ALBERTA TECH TRANSFER PROGRAM

AMERICAN FOULBROOD (PART 2 OF 2)

(Written by Dr. Meghan Milbrath)

Diagnosis of AFB: Laboratory analysis

AFB spores can be characterized through microscopic identification, culture, or molecular techniques. For more information on laboratory diagnosis of AFB, read Diagnosis of American Foulbrood in honey bees: a synthesis and proposed analytical protocols (https://naldc.nal.usda.gov/download/28123/PDF), and the COLOSS standards for American Foulbrood Research (https://www.tandfonline.com/doi/abs/10.3896/IBRA.1.52.1.11).

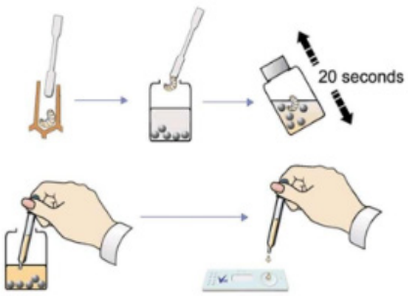
Diagnosis of American Foulbrood: Field tests

There are three field tests for identifying AFB in a hive. All three tests are highly specific, but not highly sensitive – a positive test result indicates that AFB is present, but a negative test result could be because the test missed the disease rather than the hive was truly free of AFB. A hive that produces negative field tests may still have infectious spores, so if AFB is suspected, the colony should still be dealt with appropriately. The field tests include:

1. **Commercial diagnostic kit**
2. **Match stick test**
3. **Holst milk test**

1. Commercial Diagnostic Kit

The commercially available kit looks similar to a standard pregnancy test - one line will appear to tell you the test worked, and if there is AFB, another line will appear indicating a positive result. The kits include all materials and instructions to test a hive in minutes.



www.vita-europe.com/PRODUCTS/AFB-DIAGNOSTIC-TEST-KIT

A positive result of a match stick field test, indicating that this colony has AFB. The stick was used to pierce a suspicious capping, and was slowly removed, drawing out the contents inside. No other disease will cause the larvae to string out > 2 cm as shown

2. Match stick test

The match stick test can be performed on colonies with active infection. To perform the test, use a match stick, twig, coffee stirrer, toothpick, or any sort of tool that has a rough surface and is stiff enough to pierce a capping. Pierce any suspicious capping (sunken, discolored, or perforated), and slowly draw the stick out, trying to make the larvae draw with it. If the larval goo is ropey and draws out in a string greater than 2 cm (3/4 in), then it is considered a positive result for AFB. Dead and dying larva from many different causes will look unpleasant, and many will discolor, but only AFB will cause dead larvae to string out

Don't be Mistaken!

The photobelow shows the match stick test being performed on a colony with European Foulbrood (EFB). Note that the larva still looks brown and gross, but does not form a ropy string. It may slightly rope out of the cell, but will break or snap back. AFB will hold in a string to this length. The larva must be in the correct stage of decay for the ropiness to occur, and it may be necessary to try multiple times. The failure of larvae to rope does not mean that AFB is not present.

3. The Holst milk test

The Holst Milk Test works best with active infections, but can also work on scales. The proteolytic enzymes released by AFB bacteria breaks down many proteins, including milk proteins. The Holst milk test will be positive, if a sample has enzymes that break down milk proteins— which is unique to AFB. In this test, suspect larvae are added to a weak milk solution. If AFB is present then the milk will break down and the solution will turn clear brown, losing its opaque 'milkiness'. Holst, EC (1946). For more information on the Holst milk test: A simple field test for American Foulbrood. American Bee Journal, 86: 14–34.

Items needed for a Holst milk test: sample tubes, stick (can use match stick from match stick test), and nonfat dry milk. Fresh milk may also be used and diluted with water. Kits can be made ahead of time – Place a small amount of nonfat dry milk and a stick in the bottom of each. The kits are fine to leave in your equipment until needed.

