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 Erwinia amylovora 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)	Erwinia amylovora (ERWIAM)
Detection / identification	detection
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 Erwinia amylovora (version 2)
Other information	
Other details on the test	llop 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 Erwinia amylovora (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 EPPO diagnostic protocol	yes
EPPO 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 EPPO diagnostic protocol	yes
EPPO 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 EPPO diagnostic protocol?	**yes**
Performance Criteria :	
Organism 1.:	**Erwinia amylovora(ERWIAM)**
Analytical sensitivity	
What is 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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