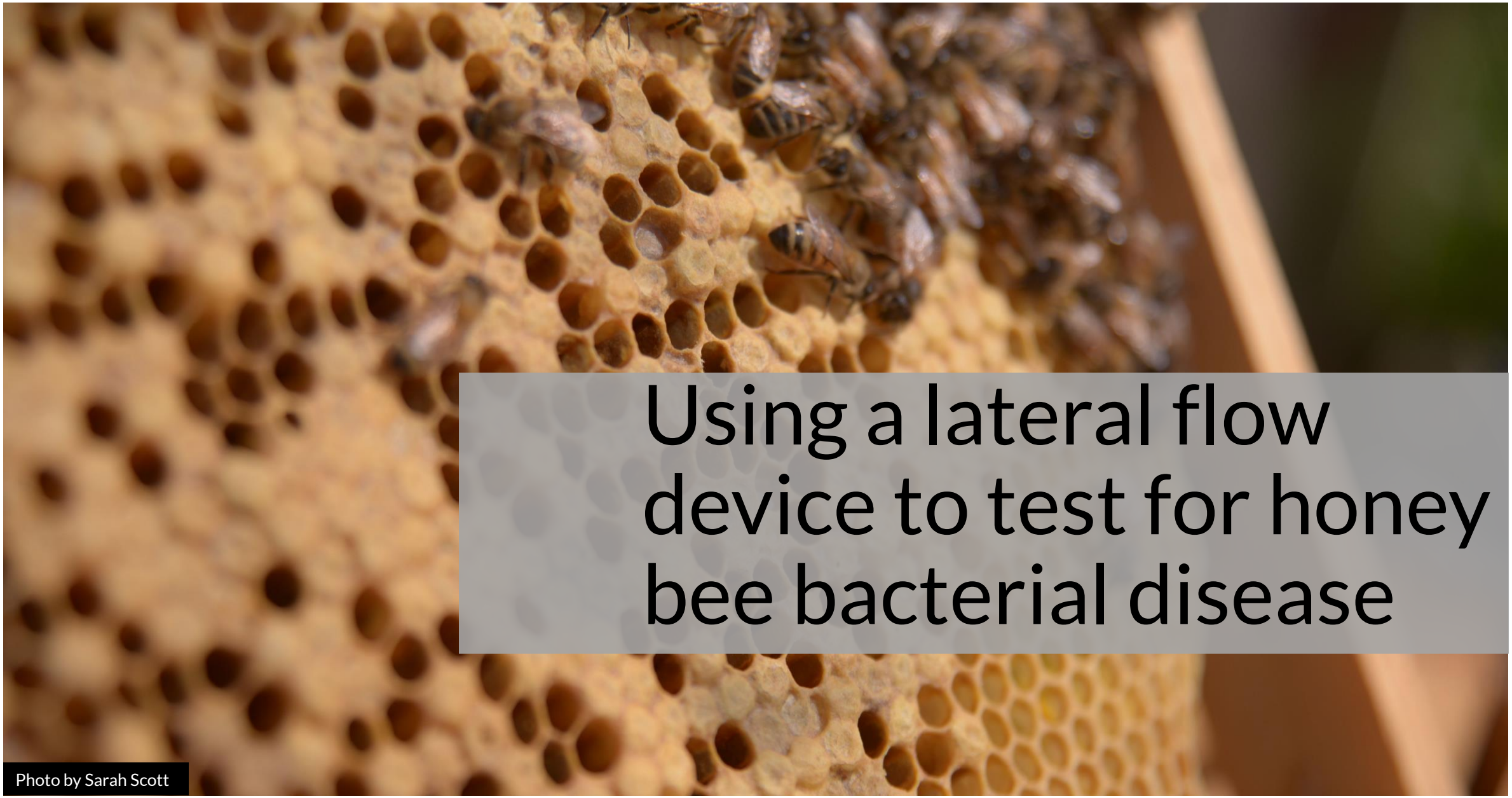


Using a lateral flow device to test for honey bee bacterial disease

Photo by Sarah Scott

Choosing the right cells

European foulbrood (EFB)

