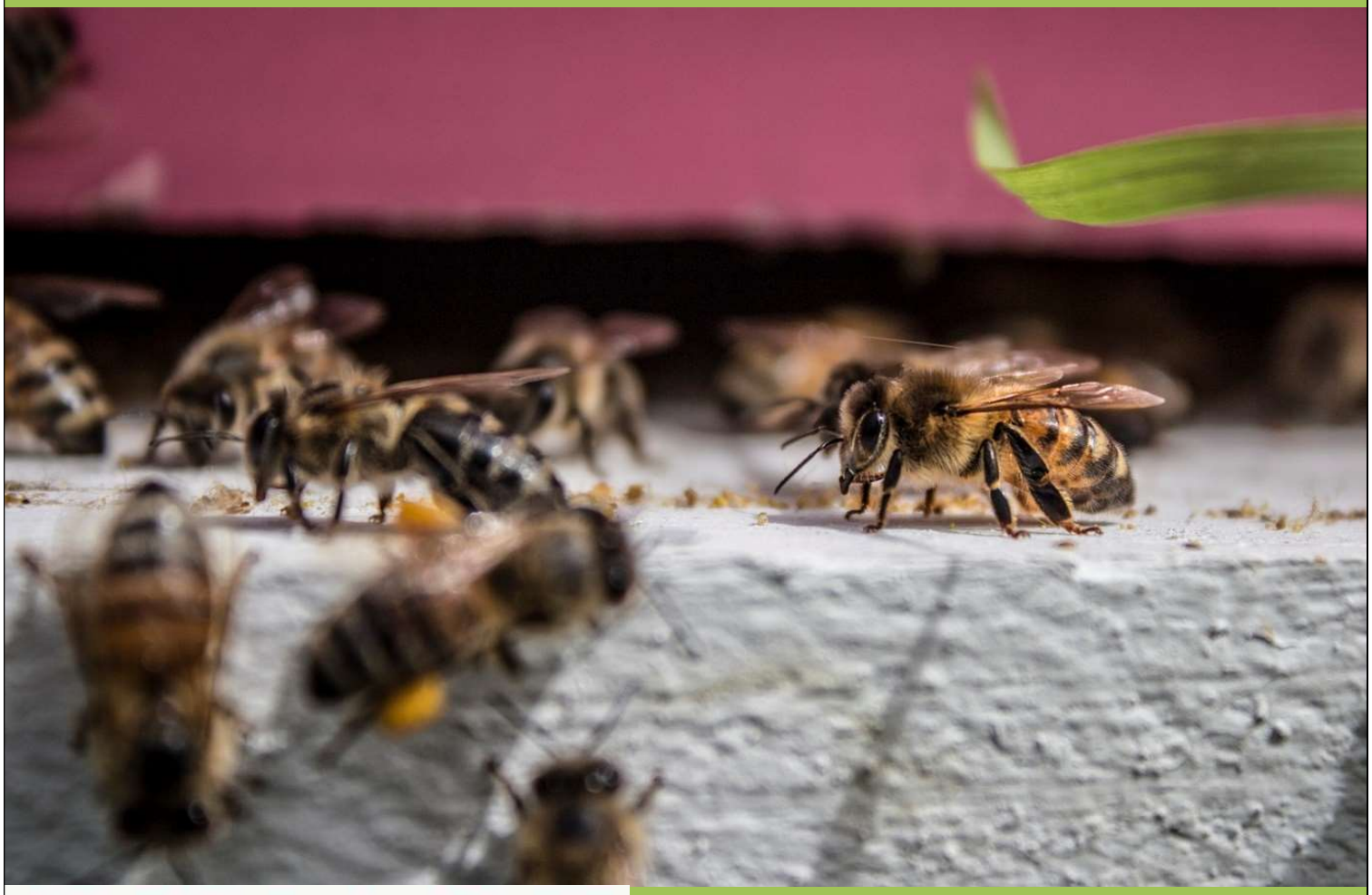
DIAGNOSING AND TREATING AMERICAN FOULBROOD IN HONEY BEE COLONIES



MICHIGAN STATE UNIVERSITY

Meghan Milbrath, 2018

DIAGNOSING AND TREATING AMERICAN FOULBROOD IN HONEY BEE COLONIES

Honey bee colonies can be infected with two bacterial diseases: **American Foulbrood (AFB)** and **European Foulbrood (EFB).** They are called 'Foulbrood' because both diseases affect the *brood* (the term for bee larvae and pupae) and these diseases cause the hive to have a particular foul odor. In this article, we will discuss how to identify and safely manage American Foulbrood, the more severe bacterial disease. Infection with AFB is serious, needs immediate attention, and may require the involvement of a veterinarian. Early diagnosis and prompt response is essential for preventing the spread of AFB.

