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	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
Short description of the test	Detection of Acidovorax citrulli by PCR in seeds
Date, reference of the validation report	2015-11-16 - 1v1.2
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?	no
Description of the test	
Organism(s)	Acidovorax citrulli (PSDMAC)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA 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 7/127 Acidovorax citrulli (version 1)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	LGC
Specify the kit used	sbeadex maxi plant
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	DNA extraction using Kingfisher and Sbeadex maxi kit for Acidovorax citrulli (LGC Genomics)
Method: Molecular real time PCR	

Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/127 Acidovorax citrulli (version 1)
Name of the test	Real-time PCR targeting the IS1002 element (Woudt et al., 2009a,b) and Contig 22
Is the test modified compared to the reference test	no
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**yes**
Performance Criteria :	
Organism 1.:	**Acidovorax citrulli(PSDMAC)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	The limit of detection at 0.95 probability is 9 cells/mL seed extract.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Not determined
Standard test(s)	No standard test available
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	168 strains
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	54 non-targets
Specificity value	Two primers sets tested - IS1002 cross-reacts with 2 of the 9 Acidovorax cattleyae isolates tested and several unknown bacteria - Contig22 cross-reacts only with 1 unknown bacteria, characterized in AFLP-study, outside of the Acit tree. Cross-reacts with both primer sets
Cross reacts with	Acidovorax cattleyae
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Diagnostic specificity: 98%
Specify the test(s)	AFLP-study
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Intralaboratory testing

Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Inter- and intralaboratory testing
Test performance study	
Test performance study?	no
The following complementary files are available online:	• Poster Koenraadt et al 2014 ISTA Seed Health Symposium • Specificity of Contig21 Taqman • Validation report Acidovorax citrulli v1.2

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