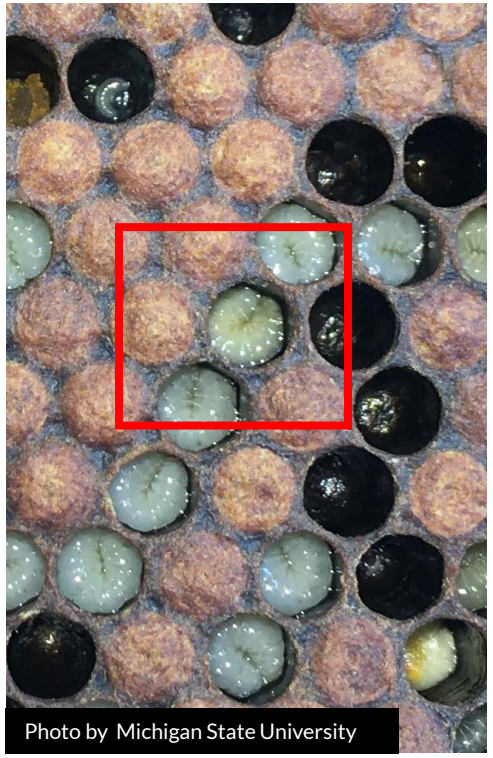
The lateral flow device (LFD) tests for the presence of the bacterium *Melissococcus plutonius*, the pathogen that causes EFB. *M. plutonius* is present in the early stages of disease but is taken over by secondary bacteria as the larva dies and decomposes. If a larva in the late stage of disease is selected for testing (dark brown, dried down, or scale) you may end up with a false negative result, When testing for EFB using a lateral flow device (LFD) choose cells that display symptoms of **early** infection (i.e. twisted, yellowed, malpositioned, etc.).

American foulbrood (AFB)

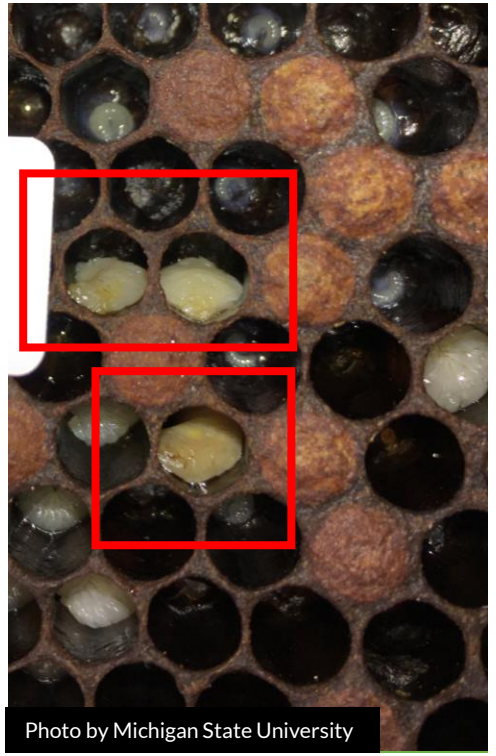
When testing for AFB, use cells with any symptoms of the disease. Since the symptoms of AFB appear later in infection, most cells that we would test with an LFD would be of **later** infection (i.e. dark, sunk cappings, perforated cappings, and dark, melty larva.)