NOTE: DIFFERENT STATES AND PROVINCES HAVE DIFFERENT LAWS AND REPORTING REQUIREMENTS FOR HONEY BEE DISEASES. MAKE SURE THAT YOU CHECK WITH YOUR STATE APIARY INSPECTOR OR LOCAL EXTENSION SPECIALIST IF YOU SUSPECT YOUR COLONY HAS A BACTERIAL DISEASE. A LIST OF STATE APIARY INSPECTORS IS AVAILABLE AT https://apiaryinspectors.org/

Inspection for disease

Take care when inspecting hives to prevent the transmission of bacteria or spores to healthy colonies. While unlikely, be aware that bacterial spores can be transmitted by hands, hive tools, smokers, and any beekeeping equipment. Wear nitrile or latex gloves when working in hives that may have foulbrood, remembering to remove and safely dispose of them before handling another hive. Do not wear leather beekeeping gloves when inspecting someone else's colonies, or when working with sick hives, as they cannot be cleaned. Wash beekeeping jackets and other equipment often.

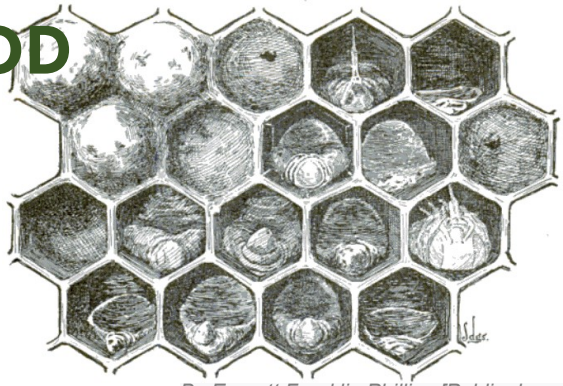
Photo by Sarah B. Scott

Keep your hands clean. *Wash your hands often and well. If water is not available at your site, use rubbing alcohol or hand sanitizer and rub vigorously. Remove all the wax and propolis from your hands, as spores can remain in these materials. Best practice is to use nitrile gloves - being careful to remove and dispose of them after handling an infected hive.*

Make sure that your hive tools are completely clean. *After working suspect or sick hives, switch to a clean tool. If water is available soak your tools in bleach solution, and use a chlorinated scrubbing cleaner like comet as well as rubbing alcohol to remove all of the propolis that can accumulate on the tool. Clean tools can be autoclaved if facilities are available. If no water is available, you can flame the tool, but some spores may still remain in any attached propolis and wax.*

Photo by Sarah B. Scott

AMERICAN FOULBROOD



By Everett Franklin Phillips [Public domain], via Wikimedia Commons

American Foulbrood is an infectious and highly contagious disease caused by a gram-positive spore-forming bacterium, *Paenibacillus larvae*. AFB has been known to affect honey bees for hundreds of years. It is found all around the world, though different genotypes predominate in different areas. AFB is considered by many to be the worst disease of honey bees. There are three reasons why this disease is so serious:

1. AFB has a highly persistent spore form that can remain infectious for decades and spread easily to other colonies.
2. AFB can devastate an otherwise healthy hive. It does not require another stressor, and colonies do not spontaneously recover – AFB generally leads to death.
3. It is highly infectious – only a few spores are needed to cause infection in an otherwise healthy colony.

The infectious spore form is incredibly stable in the environment. It can persist for decades on equipment, honey, wax, pollen, etc., and can remain infectious years later, even after freezing, droughts, and humidity. No other honey bee disease is known to be as persistent, and great care must be taken with the equipment of colonies known or suspected to be infected with AFB. If a beekeeper does not notice or appropriately deal with an infected colony, they can easily spread the spores throughout their entire operation through actions taken in routine apiary management.

Photo by Sarah B. Scott

A yard where two colonies were found to be infected with American Foulbrood. It is easy to tell what colony remained uninfected. The large colony is normal for this area at this time of year, while the two infected colonies had dwindled down to only a few frames of bees. This beekeeper faces the loss of the bees, equipment, and honey production from these colonies.

AFB spores infect the bee early in the larval stage (12 - 48 hours old), generally through infected food. Infection spreads quickly among the larvae, as nurse bees move from cell to cell during feeding. Only a few spores (<10) are required to cause disease in larvae. Adult bees can carry the spores, but are not affected. The spores germinate into the active vegetative form when they reach the larval intestine. They begin to reproduce, and the bacteria massively colonize the midgut. In the midgut, the bacteria release toxins and enzymes that digest the larval tissues. As the disease worsens, the gut epithelium (lining) is breached, and the infection spreads to all tissues, causing sepsis and death. In the strain of AFB found in the US, death occurs just as the larvae are capped (in a cocoon to commence pupation). The larvae completely breakdown into a glue-like biofilm. As conditions become unfavorable for the vegetative form, the bacteria form spores. A single dead larva may contain millions of infectious spores.

