

**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.

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

<b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b>		
<b>Pathogenicity test</b>		
<b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA profiling</b>		
<b>Morphological and morphometrical methods intended for identification</b>		
<b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>		
<b>Other</b>		
<b><u>Analytical sensitivity (= limit of detection)</u></b>		
<b>What is smallest amount of target that can be detected reliably?</b>	The limit of detection at 0.95 probability is 9 cells/mL seed extract.	
<b><u>Diagnostic sensitivity</u></b>		
<b>Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98</b>	Not determined	
<b>Specify the standard test</b>	No standard test available	
<b><u>Analytical specificity</u></b>		
<b>Specificity value</b>	Diagnostic sensitivity: 100% Diagnostic specificity: 98%	
<b>Number of strains/populations of target organisms tested</b>	168 strains	
<b>Number of non-target organisms tested</b>	54 non-targets	
<b>Cross reacts with (specify the species)</b>	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	
<b><u>Diagnostic Specificity</u></b>		
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	98%	
<b>Specify the standard test</b>	AFLP-study	

<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% Intralaboratory testing
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% Inter- and intralaboratory testing
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	No
<b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b>	
<b><u>Other information</u></b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	
<b>The following complementary files are available online:</b>	<ul style="list-style-type: none"> <li>• <a href="#">Poster Koenraadt et al 2014 ISTA Seed Health Symposium</a></li> <li>• <a href="#">Specificity of Contig21 Taqman</a></li> <li>• <a href="#">Validation report Acidovorax citrulli v1.2</a></li> </ul>