The Holst milk test: Step-by-step (photos by Sarah B. Scott)

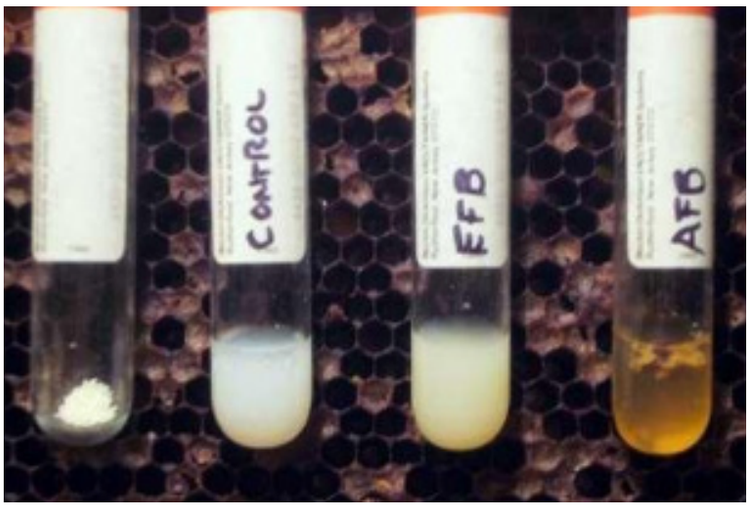
Step 1: Place a small amount of non-fat dry milk into the bottom of 2 tubes. The actual amount is not important – it should be very dilute, but still clearly have a cloudy, milky appearance. The amount in this tube was a bit much, but the results were still obvious. If you have fresh skim milk you can dilute it in half, and then half again. It is best with a dilution that is just cloudy.

Step 2: Make milk by adding water. If you are using dry milk, add water, cap the tubes, and shake to make a uniform liquid. Warmer water will allow the reaction to occur more quickly. If available use water that is warm, up to 150˚F (the temperature of hot coffee).

Step 3: Label your tubes. Mark one tube as sample, and one tube as control. In the beginning, it is good to use two tubes. As you become more experienced, a control may not be necessary.

Step 4: Add a sample to the tube marked 'sample'. You can use a capped larva, the results of your match stick test, or a dried scale. The more sample you add, the faster the reaction will occur. The enzyme is not present in young larvae, so older larvae should be used. Remember, you are looking for the presence of the enzyme that is responsible for breaking down larvae, so the grosser, the better.

Step 5: Incubate. The reaction will not be immediate, and will depend on the amount of sample, the dilution of the milk, and the temperature. Put the tubes in a warm place like your pocket for up to 20 minutes.



In our test, we used too much milk, so the results were not as obvious. The photo to the right by Randy Oliver shows a much more clear result. His website www.scientificbeekeeping.com has more resources on identifying and managing honey bee diseases.

Step 6. Examine. Examine your results. This sample was positive for AFB by the Holst milk test. Note that the control tube remains cloudy, while the sample tube started to turn a clear brown. This test could be made more obvious by using more dilute milk, warmer water, and more sample material. When less milk is used, the positive sample will take on the appearance of iced tea.

What to do if you have a hive with American Foulbrood

An infected colony should be dealt with immediately. As the colony weakens and dies, the contents will be robbed by nearby colonies, and the foragers from the sick colony can drift into nearby colonies, resulting in disease spread. Your choice of action will depend on the regulations in your state, your ability to monitor, your willingness to accept risk, the number of infected colonies, the proximity to other apiaries, the size of the hives, and the time of year. The standard recommendation is to burn the colony the evening that you identify disease. After you burn the infected colony, you will want to treat the remaining colonies in the yard with antibiotics and monitor them closely to control the spread of disease.

Remember, in some states and provinces, AFB is a reportable disease. Contact your apiary inspector to learn the regulations in your area. A list of state and provincial inspectors can be found at the site of the Apiary Inspectors of America (*https://apiaryinspectors.org/).*

Burning the hive

Some areas require that the hive and all of its contents be immediately burned. Even if you do not live in a state that requires burning, this is the best option for reducing the chance of transmission. Burning the hive is the safest way to rid the yard of the disease and to prevent transmission of the spores. Ideally, you will burn the hive on site, after dusk on the day AFB infection was identified. Waiting until after dusk will increase the number of foragers that are in the colony (so they do not return to other healthy colonies). The bees can be killed prior to burning if desired, but make sure that you do so quickly and safely.

Instructions:

1. Close up the hive to prevent bees and other hive materials from escaping during destruction.

2. Dig a hole, and start a small fire in the hole.

3. Add the hive and all its contents as well as any potentially infectious tools like gloves and brushes.

4. After the hive has burned down to ashes, bury the ashes.

Burning bee equipment is different from other fires: the wax is highly flammable, and will quickly combust; the boxes and flat surfaces of the covers and bottom boards can form a chimney effect; and the honey will not burn, but will run over the fire and the ground once the cappings melt- hence the need for burial. Some bee older bee equipment may have been painted with lead-based paint, so be careful not to inhale any smoke.