Clinical signs of American Foulbrood

The clinical signs of AFB are variable, and what you see will depend on the stage of the disease and the time elapsed since infection. At the beginning of an outbreak in a colony, only a few larvae may be infected. As the disease progresses, the population in the colony dwindles, as very few young bees survive.

Foul odor

As the infection continues, a particular foul odor will develop. In a severely infected colony, the odor may be detectible without opening the hive. Some beekeepers can smell the odor easily, and can tell there is an infected hive as they enter the yard. Other beekeepers can never smell the odor, and may not recognize it, so an absence of smell does not mean AFB is absent. The presence of a bad smell does not necessarily mean that AFB is present, as larval decay for any reason will smell unpleasant.

In addition to the foul smell, AFB causes a characteristic set of visual signs in the brood nest of a hive:

1. **Spotted brood pattern**
2. **Sunken cappings**
3. **Off center holes in cappings**
4. **Larval scale**
5. **Caramel color of dead larvae***
6. **Pupal tongue***

* These two signs (caramel color and pupal tongue are unique to AFB.

A healthy brood pattern: a few gaps in the brood are considered normal, but capped cells are generally touching other capped cells, and the cappings look dry, even, and uniform.

Signs of AFB: Spotted or "shotgun" brood pattern

In a healthy colony, the cappings over the pupae should look uniform in shape and consistently colored. The larvae are generally raised in groups of the same age, so a healthy brood pattern would appear to be largely unbroken. In a heavily infected colony, few larvae live to emerge as adults, and the pattern becomes broken as they die and are replaced in a non-regular order.

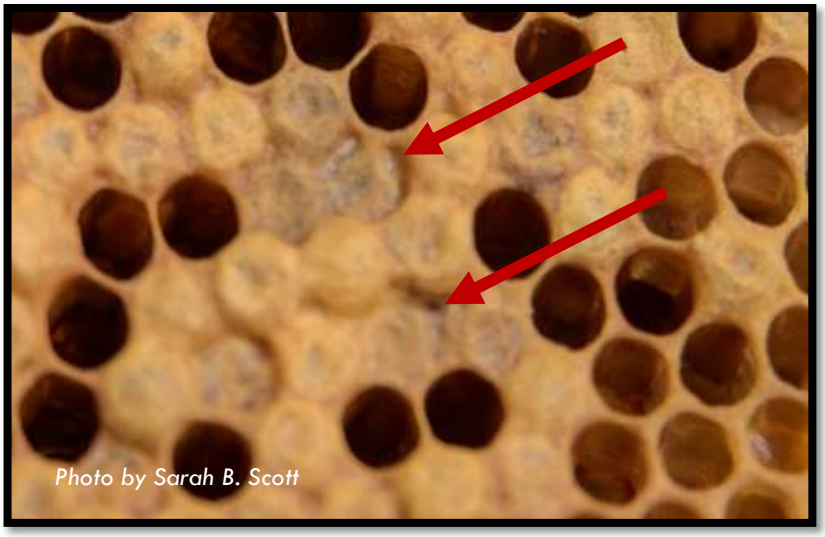
A spotty brood pattern: This pattern is often referred to as "Shotgun" because it looks like shot sprayed on the frame.

Not all spotty brood patterns are caused by AFB. Many other factors can cause spotty brood: other diseases, a poor queen, or environmental factors such as chilling or poor nutrition. In this hive, we saw plenty of eggs, and there was plenty of food available, which suggested that the brood pattern was likely due to disease.

Eggs are visible among the surviving pupae indicating that the spotty brood pattern is not due to a queen issue.

Don't be mistaken! Not all shotgun patterns indicate AFB or other disease.

This brood pattern looks spotty, but it is actually from a very healthy hive which is 'nectar bound' a condition where the beekeeper failed to provide adequate space to allow for nectar storage outside of the brood nest. The queen and larvae are fine, but the nest is too crowded with food for the queen to lay her normal pattern. This is an example of a spotty pattern that does not indicate the presence of disease.



Signs of AFB: Sunken cappings

Larvae that are infected with AFB die just after the cell is capped. The death of the larvae can cause the cap to shrink down, and appear deflated. Other diseases, including non-typical EFB can kill the larvae at this stage, so sunken cappings are usually present, but not unique to AFB.

Note the depressed edges around the cappings, indicating the dead larva underneath

A typical frame of a colony in the early stages of AFB infection. Note the sunken, discolored cappings beginning to form holes, as well as the spotty, irregular pattern. As infection progresses, it is common for these cappings to develop a wet or greasy appearance. Note also that the adult bees that are present will appear to be completely healthy.