Choosing the right cells for **EFB** testing

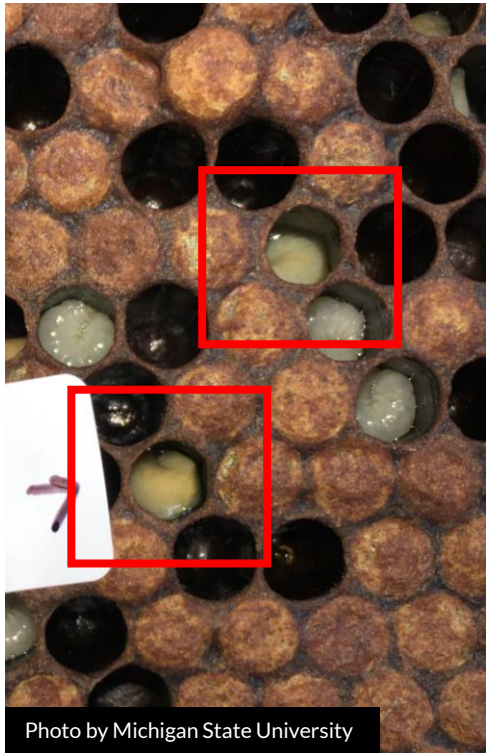
Malpositioned - twisted



Yellow larva



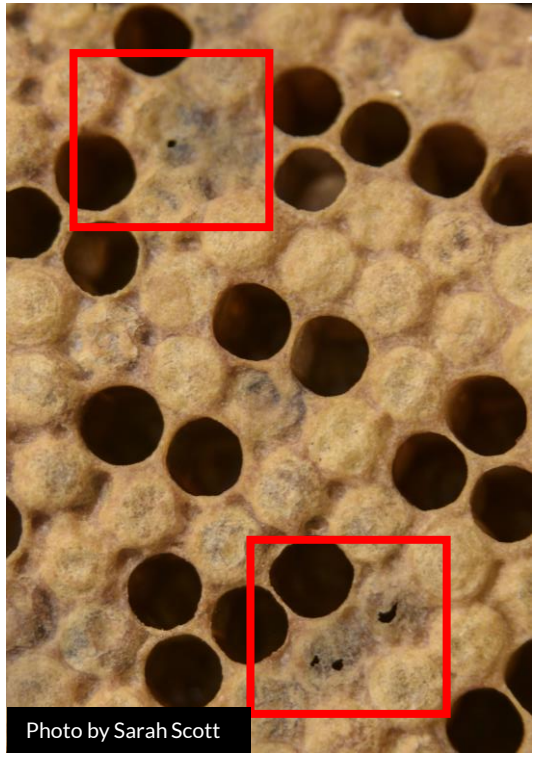
Malpositioned - blobs



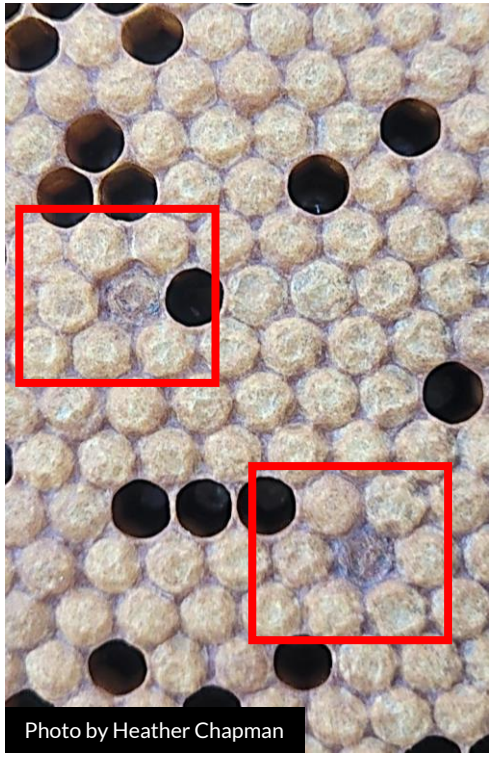
Yellowed, malpositioned larva

Each of these display signs of **early EFB** infection and are therefore appropriate for LFD testing for EFB.

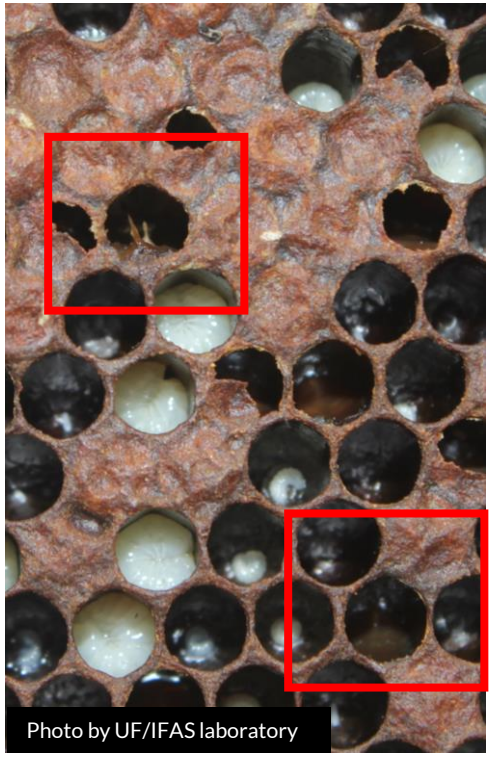
Choosing the right cells for **AFB** testing



