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.

Target Organism	Acidovorax citrulli	
Short description	Detection of Acidovorax citrulli by PCR in seeds	
Laboratory contact details	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands	
Date and reference of the validation report	2015-11-16 - v1.2	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	N/R The protocol will be included in the EPPO diagnostic protocol that is currently being drafted.	
Is the test the same as described in the EPPO DP?		
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Citrullus lanatus, Cucumis melo and other Cucurbitaceae	
Matrices tested (if relevant)	seeds and isolates	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	DNA extraction using Kingfisher and Sbeadex maxi kit for Acidovorax citrulli (LGC Genomics) followed by Taqman PCR
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		

Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
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	
Specify the standard test	No standard test available	
Analytical specificity		
Specificity value	Diagnostic sensitivity: 100% Diagnostic specificity: 98%	
Number of strains/populations of target organisms tested	168 strains	
Number of non-target organisms tested	54 non-targets	
Cross reacts with (specify the species)	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	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	98%	
Specify the standard test	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	
Include brief details of the test performance study and its output.It available, provide a link to published article/report		

Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	
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