

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.

Laboratory contact details	Bacteriology. Instituto Valenciano de Investigaciones Agrarias CV-315, km. 10.7, 46113 Moncada, Spain
Short description of the test	Detection of <i>Erwinia amylovora</i> from plant material by Real time PCR
Date, reference of the validation report	2012-03-01 - Not specified
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	<i>Erwinia amylovora</i> (ERWIAM)
Detection / identification	detection
Matrix(ces) tested	Leaves, Shoots Shoots, leaves
Plant species tested	Rosaceae
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/020 <i>Erwinia amylovora</i> (version 2)
Other information	
Other details on the test	Ilop et al. 1999
Method: Molecular Extraction DNA RNA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/020 <i>Erwinia amylovora</i> (version 2)

Other information	
Other details on the test	Taylor et al. 2001
Method: Molecular Extraction DNA RNA (3)	
Reference of the test description	
As or adapted from an EPP0 diagnostic protocol	yes
EPP0 Diagnostic Protocol name	PM 7/020 Erwinia amylovora (version 2)
Kit	
Is a kit used	yes
Manufacturer name	SIGMA-ALDRICH
Specify the kit used	RED-Extract N-Amp T Plant kit
Kit used following the manufacturer's instructions?	
Other information	
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPP0 diagnostic protocol	yes
EPP0 Diagnostic Protocol name	PM 7/020 Erwinia amylovora (version 2)
Name of the test	Real-time PCR (Pirc et al. 2009)
Other information	
Other details on the test	Pirc et al. (2009). Improved fire blight diagnostics using quantitative real-time PCR detection of Erwinia amylovora chromosomal DNA. Plant Pathology 58, 872-881.
Are the performance characteristics included in the EPP0 diagnostic protocol?	yes
Performance Criteria :	
Organism 1.:	Erwinia amylovora(ERWIAM)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	10 ³ -10 ⁴ CFU/mL plant extract after DNA extraction following Llop et al (1999), Taylor et al (2001) and RED-extract-N-Amp T kit.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	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 ⁶ CFU/mL and healthy samples in ring test 2010).
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	235 strains: all positive

Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	37 strains: all negative
Specificity value	100% Cross reactions: None
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	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 ⁶ CFU/mL and healthy samples in ring test 2010).
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	94% in IVIA assays when tested with different operators:
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	98% in IVIA assays
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output. It available, link to published article/report	Yes (14 laboratories from Europe, Morocco, USA and New Zealand) analysed 12 samples each (from 1 to 10 ⁶ CFU/mL plant extract and healthy samples). Details about ring test protocol available.

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