Signs of AFB: Holes in cappings

As the larvae continue to die, many of the cappings will darken, and develop a hole. The holes are generally jagged and off-center.

Holes in cappings of AFB-infected drone larvae (the caste is indicated by the larger size of the cell). AFB can infect workers, drones, and queens in the larval state.

Don't be mistaken by healthy holes!

Holes in the cappings are not always indicative of AFB; there are situations where you can witness holes in the cappings in a healthy hive: during the capping process, when the bees are emerging from the cells, and due to hygienic behavior.

Photo by Sarah B. Scott

Holes due to the capping process- Note that the hole is generally centralized. The larvae in cells surrounding the capping with the hole are older – filling the whole cell, and about to be capped themselves. The larvae around the capping with the whole are white, pearly, and healthy.

Photo by Andrea Villarreal

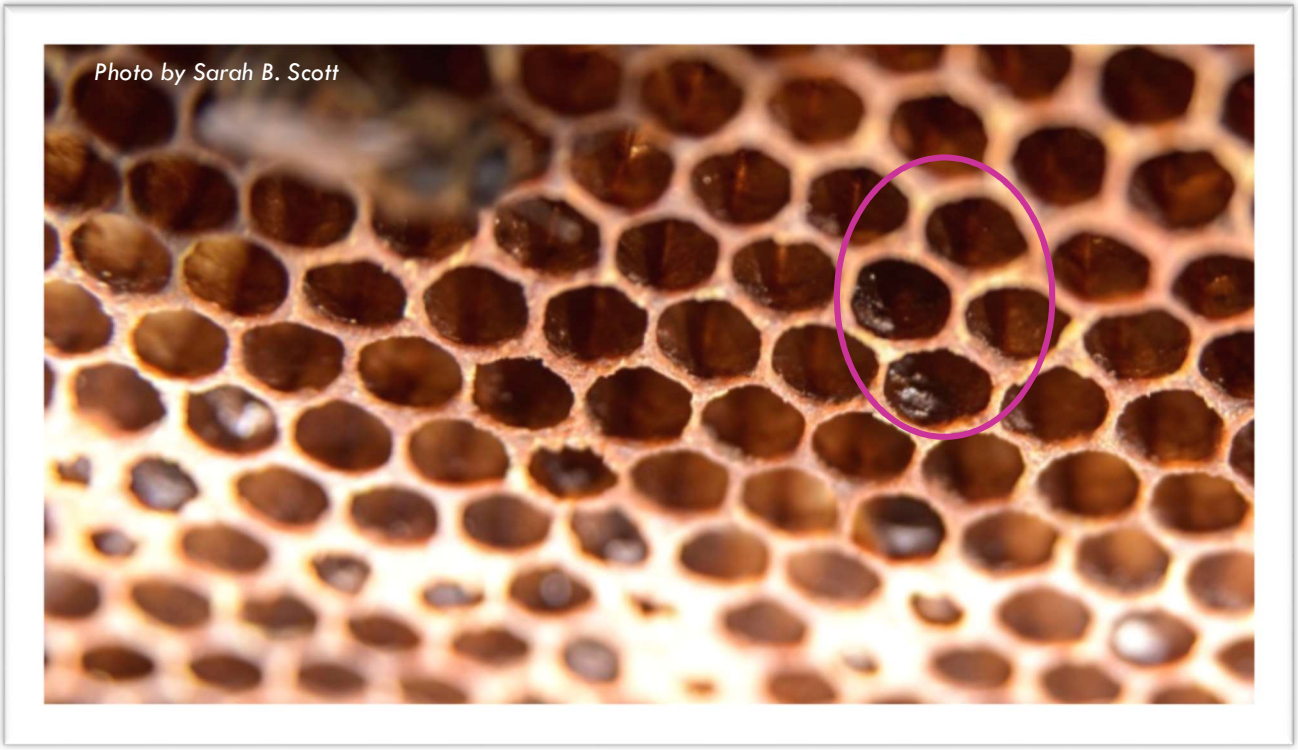
A hole from eclosion (the emergence of a newly formed adult bee from a capped cell). If you look closely, you will see antennae and movement around the hole, and the emerging bee will be alive and healthy.

Photo by Meghan Milbrath

A hole due to hygienic behavior. The bees opened the capping to inspect the pupae underneath. While the cell is not normally uncapped at this stage, but the pupae underneath appears white and healthy.

Signs of AFB: Larval scale

The larvae succumb to AFB just as they are capped. This means that signs of disease are visible in capped cells, as shown above, as well as in in the open cells of older larvae. Healthy larvae are always a brilliant, glistening, pearly white. As the larvae die, they darken and flatten against the lower cell wall, almost looking like they have melted into a goo. Newly dead larvae appear wet, and are light tan or caramel colored. They will continue to desiccate, eventually drying into a scale at the lower cell wall (the side that is parallel to the ground when the frame is in the normal position). The scale of AFB will be hard, and stuck to the cell wall.

