

National Honey Bee Pests and Diseases Survey Sampling Protocol

Overview

Detailed information about the National Honey Bee Pests and Diseases Survey, including the project plan, training videos, and all protocols, is located at the Animal and Plant Health Inspection Service (APHIS) [Survey of Honey Bee Pests and Diseases](#) webpage.

This document outlines the sampling protocol to collect adult bees and conduct *Tropilaelaps* spp. “bump” tests in eight randomly selected colonies in each participating apiary.

Please read this protocol carefully and watch the training video accessible using the link below prior to initiating sampling.

https://youtu.be/MZ_a6BJEGml?si=ZbjJfi2smF60XDTD

For additional information, email Rachel Fahey (University of Maryland) at nhbs@umd.edu or Josie Ryan (APHIS) at josie.k.ryan@usda.gov.

List of Equipment Needed for Sampling/Shipping



Figure 1: Reusable equipment needed for sampling apiaries (provided by the participating state or territory). Also needed are basic beekeeping protective equipment (e.g. coveralls, veil, etc.) and beekeeping tools (e.g. smoker, hive tool, etc.), which are not provided.

For all apiaries (to be provided by your state unless otherwise noted; Fig 1)

- ✓ 1 plastic wash tub
- ✓ 1 metal pan
- ✓ ¼ cup measuring cup
- ✓ 2 radiator funnels
- ✓ 1 gallon jug (to be filled with water)
- ✓ 1 squeeze bottle (to be filled with water)
- ✓ 1 gallon of chlorine bleach (to be used for sterilizing equipment) (not pictured)
- ✓ 1 gallon sized bucket
- ✓ mesh strainer
- ✓ binder clips
- ✓ scissors
- ✓ writing pen
- ✓ permanent marker
- ✓ 1-inch wide transparent shipping tape

Per apiary (contained in your live bee sampling kit provided by the UMD; Fig 2)

- ✓ 1 live bee shipping box
- ✓ 1 pre-addressed mailing label
- ✓ 1 data sheet
- ✓ Sample ID labels
- ✓ 1 large (500 mL) bottle with alcohol
- ✓ 1 small (125 mL) bottle with alcohol
- ✓ 1 cloth filter
- ✓ queen candy in petri dish on bottom of the live bee shipping box
- ✓ 1 capped 15 mL tube filled with water and a sponge
- ✓ brochure for the beekeeper

Procedure Summary

Before beginning, ensure all equipment in the “List of Equipment” is on hand as well as beekeeping protective gear and beekeeping tools. Remember to fill a one-gallon bottle and squeeze bottle with water.

Identify an apiary with at least ten colonies of bees (eight of which will be sampled, with two extra in case an inspector encounters dead outs or queen-less colonies during inspection). Dead outs and queen-less colonies should not be included in the survey sampling. Prioritize sampling from queen producers, package/nuc producers, honey producers, and apiaries used for crop pollination. Select apiaries in areas at high risk for exotic pest invasion (near deep water shipping ports, international airports, and high traffic areas for migratory beekeeping). Apiaries should be chosen to give as close to an equal representation of the entire state as possible. Ideally, a state will be sectioned into four quadrants with apiaries randomly chosen from each quadrant.

When sampling an apiary, it is critical to select colonies at random rather than haphazardly or regularly spaced. Colonies should **not** be preferentially selected because they seem “healthy” or “sickly”. One way to select colonies at random is to “assign” them numbers in ascending order. Use a random number generator app on your phone to select each colony you will sample.

Important: Split up your sampling trips so you are not sampling all of your beekeepers within the same season. Take samples in the spring, summer, and fall. This provides a more comprehensive pest and disease baseline for your state.

Identify the nearest US Post Office (USPS) or other shipping facility (and confirm their operating hours) to this apiary so that you can mail the live sample of bees immediately. If you plan to mail several boxes, ensure that all boxes are ready to be mailed before the Post Office or shipping facility closes.