Perforated cappings



Dark, sunken cappings

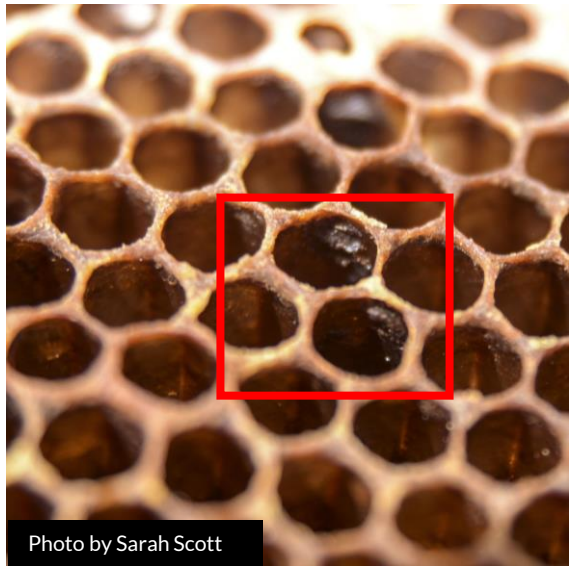


Carmel-colored, melty brood

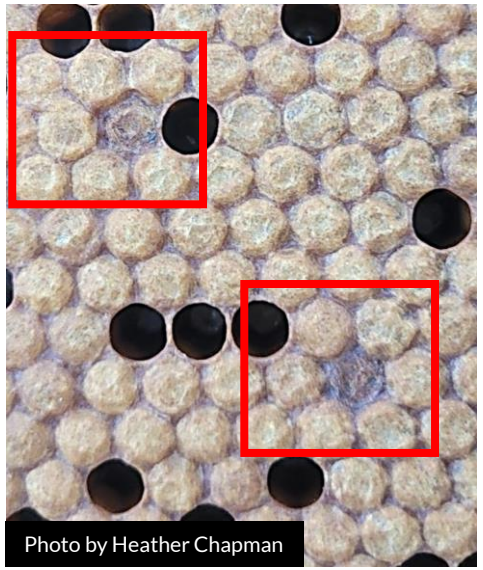
Each of these display signs of **late AFB** infection but are still appropriate for LFD testing for AFB.

Cells to avoid for LFD testing

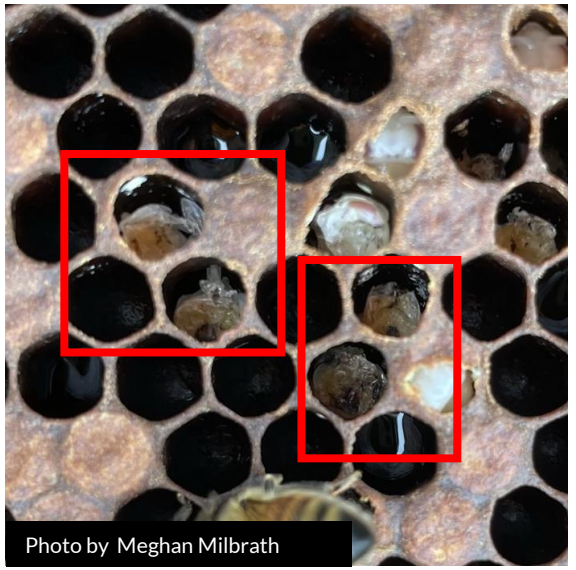
Some cells are not optimal for LFD testing. These include old infections, early infections, and ….