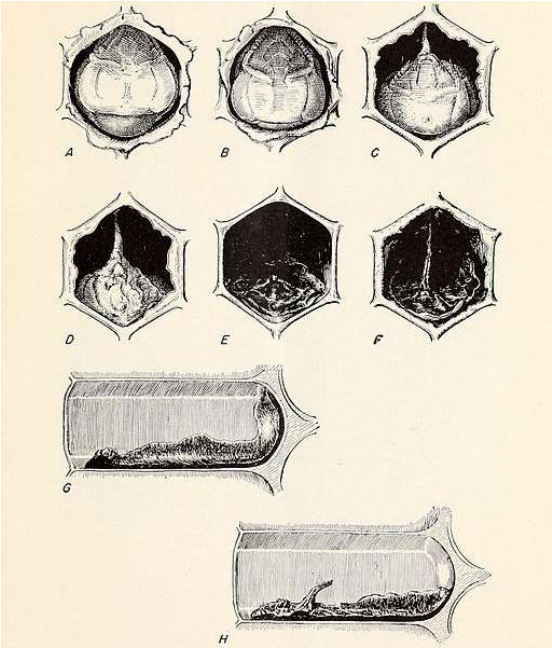


Larval scale. Note the dried, blacked larvae on the wall of the cells (this side would be towards the ground in the hive). Each of these scales can contain millions of AFB spores.

How to inspect for larval scale. Hold the frame with the top bar facing towards you with the sun over your shoulder. Look at the cell wall that is directly facing towards you to inspect for a dried black scale. Photo courtesy of Randy Oliver.

Signs of AFB: Pupal tongue

If the larvae live a bit after they are capped, then they can start to pupate. Larvae that die at this stage often leave what is called a 'pupal tongue' - the larvae melt, but the embryonic proboscis structure, which is more firm, does not melt, and can be retained as a visible point. This sign is less commonly seen, but is unique to AFB. If pupal tongue is not visible, you may still have AFB, but if you do see it, then you very likely are dealing with an AFB infection.



Decay of pupae infected with American Foulbrood. From 'Diagnosing bee diseases in the apiary' By C.E. Burnside. Panel A shows a healthy pupa, while panels B-F show the various stages of decay. The point visible in panel F is commonly referred to as the 'pupal tongue'. via Wikimedia Commons by Burnside, C. E.; Sturtevant, Arnold Parker [Public domain]

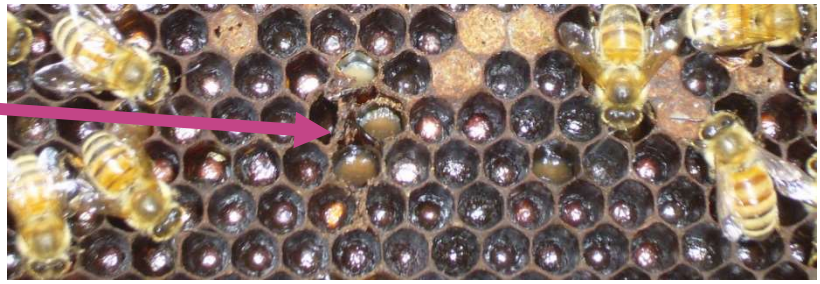
Photo by Sarah B. Scott

Don't be mistaken! *This hive was sick, but not with AFB. This is an adult tongue, not a pupal tongue.*

The photo on the left shows a bee that died just as it emerged from the cell. The tongue is visible, but this is NOT the same as pupal tongue. Note that the entire developed head is present. With AFB, only the tongue is identifiable as a single point, emerging from a scale. The bee on the left likely died from varroa-associated viruses.

Signs of AFB: Caramel color

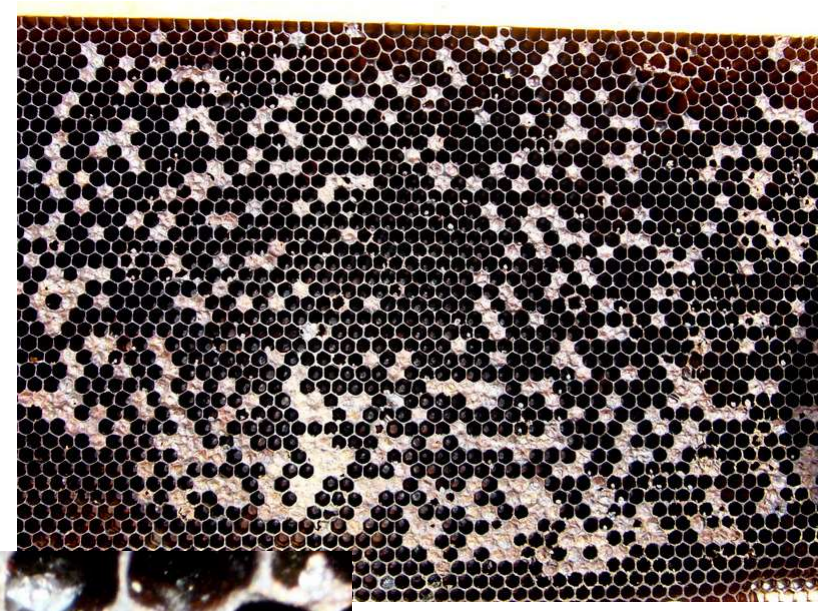
As the bacteria breakdown the larvae, they form a caramel color biofilm. This may not always be visible as this stage is only temporary. This color is characteristic of AFB. Other diseases cause larval discoloration, but they generally range from yellow to gray.