- a. It is best to ship live bees between Monday and Wednesday so they do not arrive at the molecular labs (University of Maryland (UMD) Honey Bee Lab or Washington State Department of Agriculture (WSDA) Molecular Diagnostic Lab) on the weekend when there is no one available to receive them. **Please do not sample and ship live bees on Thursday or Friday.**
- b. It is legal to mail live bees by ground transportation through USPS. Please tape the box well and ensure the air vents are not covered.. We will contact you if your bees are dead on arrival (unsuitable for molecular testing). If difficulty arises, there is an alternative solution available which requires additional training. Please contact Rachel Fahey for more information on alternative sampling methods (nhbs@umd.edu).
- c. Go to the [USPS webpage for Mailable Live Animals](#) for more information on the postal service regulations.

Once you have identified the apiaries that you will sample, explain the sampling process to the beekeeper. Show the beekeeper the brochure and answer any questions they may have.

Set-up

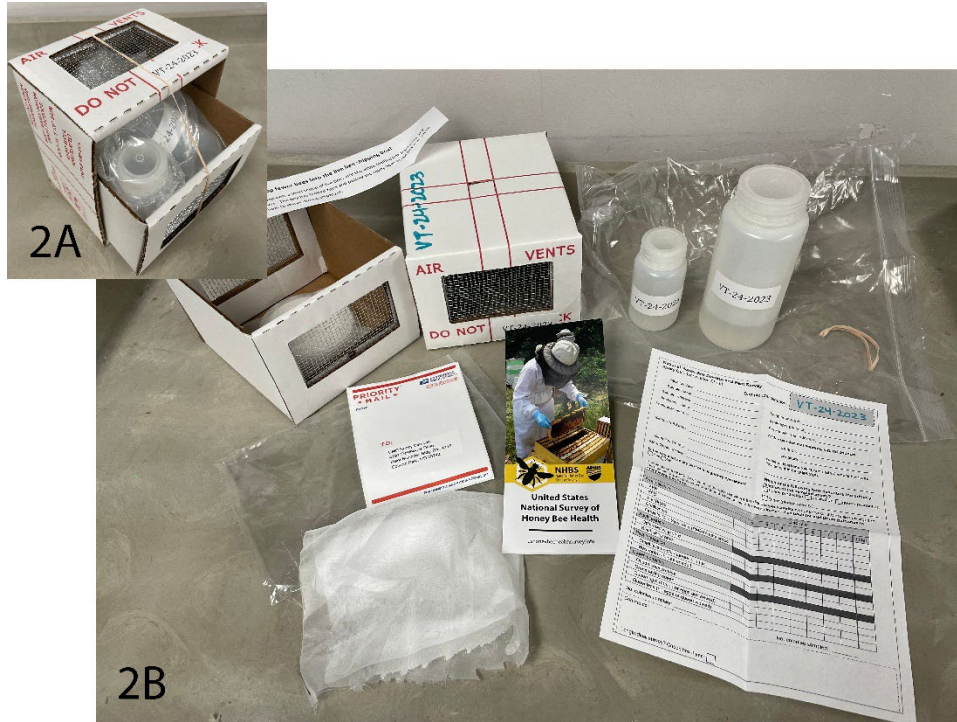


Figure 2(A and B): A live bee shipping box which contains all the materials provided by UMD to collect and ship samples in one apiary.

1. Open one live bee mailing box (Fig 2), it should contain:
 - a. Data sheet.
 - b. Sample ID labels.
 - c. Large bottle and small bottle containing ethanol.
 - d. Petri dish glued to box floor containing queen candy.
 - e. Capped 15 mL tube filled with water and a wicking sponge.
 - f. Cloth filter.
 - g. Mailing label.
2. Place sample identifying labels onto (Fig 3):
 - a. Data sheet.
 - b. Large collection bottle containing ethanol.
 - c. Small collection bottle containing ethanol.
 - d. Live bee shipping box.

