



Intended Use:

This ImmunoStrip test is a rapid means of screening tomatoes and bacterial samples for *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), the causal agent of bacterial canker in tomatoes. ImmunoStrip tests require no equipment or expertise to run. Results can be obtained in 30 minutes or less, making ImmunoStrips perfect for use in the field or greenhouse.

Kit Storage:

Kit components should be stored refrigerated (2 - 8 °C) between uses, and ImmunoStrips should be tightly sealed in the desiccated container at all times.

Before use, allow all kit components to warm to room temperature (18 - 30 °C).

Limitations:

Cross-reactions have been observed with other *Clavibacter* and *Xanthomonas* subspecies as well as non-pathogenic soil bacteria from the *Ochrobactrum* and *Microbacterium* genera. For the full list of cross-reactors, see the validation report. For more information regarding cross-reactions, see page 3.

PERFORMING THE ASSAY (*Special Attention Required)

Prepare Sample

1. Take a sample from symptomatic leaves, petioles, or stems when possible. When working with stems cut two cross section pieces at the first two internodes from the crown. The stem can be cut into smaller sections for easier grinding. Agdia sample extract bags **contain 3 mL** of extraction buffer, requiring 0.15 g (approximately 1 inch² or the size of the bottom of the ImmunoStrip container) of tissue for the optimal 1:20 dilution. Please note that thick or dense tissues can alter the targeted 1:20 dilution. (Figure 1)

Bacterial samples: Use a toothpick to remove a colony of bacteria from a culture plate. Stir into a microcentrifuge tube containing 300 µL of SEB4 extraction buffer. If using cell culture broth, add 50 µL of the cell suspension to a microcentrifuge tube containing 250 µL of SEB4 sample extraction buffer. Skip to step 5.

Note: If reusing cutting tools, disinfect them with a 10 % bleach solution between every sample.

2. Cut open the sample extraction bag near the bottom of the label. Be careful not to spill the buffer. *SEB4 buffer is required to perform this assay. (Figure 2)

3. Insert the sample between the mesh linings near the bottom of the sample extraction bag.

4. Extract the sample by thoroughly macerating it with an Agdia tissue homogenizer or a blunt object such as a pen or marker. (Figure 3)

An adequately extracted sample will result in a homogenous green or light brown colored solution.

Allow the resulting solution to settle for 3 minutes before inserting the ImmunoStrip.

Perform Assay

5. Remove an ImmunoStrip then reclose the container. When handling the ImmunoStrips, always grasp the top of the ImmunoStrip marked with Agdia's name. Do not remove the protective covering.

Insert sample end of the ImmunoStrip into the channel portion of the bag (no mesh) until submerged in the extract **up to the white line approximately ¼ inch from the bottom. Do not allow the side of the ImmunoStrip to come into contact with foam or bubbles (if present).** (Figure 4)

6. Place the bag in a letter holder or another device in an **upright** position. Allow the ImmunoStrip test to remain in the sample extract **for 30 minutes**. Positive results may be visible in as few as 5 minutes. Lower titer samples may take up to 30 minutes.

Contents of Kit:

- ImmunoStrips
- *SEB4 sample extraction bags

Not Included but Required:

- Scissors, knife, or razorblade
- Extraction tool
 - » Agdia tissue homogenizer ([ACC 00900](#)), marker, or pen
- Letter holder or another device to hold sample extraction bags upright

*Not included if ordering STX only



Figure 1



Figure 2



Figure 3

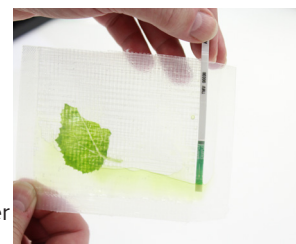


Figure 4

Interpret Results

7. Remove the ImmunoStrip from the extract and interpret the results. Use the images provided as a guide to determine results. If storing the ImmunoStrips as a permanent record, immediately cut off the sample pad, then press the remaining ImmunoStrip between paper towels to remove any excess liquid.

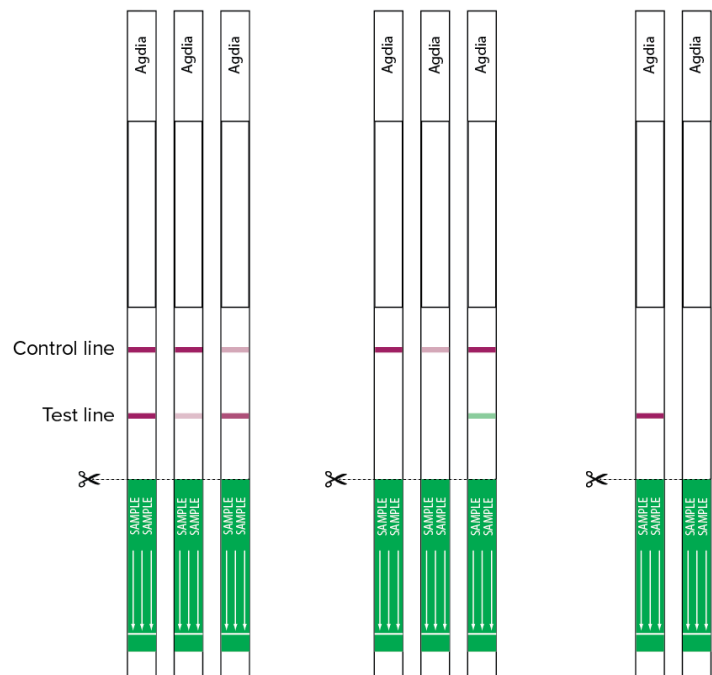
If only the control line is visible, this indicates a negative result.

