

Holst Milk Test

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Introduction

The Holst milk test is a field test used to diagnose American foulbrood (AFB) that works on both active infections and scales from previous infections. The Holst milk test is performed by adding a sample of infected brood or scale to a tube of diluted milk. The proteolytic enzymes released by the AFB-causing bacteria *Paenibacillus larvae* during sporulation can break down the milk proteins, causing the milk to become clearer and lose its 'milky' appearance. No other pathogens are known to have this effect, so this test is highly specific.

Indications

A Holst milk test is used when American foulbrood is suspected, or any scale is observed on old comb (Figure 1).

Choosing the Right Cells

For this test, it is important to choose cells that exhibit visual signs of American foulbrood (AFB) (Figure 2). Look for 1) brood cells with cappings that appear dark in color, sunken, or perforated, 2) open brood cells containing caramel-colored brood that appears melted, or 3) open cells containing dark, brittle scale.

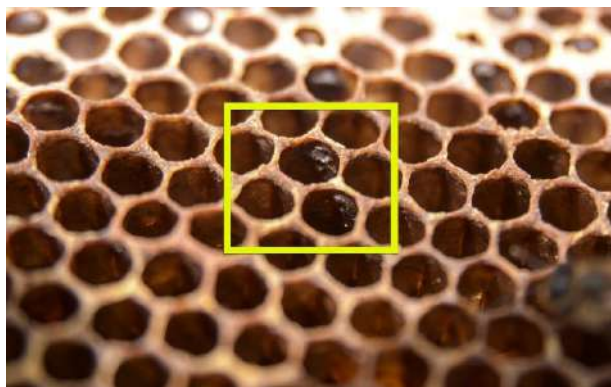


Figure 1: Scales can be seen in multiple cells of this comb. A Holst milk test would be appropriate to test with these samples. Note: this frame is being viewed from the top of the frame. Photo by Sarah Scott.



Figure 2: Brood cells showing signs of AFB, including a perforated capping and cells containing caramel-colored brood that appears melted. Photo by Brandi Stanford, UF/IFAS Honey Bee Research and Extension Laboratory.

SUPPLIES NEEDED

Two small sample tubes
(Glass vial, 15 ml falcon tube, or similar)

Marker for labeling tubes

Stick or other similar object for sampling
(Matchstick, toothpick, twig, coffee stirrer, etc.)

Tweezers or forceps

Non-fat milk powder or fresh skim milk

Water

Brood frame exhibiting visible signs of AFB

Completing the Holst Milk Test

1. Place water (warm water works best) into two tubes. One tube will be for the test sample and the other will act as a control to compare if the test undergoes a visible change. Label tubes to differentiate test/sample from control.
2. Add a small amount of nonfat dry milk or fresh skim milk until the water is visibly cloudy/ milky (Figure 3). The exact amount does not need to be precise, just enough to visualize a difference in cloudiness.

If using fresh skim milk, dilute in half with water twice; fill one tube half full with skim milk, top off with water, then split this diluted milk evenly into the two tubes and top off again with water.



Figure 3: Two tubes filled with dilute milk - one for the test and one as a comparative control. Photo by Sarah Scott.

3. Set the control tube to the side.
4. In the other tube (test), collect suspect brood from multiple cells exhibiting visual signs of disease.

When removing larvae, use a stick such as a coffee stirrer or toothpick. Because the brood are often liquid, it is often difficult to remove from the stick. You may leave the collection stick in the sample tube. Note that this test relies on the presence of *Paenibacillus larvae* spores, therefore sampling early-stage larvae, which more likely contain the vegetative state of the bacteria, may result in false negatives.

When removing scales, use tweezers/forceps. Scales may be stuck to the wall of the cells, and you will likely have to remove the entire cell. You can collect the entire cell into the tube.

5. Place both tubes in a warm location, ideally at 37°C/99°F (e.g., warm pocket, water bath) for 15 to 20 minutes. Agitate the tubes regularly throughout this time.

Interpreting the Results

Compare the solutions in the two tubes. The solution in the control tube should still have a uniform cloudy appearance. If the solution in the sample tube appears the same, then the sample is not positive for AFB. However, if the sample solution has become clear brown, this indicates a positive diagnosis of AFB in that sample/colony (Figure 4). Note that if you use too much milk in your sample tubes, the solution may still be somewhat cloudy even with an AFB-positive sample. However, in this case, the sample solution will still be clearer and darker in color than the control solution.

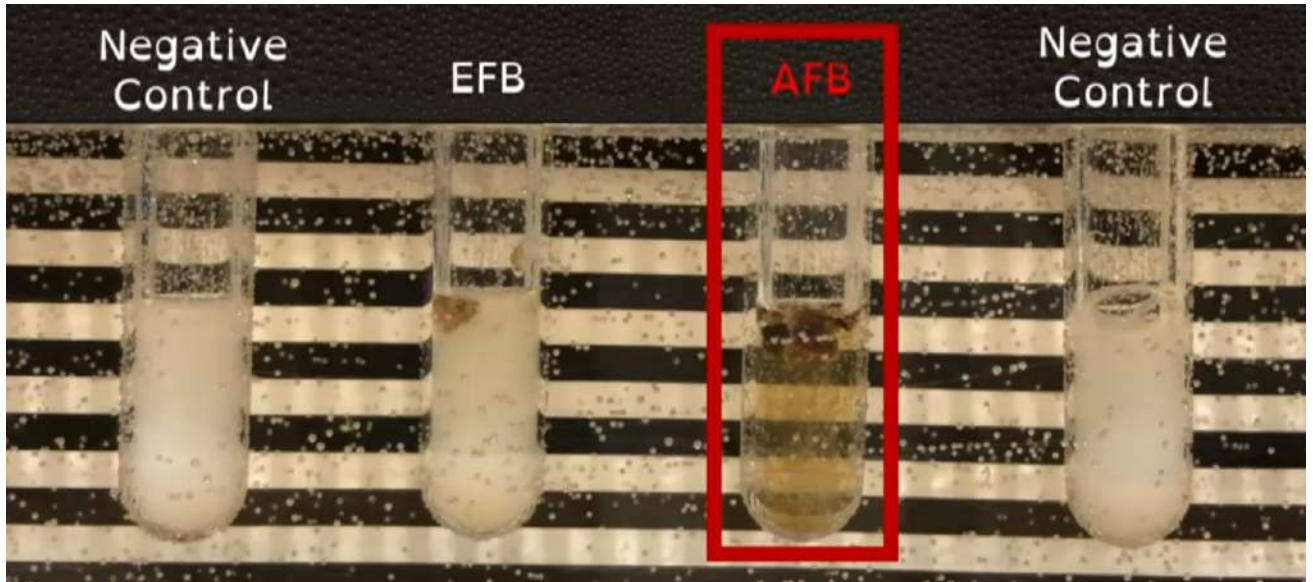


Figure 4: The results of a Holst milk test. Notice how the two control tubes and the tube containing EFB-infected brood remain cloudy, while the tube containing AFB-infected brood is less opaque and is brown in color. Image by Peter Fowler.

Common Errors to Avoid

Avoid mixing the milk too concentrated – it may be difficult to witness a change, resulting in a false negative result.

Avoid sampling pollen. While there are no known pathogens besides *P. larvae* that have the effect of breaking down milk proteins, similarly functioning enzymes may be found in pollen, resulting in a false positive result.

This test will not work at room temperature. The samples must be incubated around 37°C/99°F for AFB-positive samples to clear.



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