Information pest: *Clavibacter michiganensis* subsp. *michiganensis*

Clavibacter michiganensis subsp. *michiganensis* (*Cmm)* is the causative agent of bacterial wilt and canker of tomato. The main host of economic importance is tomato plant but natural infection has also been reported on *Capsicum annuum,* aubergine (*Solanum dulcamara*) and several *Solanum* weeds (e.g. *Solanum douglasii*, *S. nigrum* and *S. triflorum*)

The pathogen is frequently seed transmitted, both internally in seed and on the seed surface. The pathogen can be spread long distances because of its association with seeds. Warm temperature in the range of 23-28°C and high relative humidity (>80%) are optimal environments.

Cmm causes systemic infection of tomato plants. The pathogen can also cause spots on leaves, petioles, peduncles and fruits as a result of a local infection. There is a wide range of symptoms which vary depending on the place of production, the age of the plant at the time of infection, cultural practices, cultivar, etc.

Cmm is a regulated plant pathogen included in European legislation as a quarantine pest.

Introduction

The PCR *Cmm* set has been developed and optimized by Qualiplante according to Pastrik & Rainey, 1999 that is based on the amplification of the PSA-8/PSA-R primers. A verification was performed by Qualiplante (data not published) and the performance characteristics of the set are the same as the original publication.

It was possible to detect the pathogen in DNA extracts from 50 seeds containing 1x10^3 bacteria.

The PCR test from Pastrik & Rainey, 1999 is recommended by the European and Mediterranean Plant Protection Organization (www.eppo.int). in the Appendix 5 of the PM7/42 (3) (2016) Bulletin 46, 202–225.

This product should be used only for research purposes.

Intended use

The End-Point PCR set is validated for the detection of *Clavibacter michiganensis* subsp. *michiganensis* in End-Point PCR.

Suitable samples are seeds and bacterial isolates from infected plants and seeds.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7Cmm--P2	PCR *Clavibacter michiganensis* subsp. *michiganensis (Cmm)* set 24
7Cmm--P9	PCR *Clavibacter michiganensis* subsp. *michiganensis (Cmm)* set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7Cmm--P2-DM-	2x48 tests 7Cmm--P9-DM-
Positive Control	3 tests 7Cmm--P2-PC-	8 tests 7Cmm--P9-PC-
Negative Control	3 tests 7Cmm--P2-NC-	8 tests 7Cmm--P9-NC-

Storage conditions

This set can be shipped at room temperature but upon receipt it should be stored immediately at the recommended storage temperature: **from -30 ° C to -10 ° C**.
Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the set is correctly stored, at constant-temperature freezer, its performance is guaranteed until the expiration date indicated on the tubes label.

Materials and equipment (not provided)

- DNA extraction tools and reagents
- Nuclease-free filter tips and micropipettes
- Optical grade nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- DNA ladder and loading-dye buffer
- PCR thermal cycler
- Agarose gel reagents and apparatus

Nucleic acids extraction

Extract DNA from samples according to your usual protocol. Upon request, Qualiplante can recommend you an extraction method.

Reaction set-up

a) Slowly thaw Direct Master Mix by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and spin down the liquid.
c) Add 18 µl of Direct Master Mix (without DNA template) to each PCR tubes or wells of an optical-grade PCR plate.
d) Add 2 µl of DNA template to the Direct Master Mix. Do not forget to prepare a PCR tube or well of a

Qualiplante SAS
Cap Alpha – Avenue de l'Europe
FR-34830 Clapiers

www.qualiplante.eu
info@qualiplante.eu

PCR plate for the Positive Control and the Negative Control.

Components	Volume/PCR tube or well
DNA template or Positive control or Negative control	2 µl
Direct Master Mix	18 µl
Total Volume / PCR tube or well	20 µl

In order to confirm the absence of any reagent's contamination, we strongly recommend including a no-template control (e.g. DEPC water) in the assay.

