

**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.

<b>Laboratory contact details</b>	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
<b>Short description of the test</b>	Detection of Acidovorax citrulli by PCR in seeds
<b>Date, reference of the validation report</b>	2015-11-16 - 1v1.2
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	Acidovorax citrulli(PSDMAC)
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/127 <i>Acidovorax citrulli</i> (version 1)
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	LGC
<b>Specify the kit used</b>	sbeadex maxi plant
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	DNA extraction using Kingfisher and Sbeadex maxi kit for Acidovorax citrulli (LGC Genomics)
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	

<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/127 <i>Acidovorax citrulli</i> (version 1)
<b>Name of the test</b>	Real-time PCR targeting the IS1002 element (Woudt et al., 2009a,b) and Contig 22
<b>Is the test modified compared to the reference test</b>	no
<b>Other information</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	yes
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Acidovorax citrulli(PSDMAC)</b>
<b>Analytical sensitivity</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>Diagnostic sensitivity</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>Standard test(s)</b>	No standard test available
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	168 strains
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	54 non-targets
<b>Specificity value</b>	Two primers sets tested - IS1002 cross-reacts with 2 of the 9 <i>Acidovorax cattleyae</i> 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>Cross reacts with</b>	<i>Acidovorax cattleyae</i>
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Diagnostic specificity: 98%
<b>Specify the test(s)</b>	AFLP-study
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% Intralaboratory testing
<b>Repeatability</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>Test performance study</b>	
<b>Test performance study?</b>	no
The following complementary files are available online:	
	<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>

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