Frame with AFB with characteristic caramel coloring. Photo by Randy Oliver.

A note on old equipment

Many new beekeepers are tempted to purchase used equipment or to use old equipment from a friend or family member. A common **high-risk** scenario is a beekeeper who wants to start beekeeping using their grandfather's equipment that they found out in the barn. If the grandfather stopped keeping bees in the 1930s - 1980s there is a real chance that they stopped beekeeping after they lost their bees to American Foulbrood, as the disease peaked during that era. It is highly likely that this equipment can still contain viable spores of AFB, and pose a threat to bees. It is impossible to use any of the field tests to determine if the old equipment is safe. If a beekeeper wants to use old equipment of unknown origin or uncertain history, the frames should all be burned, and the rest of the woodenware should be sterilized as explained later in this document. If the used equipment is from a new beekeeper who quit beekeeping after 1-2 years of failure (which is also common), there is still a risk of AFB spores, but it is not likely. The current main epidemic is varroa-associated viruses, which are not known to be as environmentally stable. When starting new hives, the best practice is to always use new equipment and to carefully inspect any incoming equipment, such as during the purchase of nucleus colonies.



A typical looking frame from a colony that had died from AFB. Spores may still be present on this frame, and using it in a hive with a new colony will likely lead to infection of that colony. Photo by Randy Oliver

Visible AFB scales in an old frame. While other diseases may cause scales (e.g. non-typical EFB), the risk of AFB infection is too high to risk using these frames. Any frames with visible scales should be immediately burned. Photo by Randy Oliver

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).

Taking a sample for diagnosis at the National Laboratory

The USDA-ARS runs a honey bee disease diagnostic service in Beltsville, MD that can test for AFB, EFB, and other diseases. You should submit a sample from any hive where AFB is suspected. Even if your field tests indicate a negative result, it is still useful to send a sample. The lab can track antibiotic resistance and it is useful data for them to see where infections are occurring.

In the spring of 2017 the honey bee laboratory experienced a staffing crisis and was not accepting samples, and has similarly been closed during government shut downs. Check their website before shipping samples.

https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-agricultural-research-center/bee-research-laboratory/docs/how-to-submit-samples/

How to send brood samples:

- A comb sample should be at least 2 x 2 inches and contain as much of the dead or discolored brood as possible. NO HONEY SHOULD BE PRESENT IN THE SAMPLE.
- The comb can be sent in a paper bag or loosely wrapped in a paper towel, newspaper, etc. and sent in a heavy cardboard box. *A small flat rate mailer box from the post office is just the right size, currently $7.25 postage cost.*
- AVOID wrappings such as plastic, aluminum foil, waxed paper, tin, glass, etc. because they promote decomposition and the growth of mold.
- If a comb cannot be sent, the probe used to examine a diseased larva in the cell may contain enough material for tests. The probe can be wrapped in paper and sent to the laboratory in an envelope. In a worst-case scenario, a diseased larva can be smeared on a 3 x 5 index card, folded, and sent for sampling.

Send samples to

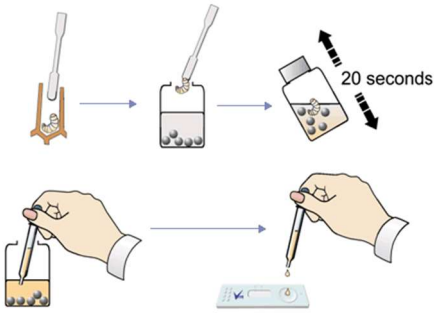
Bee Disease Diagnosis
Bee Research Laboratory
10300 Baltimore Ave. BARC-East
Bldg. 306 Room 316
Beltsville Agricultural Research Center - East
Beltsville, MD 20705

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

AFB field tests: 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. (HTTP://WWW.VITA-EUROPE.COM/PRODUCTS/AFB-DIAGNOSTIC-TEST-KIT/)

Below are the methods used in the summer of 2017 – read the manufacturer's instructions carefully, as the process may change. Photos by Sarah B. Scott

1. Lay out all of the kit contents and pull a frame with suspect larvae.

2. Using the included spatula from the kit find a small amount of symptomatic larvae. You don't need a lot, and too much may clog the device. Only a tiny amount is needed.