Run and thermal cycling

a) Seal carefully the PCR tubes or PCR plate. Centrifuge briefly to collect components at the bottom of the PCR tubes or wells of the plate. Protect from light before thermocycling.
b) Load the PCR tubes or plate into the PCR thermal cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
Initial denaturation	94°C	2 min	1
Denaturation	94°C	30 sec	35
Annealing	63°C	20 sec	
Elongation	72°C	45 sec	
Storage	4°C	∞	-

Agarose gel electrophoresis

Prepare an agarose gel **at 1,8% w/v in 0,5X-TBE buffer.**

Gel loading:
- load the DNA ladder (for example 100-1.000 bp DNA step ladder).
- load 10 µl of PCR products from the previous step adding the loading dye buffer (*not provided in the set)*.

Run: run the gel electrophoresis for 30-40 minutes at 80V.

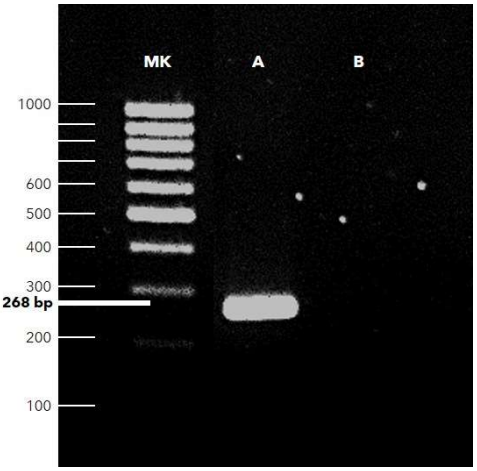
Results analysis

ANALYSIS VALIDATION

Clavibacter michiganensis subsp. *michiganensis* is detected when a 268 bp DNA fragment is observed.

The analysis is validated when:
- ✓ 1 DNA fragment of 268 bp is visible in the positive control lane.
- ✓ No DNA fragment is visible in the negative control lane.

The picture below represents a 0,5X-TBE 1,8% agarose gel showing the DNA amplification in a sample infected by *Cmm*.



MK: DNA ladder - **A**: *Cmm* positive sample or Positive Control of the set - **B**: healthy sample or Negative Control of the set

RESULTS INTERPRETATION

The specific product of *Clavibacter michiganensis* subsp. *michiganensis* is a 268 bp fragment.

- ✓ A sample is **positive** when a 268 bp specific DNA fragment is present in the PCR reaction.
- ✓ A sample is negative when no fragment is present in the PCR reaction.

The table below summarizes the results interpretation:

Fragment size **268 bp**	Interpretation
-	NEGATIVE
✓	**POSITIVE** *Clavibacter michiganensis* subsp. *michiganensis*

POSITIVE: infected sample – NEGATIVE: healthy sample

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Special handling instructions

This set was designed to be used by laboratory staff trained to follow the usual molecular biology precautions. Always perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing DNA/RNA and the components of the set. Do not touch any set components with an ungloved hand. Use appropriate laboratory disposable parts. Use nuclease-free tubes and filter tips to avoid degradation and cross-contamination. Do not use components from sets with different batch numbers in the same test procedure. Do not interchange reagents with other sets. To avoid cross-contamination, use separate rooms for (a) nucleic acids extraction, (b) preparation of the Master Mix and (c) amplification. To avoid cross-contamination and obtain reliable results, it is essential to strictly follow the protocol in this manual. Avoid unnecessary freeze-thaw cycles of the set components. Do not use reagents after their expiration date.

Troubleshooting

Post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal:

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate	Repeat the test using the appropriate tools to correctly seal the plate
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the thermal cycler supplier
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In any doubt, please, contact us
Abnormal amplification	Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles

No amplification reaction is observed in the positive control well, while other samples are positive:

Possible causes	Corrective actions
The positive control provided with the set was not added into the reaction well	Repeat the test. If the problem persists, please, contact us

An amplification plot is observed in the negative control well:

Possible causes	Corrective actions
Contamination of the negative control or the Master Mix with target-positive nucleic acid	Repeat the test by applying appropriate quality procedures to prevent contamination. Seal the plate correctly

Warranty and Responsibilities

Qualiplante SAS
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FR-34830 Clapiers

www.qualiplante.eu
info@qualiplante.eu