Be aware of all burn regulations in your area, and prepare the site well. If you are in an area where it is physically impossible to burn the hive (e.g. there is a burn ban, or it is not legal in your area), keep in mind that the goal is to make the equipment inaccessible for all other bees, and to eliminate the chance of robbing. You can double bag the equipment in thick contractor bags, and take the equipment to a landfill or commercial incinerator. Be extra careful when you transport infectious equipment – it can overheat in bags, causing wax to melt, and spore-laden honey to leak out.

2. Shake the bees from the old equipment onto the new hive.

3. Destroy the frames.

4. Treat the colony with antibiotics.

In a successful frame exchange, all of the infectious larvae are removed from the hive. There will be a break in the brood cycle, and a lack of susceptible larvae for a few days as the bees draw the wax and the queen lays eggs. Some of the adult bees may still have infectious spores in and on their bodies, which is why antibiotics are necessary with this method to prevent reinfection.

Shook swarm method: Step by step

1. Set up a hive with all new equipment. You can use old boxes, but frames with brand new foundation should be used. You want the bees to have a full break in the brood cycle and to take time to draw out the wax. Be prepared to feed them with 1:1 sugar water as they will not have food stores, and will be drawing out wax. If it is too late in the season for the colony to draw out wax, then this method should not be used, and the colony should be destroyed.

2. Make sure that the bees cannot access any frames that need to be destroyed, especially if they are moved to a new location for destruction. Here, we were not able to burn on site - these boxes are tightly closed to prevent bees from robbing them while they are moved. Strong contractor-style trash bags can also be used to keep the frames from being robbed while in the yard.

3. Shake the bees off the old frames onto or in front of the hive with new, clean equipment. Use strong shakes to dislodge the bees, or brush the bees off the frames. Because AFB spores can be present in honey, pollen, nectar, and brood, all frames should be removed and replaced from the hive. If you use a brush, burn it with the frames.

Use firm, swift shakes to dislodge the bees from the old frames and drop them into the new equipment. Try to complete this process as quickly as possible to minimize robbing from neighboring hives.

4. Dispose of the equipment properly. Frames should be burned, while boxes and lids can be sterilized as explained later. AFB does not cause disease in humans when it is consumed in honey. While you technically can save honey from hives that have AFB for human consumption, you really need to consider the risk of contaminating your equipment--the spores can contaminate your honey extraction equipment, putting the rest of your operation at risk in the future.

5. Provide the infected colony and all other colonies in your apiary with antibiotics. The adult bees may still have some infectious spores, and antibiotics are used to control re-infection and spread in the yard.

6. Monitor all colonies in the yard frequently to ensure that you catch signs of re-infection early. Treat any yard where AFB has been identified as a quarantine yard. Do not sell bees or products from this yard, and do not use honey supers from this yard for use in other yards

Sterilizing equipment

Any equipment that is not burned can be sterilized to kill spores. AFB spores can be killed by 30 minutes at 130˚C (266˚F) dry heat, by 1.5% bleach, 1.5% caustic soda in boiling water, gamma rays, and flames. It is important to remove all potentially spore laden materials including wax and propolis. Simply soaking the boxes in bleach will not be sufficient. Disinfection of hive bodies /supers (boxes), bottom boards, and lids can be performed by scorching, followed by spraying bleach or caustic soda, and finally by immersion in hot miccrocrystaline wax. Remember to keep all potentially spore laden equipment away from bees before it is properly sterilized.

Commercial sterilization: Some beekeepers live in areas where sterilization facilities are available. This is the only method where drawn comb can be safely reused. Many facilities have a minimum limit, so it is good to see if your bee club knows of any facilities and organizes a trip to sterilize equipment in bulk.

Scorching: Boxes can be sterilized by scorching using a propane torch. Scrape off any wax or propolis on the surface, and make sure that it is burned properly. Slowly move the flame over all surfaces, ensuring that the wood is sufficiently browned and that all propolis and was should be heated until it bubbles and melts into the wood. Make sure to pay close attention to corners and crevices. When scorching boxes, be aware of the chimney effect, and always keep a lid and fire extinguisher close by to stop a fire. Let the boxes cool completely or use a hose to wet them down before putting them into your garage or shed. Even if boxes are scorched carefully, it is impossible to ensure that every pore in the wood is completely sterile, so it is advised to use a second method of sterilization.