If the control line is visible and the test line is also present at any intensity of pink**/purple, this indicates the presence of the target pathogen (or in some cases, a closely related pathogen). Visit the product webpage to see if any other pathogens are known to cross-react with this test.

The control line assures that the test is working properly. If the control line does not appear, the test is invalid, even if a test line is visible (see troubleshooting).

As with all diagnostic tools, Agdia recommends confirming all results with a secondary detection method before making any economic decisions (ex: discarding plants due to positive test results, etc.).

Positive Results Negative Results Invalid Results



SAFETY

Agdia recommends reading all relevant SDS sheets before using assay components: <http://docs.agdia.com/datasheets.aspx>.

TROUBLESHOOTING

Control line did not develop.	<ul style="list-style-type: none"> Submerging the ImmunoStrip past the white line in the sample extract. (Step 5) ImmunoStrip inserted before the 3 minute sample extract settling period. (Step 4)
Test runs very slow or not at all.	<ul style="list-style-type: none"> Extracting more tissue than is required. (Step 1) Further dilute sample extract 1:10 in SEB4 and repeat test. Components were not warmed to room temperature before use. (Kit Storage) Check kit and components expiration dates.
Test has a green or pigmented test line.	<ul style="list-style-type: none"> Extracting more tissue than is required. (Step 1) Green lines should be considered a negative result. (Step 7) **In rare cases, red, orange, or purple fruits and tissues may cause what appears to be a positive test line. Contact Agdia before testing these types of samples. (Step 7)
Test and/or control line is weak.	<ul style="list-style-type: none"> Components absorbed moisture. (Kit Storage) Moisture can cause the membrane to wick without test components and fail to produce lines. Low pathogen titer in the sample. (Step 7) Check kit and components expiration dates.

QUESTIONS OR TECHNICAL SUPPORT:

Phone: 800-622-4342 (toll-free) or 574-264-2014

Fax: 574-264-2153

E-mail: info@agdia.com for sales and general product information
techsupport@agdia.com for technical information and troubleshooting

Web: www.agdia.com

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To Video



Cross-reaction

Five bacterial isolates, not related to *Cmm* and not pathogenic to tomato plants, have been confirmed to produce strong positive reactions on Agdia's *Cmm* ImmunoStrip, catalog number, ISK 44001 and STX 44001. Four isolates have been identified by sequencing as *Ochrobactrum* species; one isolate has been identified as *Microbacterium paraoxydans* [1]. Agdia has received copies of these isolates and has confirmed the positive reactions observed with the *Cmm* ImmunoStrip.

If you observe a suspicious positive ImmunoStrip result that you believe may be due to a cross-reaction with a saprophytic bacteria, it is suggested to re-test the plant as follows: re-sample the same area of the plant, rinse the leaf with deionized or similar purity water and then dry the leaf before extracting the sample. Proceed with the ImmunoStrip as instructed.

The above observations demonstrate the continuing need to confirm positive ImmunoStrip results before making important crop management decisions. ImmunoStrip results may be confirmed by [AmplifyRP® XRT for Cmm](#), by PCR, by sequencing, by cultural tests and bioassays. ELISA is not a useful way to confirm ImmunoStrip tests since they both depend on the use of antibodies.

Agdia's *Cmm* ImmunoStrip detects the extra-cellular polysaccharide [EPS] associated with *Cmm* and not the intact bacteria. It is well known that this ImmunoStrip test also detects other *Clavibacter michiganensis* species that possess common EPS epitopes. The new observations suggest that the ImmunoStrip test also detects polysaccharides associated with *Ochrobactrum* sp. [2] and *Microbacterium* sp.

Many *Ochrobactrum* species are found globally at high concentration in soil. The bacterial isolates were negative in pathogenicity tests with tomato seedlings, and the bacteria did not appear to grow at any plant inoculation site [1].

References:

[1] Chet Kurowski and Matthew May, Monsanto, personal communication.

[2] Michael Lebuhn et al, Taxonomic characterization of *Ochrobactrum* sp. isolates from soil samples and wheat roots, and description of *Ochrobactrum tritici* sp. nov. and *Ochrobactrum grignonense* sp. nov., International Journal of Systematic and Evolutionary Microbiology (2000), 50, 2207–2223.