Larva that have degraded to scales. These are too old of an infection to successfully test on an LFD



Dark, sunken cappings should not be used for EFB LFD testing, though these are sufficient for AFB testing.



Pupa that have been chewed down. This is not indictive of EFB or AFB.

Supplies needed

Commercially available lateral flow device for AFB or EFB

Photo by Sarah Scott

Brood frame exhibiting visual signs of AFB or EFB

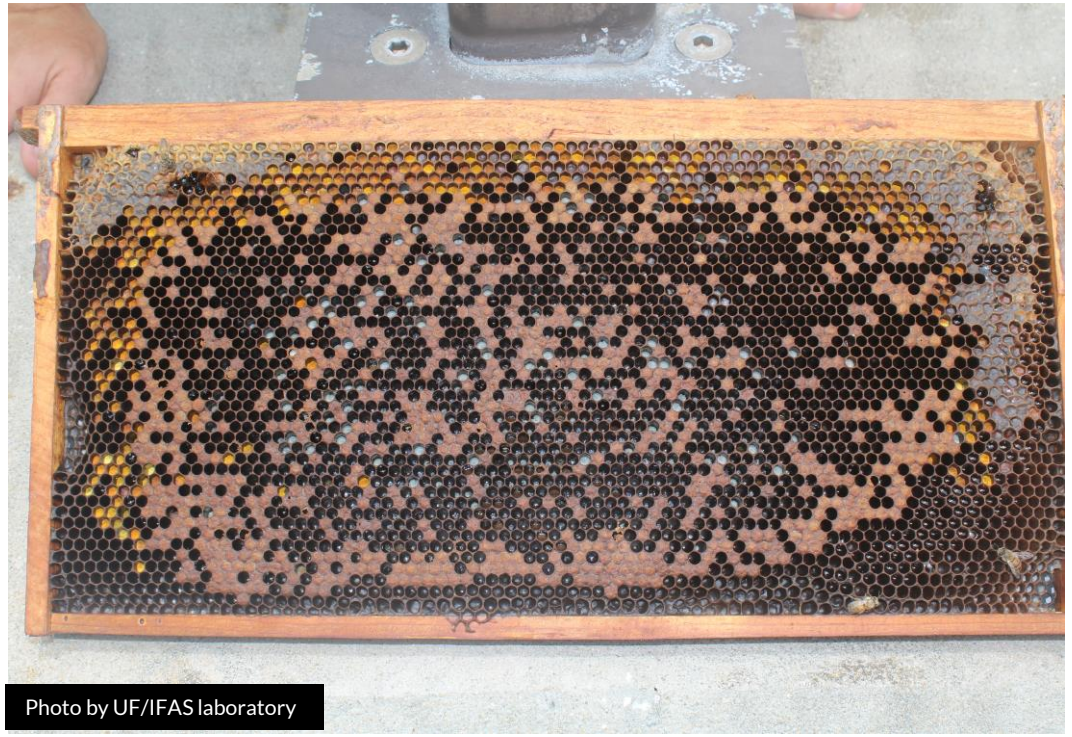


Photo by UF/IFAS laboratory

Step 1. Extract larva from frame

Extract a larva showing suspicious symptoms with the spatula. You can sample several larvae, BUT don’t put too much organic material in the sample bottle – it may clog the device.

Photo by Sarah Scott

Step 2. Deposit sample in bottle

Unscrew lid from the Extraction Bottle. Use the spatula to deposit sample in the bottle.

Photo by Sarah Scott

Step 3. Shake bottle

Replace the lid and shake vigorously for about 20 seconds. BEWARE – the bottle contains buffer and sodium azide.

Photo by Sarah Scott

Step 4. Remove test device

Remove a Test Device from foil pack.

WARNING: Do not touch viewing window.

Photo by Sarah Scott

Step 5. Remove a sample from bottle

Unscrew lid of Extraction Bottle and use the supplied pipette to remove a sample from the bottle.

For best results, remove the sample immediately after shaking to prevent bacteria from settling out of suspension.

Photo by Sarah Scott

Step 6. Apply two drops to device

Hold the Test Device horizontally and gently squeeze two drops onto the sample well of the device.