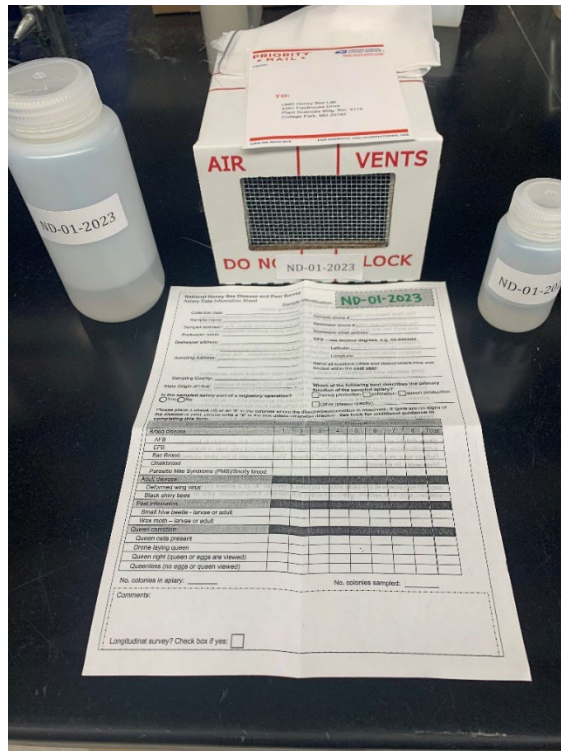


Figure 3: Place identification label onto data sheet, large bottle, small bottle, and mailing box.

3. Fill out the data sheet. An electronic version of the datasheet is available at the [APHIS Honey Bee webpage](#). Please scan and email the completed data sheet to nhbs@umd.edu.
4. Set up sampling equipment:
 - a. Remove and discard the plastic top of petri dish and the wax paper covering the queen candy (Fig 4).
 - b. Remove and discard the cap from the 15 mL tube containing the wet sponge to allow bees access to the water source. Place tube in live bee box (Fig 5).
 - c. Close the shipping box, making sure the two sides with metal screens match up to ensure proper ventilation for live bees, and place a funnel into the hole on the top of the box (Fig 6A).
 - d. **BE SURE TO KEEP THE LIVE SHIPPING BOX IN THE SHADE AND OUT OF DIRECT SUNLIGHT AND WIND**, otherwise the sampled bees may overheat and die.
 - e. Open the large alcohol bottle and place a second funnel into its mouth so it can receive bees (Fig 6B). It may be helpful to place the alcohol bottle in the center of a roll of duct tape to stabilize the bottle.



Figure 4: To ensure queen candy does not dry out before use, it has been sealed with wax paper in the petri dish. Remove and discard the petri dish cover and the wax paper before placing bees into the live shipping box.

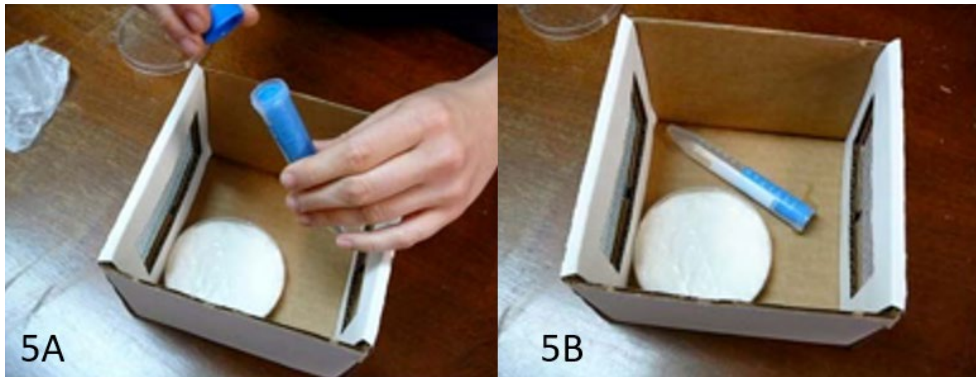


Figure 5(A and B): Bees require water during transport, so the test tube with the sponge and water should be UNCAPPED before placing bees into the live shipping box.