Wax dipping: Wax dipping is a good option as a follow up to scorching equipment as it can bring heat deep into the wood, reaching places that cannot be reached by flames. To kill spores, the wood must be submerged for at least 10 minutes at 160˚C (320˚F). This tank is filled with 2:1 paraffin wax to microcrystalline wax (though other combinations can be used). The boxes are kept submerged for at least 10 minutes, and the temperature is frequently checked.

Use of antibiotics

Antibiotics are used to control disease. Antibiotics only work to halt replication in the vegetative form of AFB, and are NOT effective against spores. The use of antibiotics is to control

replication and infection from spores present on adult bees after the affected equipment has been removed. If you use antibiotics without removing infected larvae, the colony will be re-infected after you stop antibiotic treatment. If you remove the infected frames or hives, but don't follow up with antibiotics, other colonies can be infected from spores on or in adult bees. It is recommended to treat all colonies with antibiotics in a yard where active AFB infection was present to prevent spread of disease from drifting adults.
It is essential to follow the label precisely. Apply the antibiotics with the frequency and method outlined on the label, leaving the necessary time after conclusion of treatment before honey supers are added.

Misuse and overuse of antibiotics can lead to antibiotic resistance. Some resistance has already been seen to Oxytetracycline, likely due to the widespread off-label prophylactic use of this drug. For this reason, many beekeepers and veterinarians prefer to use tylosin. However, tylosin has a longer withdrawal period, so it can only be used when there is sufficient time before honey supers are to be used.

Transitioning from prophylactic use

Many beekeepers began using antibiotics regularly in the mid-part of the 20th century, when American Foulbrood was a serious concern in many states. It is still common for beekeepers to apply antibiotics in the spring and/or the fall every year. If a beekeeper were to suddenly stop using antibiotics after years of continuous regular use, there is a high risk of devastating infection – the equipment may be laden with spores and scales, and the antibiotics may have been working to suppress disease. A beekeeper in this situation can safely transition away from antibiotics through a system of close monitoring, and taking care with how equipment is used and moved. Antibiotic use is stopped, and colonies are closely monitored for disease. Infected colonies are disposed of as they are found and other hives in that yard are closely monitored. No equipment would be taken from that yard and used in other parts of the operation. In larger operations, this can be done on a yard-by-yard basis. Antibiotic use would be stopped in on part of the operation, and this part would be monitored closely for any sign of infection. During this time, no used equipment would be brought in or out of this part of the operation. After it has been determined that AFB is not present (due to no visible disease), this yard would not need further antibiotic treatment, and the equipment can be used safely. A second portion of the operation would then be stopped, so that the beekeeper can take the same precautions to ensure that disease does not appear.

Colonies can often be saved if infection is found early, so it is important to monitor for AFB signs on a regular basis. Monitor with increased vigilance in high risk scenarios including the following: colonies that have previously been infected (if not destroyed), colonies that have been in a yard where AFB has been identified, colonies in home-sterilized equipment, and colonies that have been previously treated prophylactically with antibiotics (including bees purchased from commercial beekeepers). No equipment should be moved from yards where AFB has been identified.

Innovative Design Solutions To Practical Beekeeping Challenges

By Alberta Beekeepers Commission

The ABC recently had the opportunity to partner with five teams from the University of Alberta's (UofA) 4th year Mechanical Engineering program to research and design five different innovative solutions to five unique practical beekeeping challenges.

Project ideas were collected from Alberta beekeepers and a total of nine projects ideas were submitted to the UofA students. Five of the nine projects were selected, and the teams set to work researching, interviewing beekeepers, and developing creative solutions to these challenges. Students worked on the projects throughout the semester and presented their reports via poster and PowerPoint on December 6, 2020.

The five projects include:
1. Queen shipping container
2. Honey House designed to maintain RH and Temperature
3. Automated Filling Machine
4. Tote Flipper
5. Spin Float design to reduce foam

We are pleased to feature the posters from these projects in bee news, with the first project featured being the **Design of a Queen Bee Shipping Container.**

Full reports available on our website at www.albertabeekeepers.ca