Step 7. Read results

Keep device horizontal until extract is absorbed (~30 seconds) and a blue dye appears in the viewing window.

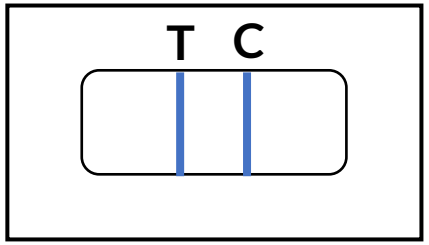
Wait until the control line appears (labelled C) and read the result (~1-3 minutes)

Photo by Sarah Scott

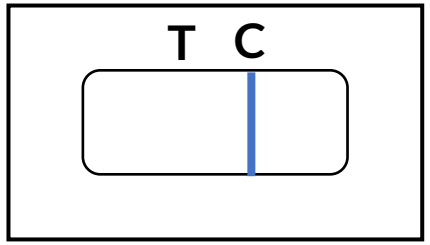
Interpreting the results

A positive result (two lines show up – both Test and Control, see below) indicates that the target pathogen is present in the test sample, that is that the cell sampled contained infection by American Foulbrood.

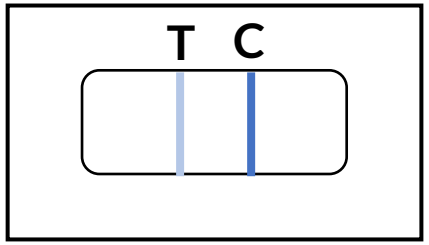
A negative result (Control line shows up only, no Test line) indicates that the AFB pathogen has not been detected in the test sample.



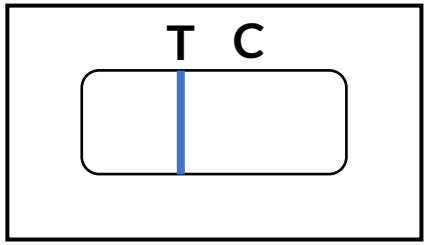
Positive result



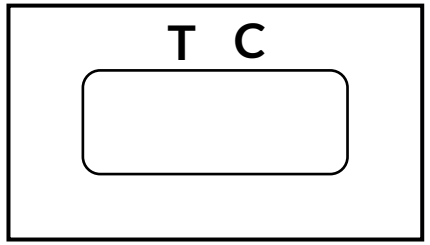
Negative result



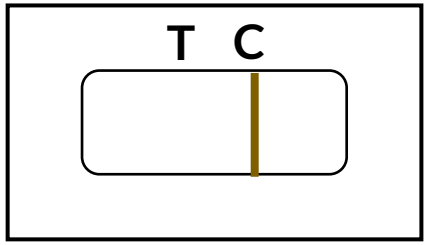
Possible positive result



Test failed



Test failed



Test failed

Test notes

If the test has failed it is recommended to carry out another test using a new sample from the same original comb with a new device.

Note: As with all diagnostic testing a negative reaction does not necessarily indicate that the target pathogen is absent. A faint or absent line may indicate a low concentration of the pathogen or recent infection.

Photo by Sarah Scott

Common errors to avoid

- Using an expired device. Note the expiration date on the test package.
- Using the incorrect kit for the pathogen. The kits are pathogen specific, and the correct kit must be used.
- Using too much material in the test. Using too much sample may clog the device.
- Selecting a larva that is too far decayed for an EFB test. When possible, chose a larva that is showing early signs of disease.

Photo by Meghan Milbrath

Photo by Sarah Scott

Additional Resources

- AFB Diagnostic Test Kit | Vita Bee Health (vita-europe.com)
- EFB Diagnostic Test Kit | Vita Bee Health (vita-europe.com)
- A Rapid Antigen Test for European Foulbrood - American Bee Journal
- Diagnosing and Treating American Foulbrood in Honey Bee Colonies – Dr. Meghan Milbrath, Michigan State University
- Identifying and Mitigating Foulbrood in Honey Bee Colonies and Reducing the use of Antibiotics – Honey Bee Health Coalition

Photo by Katie Lee

Acknowledgments

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