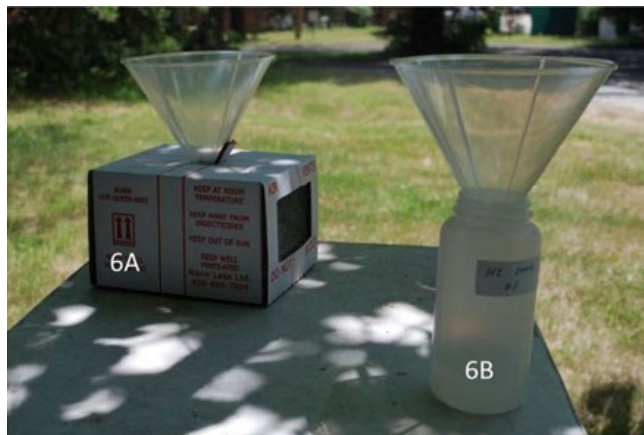


Figure 6A: Live bee shipping box with funnel inserted to receive bees. Figure 6B: Large bottle with funnel ready to receive bees.

Important: The goal is to sample at the apiary level. You will be collecting two scoops of live bees from each of the eight colonies. You will place one scoop in the live bee box and the other scoop in the large bottle of alcohol. You will also “knock” a single brood frame from each colony over the large metal tray and then wash the resulting debris into the smaller bottle of alcohol. It is critical to execute the “knocking” procedure effectively because this debris sample is used to screen for the invasive mite *Tropilaelaps*. Samples from all colonies are being placed in the same live shipping box and large alcohol collection box, and debris from 8 frames from 8 different colonies are being knocked into the same metal tray.

Live Bee Sample

5. Open the colony to the brood nest and examine for disease and queen status/condition. Record any disease/queen conditions on the data sheet.
6. Find a frame containing at least some uncapped brood (Fig 7). Ensure that the queen is not present on this frame. If no brood is present, and another colony is available, do not sample the brood-less colony. If no other colony is available, take an adult bee sample from a frame in the center of the brood nest.



Figure 7: A frame containing sufficient adult bees, uncapped and capped brood for sampling.

7. Shake bees from the frame into the wash tub (Fig 8).
8. Knock the wash tub to gather bees into one corner of the tub (Fig 9).



Figure 9

Figure 8: Shake bees from frame into wash tub. Figure 9: Knock bees into corner of tub to facilitate easy removal of ¼ cup samples of live adult bees.



Figure 10: A brimming ¼ cup of bees.

9. Scoop ¼ cup of adult bees (Fig 10) into the funnel inserted into the lid of the live bee shipping box (Fig 11). Gently tap the box to force the bees from the funnel into the box (Fig 12). Do not collect more than ¼ cup of bees because overcrowding will result in mortality during shipping.
 - a. **BE SURE TO KEEP THE LIVE SHIPPING BOX IN THE SHADE AND OUT OF DIRECT SUNLIGHT AND WIND.**



Figure 11: Place bees from cup into funnel inserted in the live bee shipping box. Figure 12: Gently tap live bee shipping box and funnel so bees are forced into the collection box.

Alcohol Bee Sample

10. Scoop another $\frac{1}{4}$ cup of bees from the tub into the funnel inserted in the large bottle containing alcohol; tap the bottle and funnel to force bees into the bottle (Fig 13).

Pro Tip: It may be helpful to put the lid from the 500 mL alcohol bottle over the opening of the funnel to reduce the risk of live bees escaping. This may also help you keep track of the lid.



Figure 13: Place a second $\frac{1}{4}$ cup of bees into large bottle of alcohol.

***Tropilaelaps* Sample**

11. Examine the frame of brood from which the bees were removed in Step 8 and look for any mites that could be scurrying across the surface. *Tropilaelaps* mites move quickly in comparison to *Varroa* and are 1/3rd of their size. If you observe this, please take photos, make note of it on the Notes section at the bottom of the data sheet **and contact UMD immediately.**
12. Hold the frame of brood over the metal collection pan with one surface of the frame facing down (Fig 14). Firmly knock the outer edge of the frame in the pan to dislodge mites, beetles and other hive debris from the frame into the collection pan (Fig 15). **Furthermore, please inspect the debris in the pan as you bump each frame. If there is anything out of the ordinary (mites that should not be there) and if you view what is a suspected *Tropilaelaps* mite, please photograph it, collect the suspect mite, and contact UMD immediately.**



Figure 14: Firmly knock the frame over the metal collection pan by allowing one end bar to hit the metal collection pan. Figure 15: Debris dislodged from frames after being knocked in collection pan.

