

**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>	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
<b>Short description of the test</b>	Detection of <i>Clavibacter michiganensis</i> <i>Clavibacter michiganensis</i> by Molecular Conventional PCR in Tomato Seeds
<b>Date, reference of the validation report</b>	2025-09-29 - Detection of <i>Clavibacter michiganensis</i> by molecular assay from tomato seeds
<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>	Other_project
<b>If yes, please specify</b>	ASPROPI (MIPAAF funded project)
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Clavibacter michiganensis</i> (CORBMI)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Conventional PCR
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/042 <i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> (version 3)
<b>Name of the test</b>	Conventional PCR test (adapted from Pastrik & Rainey, 1999)
<b>Is the test modified compared to the reference test</b>	yes The test was adapted for detection of CORBMI from tomato seeds
<b>Kit</b>	
<b>Is a kit used</b>	no

<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	Evaluation performed using the Taq DNA polymerase "Hot start" Immolase TM
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Clavibacter michiganensis(CORBMI)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	The limit of detection (LOD) was evaluated for seed extract on samples of 2000 seeds. The LOD was 1 C. michiganensis-infected seed on 2000. These data were generated by testing three series of five samples, each consisting of 2.000 seeds with varying levels of infection (1, 2, 5, 10, or 0 infected seeds per series).
<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>	81%
<b>Standard test(s)</b>	Comparison with samples of known status
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	12 different C. michiganensis strains covering different Italian geographic areas
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	11 strains of which: 3 strains of other genera (Pseudomonas corrugata, P. mediterranea, Ralstonia solanacearum), 4 C. michiganensis look-alikes isolates, 3 Clavibacter strains from different host plants (C. tessellarius, C. nebraskensis and C. sepedonicus).
<b>Specificity value</b>	100%
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	86.1%
<b>Specify the test(s)</b>	Comparison with samples of known status
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	70.5%- Evaluated following Langton et al., 2002 (Langton, S.D.; Chevennement, R.; Nagelkerke, N.; Lombard, B. Analysis collaborative trials for qualitative microbiological methods. Int. J. Food Microbiol. 2002, 79, 175-181)
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	79.8% - Evaluated following Langton et al., 2002 (Langton, S.D.; Chevennement, R.; Nagelkerke, N.; Lombard, B. Analysis collaborative trials for

	qualitative microbiological methods. Int. J. Food Microbiol. 2002, 79, 175–181)
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Performance criteria was obtained by involving nine Italian laboratories in a national test performance study. Each laboratory tested 11 tomato seed samples, each consisting of 5,000 seeds. The infected samples included: 2 samples with 1 infected seed, 2 samples with 3 infected seeds, and 2 samples with 5 infected seeds. Non-target samples were also evaluated, specifically: 2 samples of 5,000 non-infected seeds and 2 samples of 5,000 seeds contaminated with 5 seeds of <i>Pseudomonas corrugata</i> .
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">TPS CMM Pastrick and Raney 1999</a></li> </ul>

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