3. Put the suspect larvae in the jar and screw the lid on tightly. Be careful not to tip or spill the jar as the contents (sodium azide) are toxic to humans.

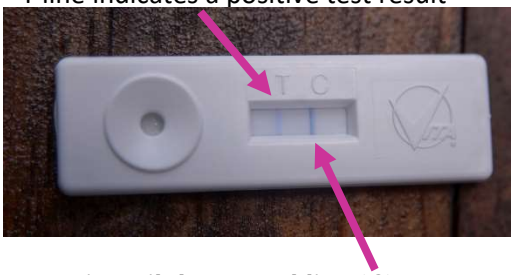
4. Shake the jar vigorously for 20 seconds.

5. Remove the test device from the foil pack--be careful to NOT touch the viewing window or to leave it out before adding the sample.

6. Use the pipette to remove a sample from the bottle.

7. Gently squeeze two drops onto the sample well of the device.

T line indicates a positive test result



8. Wait until the control line (C) appears, and read the result in 1-3 minutes. Even a faint test line (T) indicates a postive result.

AFB field tests: 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 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.

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.

Don't be Mistaken!

The photo to the right 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.

AFB field tests: 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.

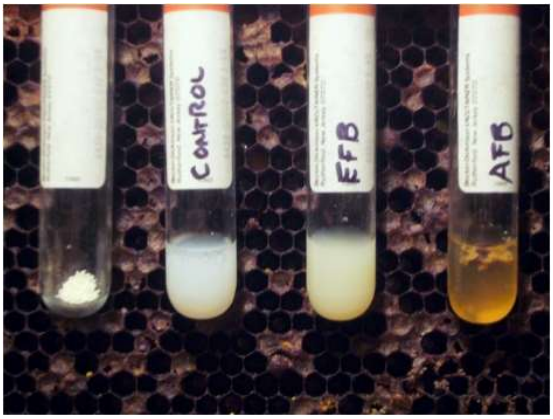
Step 4: Add a sample to the tube marked 'sample'.
You can use a capped larva, the results of your match stick test, or a dried scale. The more sample you add, the faster the reaction will occur. The enzyme is not present in young larvae, so older larvae should be used. Remember, you are looking for the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

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.

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.



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.


Photo by Randy Oliver

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.


Photo by Sarah B. Scott

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.

Frame exchange / Shook swarm

Many beekeepers are resistant to burning their bees, and in many cases, it is not necessary to burn all of the equipment. If you live in an area that does not require burning, the colony is large, and it is early enough in the season for the bees to draw wax, you can make a 'shook swarm'. When making a shook swarm, the bees are shaken onto new equipment, emulating the swarming/ absconding behavior of a colony that leaves a nest when disease pressure is too high. This process of exchanging out all the frames is only recommended in states where there is no requirement to burn infected hives and there is enough time left in the season for the colony to rebuild and recover to wintering strength. If it is late in the season (fall) then the colony should be euthanized, and the equipment destroyed.

How to do a frame exchange/ shook swarm:
In this method you must be very careful to keep track of what you touch. Deal with infectious materials first, then change gloves/wash your hands and use new tools when handling the new equipment. Repeat anytime you touch anything that may be contaminated with spores.

1. Set up a new hive with new equipment
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 *(Photos by Sarah B. Scott)*

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.

Typical method of antibiotic application. The antibiotic is mixed with dried sugar and applied to the top of the frames in the brood nest according to the rate indicated on the label. The label indicated that this specific antibiotic required 3 successive weekly applications which allowed for subsequent feeding and close monitoring.

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

Photo by Meghan Milbrath

Some beekeepers live in areas where sterilization facilities are available. In the photo to the left, hive bodies with frames are wrapped and ready for irradiation at the Iotron facility in Indiana. 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.

Photo by Sarah B. Scott

Wax dipping

Photo by Kirk Mason

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

Three antibiotics are currently available for use against AFB: Oxytetracycline Hydrochloride (Terramycin), Tylosin Tartrate (Tylan), and Lincomycin. As of 2017, antibiotics for honey bees, like other food-producing animals are only available through the care of a licensed veterinarian. A veterinarian will provide an order - either a prescription or a form called a veterinary feed directive (VFD). The type of antibiotic order used depends on the label of the antibiotic (A listing of current (2018) antibiotic formulations and the required order is in appendix 1). The type of order will determine where the antibiotic can be purchased and how it can be used. Prescriptions are filled at pharmacies, while VFD drugs can be purchased at licensed VFD distributors. The FDA maintains a list of VFD distributors by state.

