostics using quantitative real-time PCR detection of Erwinia amylovora chromosomal DNA. Plant Pathology 58, 872-881.
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>yes</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Erwinia amylovora(ERWIAM)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	10 <sup>3</sup> -10 <sup>4</sup> CFU/mL plant extract after DNA extraction following Llop et al (1999), Taylor et al (2001) and RED-extract-N-Amp T kit.
<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>	Proportion of true positives/total number of samples: 0.67; 0.58 and 0.71 after DNA extraction following Llop et al (1999), Taylor et al (2001) and RED-extract-N-Amp T kit, respectively (in samples from 1 to 10 <sup>6</sup> CFU/mL and healthy samples in ring test 2010).
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	235 strains: all positive
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	

<b>Number of non-target organisms tested</b>	37 strains: all negative
<b>Specificity value</b>	100% Cross reactions: None
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Proportion of true negatives/total number of samples: 0.93; 0.93 and 0.98 after DNA extraction following Llop et al (1999), Taylor et al (2001) and RED-extract-N-Amp T kit, respectively (in samples from 1 to 10 <sup>6</sup> CFU/mL and healthy samples in ring test 2010).
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	94% in IVIA assays 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>	98% in IVIA assays
<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>	Yes (14 laboratories from Europe, Morocco, USA and New Zealand) analysed 12 samples each (from 1 to 10 <sup>6</sup> CFU/mL plant extract and healthy samples). Details about ring test protocol available.

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