More information on changes in antibiotic access for honey bees is available at Michigan State University's website: https://pollinators.msu.edu/programs/bees-need-vets/

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.

AFB Quick Sheet

American Foulbrood is a highly infectious bacterial disease of honey bees. It has an environmentally stable spore form that can re-infect colonies after many years. It is essential to remove infected materials and to practice good hygiene when AFB is suspected or confirmed.

Signs of American Foulbrood:

1. Characteristic smell
2. Spotty brood pattern
3. Sunken cappings
4. Holes in cappings
5. Caramel color of dead larvae*
6. Pupal tongue*
7. Larval scale

*These signs are not always present, but are unique to AFB.

Some of these signs are also seen in other diseases, especially European Foulbrood and Parasitic Mite Syndrome. It is important to understand the signs of these diseases as well in order to correctly diagnose and appropriately respond.

If you see signs of AFB, you can use a field test. The following can support a diagnosis of AFB, but only work during active infection – there is no field test that can detect the spore form.

Field tests:

1) Match stick test
2) Holst milk test
3) Commercially available ELSIA test

Regardless of your field test results, it is important to submit samples to the national laboratory in Beltsville, MD. They can do further tests, and can also identify if the bacteria are resistant to antibiotics.

What to do if you have a colony with American Foulbrood:

1) Make sure you know the regulations in your state. It may be a reportable disease or you may be required to burn the colony.
2) The best practice is to burn the colony and all the components immediately, and to bury the ashes.
3) If you cannot burn in your area, the equipment should be kept out of reach of bees until it can be burned or double bagged and disposed of in a landfill.
4) The frames should always be disposed of from an infected colony. Other equipment can be sterilized using extended high heat, but colonies in sterilized equipment should be closely monitored.
5) Antibiotics should be provided to all colonies in an apiary where AFB has been identified.
6) All colonies in an apiary where AFB has been identified should be watched closely, and equipment from that yard should be kept quarantined from other beekeeping equipment.

Appendix 1: There are 11 applications approved by the FDA for used in bees, containing one of 3 antimicrobials – oxytetracycline, tylosin, or lincomycin. For more resources on antibiotic use in bees visit https://pollinators.msu.edu/programs/bees-need-vets/bee-vet-resources/

[Abbreviated] New Animal Drug Application #	Marketing Status (Antibiotic order needed. Rx = Prescription, VFD = Veterinary Feed Directive)
Oxytetracycline - N-008-804	VFD
Oxytetracycline - N-095-143	VFD
Oxytetracycline - N-138-938	VFD
Oxytetracycline - N-008-622	Rx
Oxytetracycline - A-200-247	Rx
Oxytetracycline - A-200-146	Rx
Oxytetracycline - A-200-026	Rx
Tylosin - N-013-076	Rx
Tylosin - A-200-473	Rx
Tylosin - A-200-455	Rx
Lincomycin - N-111-636	Rx

The specific conditions of use and other information for each of these applications may be viewed by clicking the hyperlinks above or entering the application number at Animal Drugs @ FDA.

Table taken from: https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm589399.htm

Appendix 2: Items that would be useful in a diagnostic veterinary kit

- ☐ Field Guide
- ☐ Hand sanitizer/alcohol
- ☐ Jug of water
- ☐ Box and labels to send samples to Beltsville
    - Newspaper to wrap comb samples
- ☐ Mite check kit
    - jar with size 8 hardware cloth lid
    - ½ cup scoop
    - Powdered sugar or alcohol
- ☐ Holst kit
    - 2 tubes
    - Coffee stir sticks
    - Nonfat dry milk
- ☐ Nitrile gloves
- ☐ Commercial Elisa kit
- ☐ Flashlight
- ☐ Magnifying glass
- ☐ Lighter
- ☐ Contractor bags
- ☐ Small trash/ziplock bags for used gloves or dirty hive tools
- ☐ At least 2 hive tools
- ☐ Veil

Other resources

- Diagnosis of Honey Bee Diseases United States Department of Agriculture Agricultural Research Service Agriculture Handbook Number 690 https://www.ars.usda.gov/is/np/honeybeediseases/honeybeediseases.pdf
- A Field Guide to Honey Bees and Their Maladies, Penn State Extension http://extension.psu.edu/publications/agrs-116
- Bee Informed Partnership Blog on American Foulbrood https://beeinformed.org/2013/10/21/american-Foulbrood-afb/
- Diagnostic kits - http://www.vita-europe.com/products/afb-diagnostic-test-kit/
- https://agdev.anr.udel.edu/maarec/honey-bee-biology/honey-bee-parasites-pests-predators-and-diseases/

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