13. Knock the frame a second time.
14. Flip the frame so that the frame surface facing down in Step 13 is now facing up.
15. Knock the frame twice in the same manner outlined in Steps 13 and 14.
16. Switch the frame so you are now holding the opposite end of the frame and repeat Steps 13-16. Each frame should be flipped 2 times, so that the frame is knocked a total of eight times (two knocks, flip over, two knocks, switch the frame end to end, two knocks, flip over, two knocks).
17. Place the brood frame back into the colony and move any bees remaining in the collection wash tub back to the colony.
18. Close the colony.
19. Repeat Steps 5 through 19 until eight colonies have been sampled.

Sample Preparation



Figure 16: Close the large sampling bottle containing 2 cups of bees (1/4 cup from eight different colonies), tightly seal the lid of the container, and tip the bottle several times to ensure all bees are damp with alcohol.

20. Close the large bottle containing alcohol and tip the bottle several times, dampening the bees inside (Fig 16).
21. Attach the cloth filter into the strainer using binder clips (Fig 17).
22. Place about two cups of water into the metal collection pan and gently swirl the contents (Fig 18) before pouring the water and debris through the cloth filter (Fig 19). Be sure to not let any water flow over the edge of the cloth filter.



Figure 17



Figure 18



Figure 19

Figure 17: Secure cloth filter to the strainer using binder clips.

Figure 18: Place water into metal collection pan to dislodge debris.

*Figure 19: Pour water and debris from metal collection pan through the cloth filter. Inspect the debris as you rinse for any signs of *Tropilaelaps* mites.*



Figure 20



Figure 21



Figure 22

Figure 20: Using squeeze bottle, ensure last of debris in metal collection pan is filtered through cloth filter.

Figure 21: After allowing filter to drip dry for a few minutes, gently fold filter so debris is secure.

Figure 22: Place folded filter into small bottle with alcohol. Secure the bottle lids.

23. Repeat Step 23 until most of the debris in the collection pan has been removed, then using the squeeze bottle remove what debris remains and pass this through the cloth filter (Fig 20). **Again, please inspect the debris as you wash it into the filter to determine if there is anything out of the ordinary. If you view what is a suspected *Tropilaelaps* mite, please photograph it, collect the suspect specimen, and contact UMD immediately.**
24. Allow the cloth filter to drip dry for a couple of minutes, and then gently remove it from the strainer, folding it so that the filtered debris remains secure in the center of the filter (Fig 21). This debris will be examined for mites, specifically *Tropilaelaps*, upon arrival at the UMD Honey Bee Lab.
25. Place the entire gently folded cloth filter into the small bottle containing alcohol and seal the bottle tightly to avoid spills during transport (Fig 22). Please make sure all of the filter material is in the main part of the bottle and none is on the neck of the bottle. Save the binder clips to be reused at subsequent apiaries.

Shipping Samples

26. Double check the lids of the small and large collections to make sure they are tight. Place both the small (containing the cloth filter) and large bottle (containing bees) into large Ziplock bags to contain any leaks from the alcohol before placing them into the large Priority Mail flat rate box. The samples should be mailed as soon as possible so they can be examined for invasive threats to U.S. honey bees. Prior to mailing, place one of the pre-addressed shipping labels (**UMD Honey Bee Lab, 4291 Fieldhouse Drive, Plant Sciences Building Room 4112, College Park, MD 20742**) at the appropriate place on the box. Write "FROM" and your return address on the upper left corner of the box.



Figure 23: After ensuring the data sheet has been filled out completely, and after making a copy of this sheet for your records, place it in its own Ziplock bag and place this Ziplock in the prepaid mailing box containing the sample bottles. (Note: Do not put the data sheets in the same Ziplock bag as the sample bottles, as the bottles may leak and make the writing illegible.) When ready to ship samples, double check all lids are securely fastened, put the sample bottles in Ziplock bags and add packing material (e.g., newspaper), if necessary, so bottles are not damaged during shipment. Add sealed Ziplock bag with data sheets and seal box with binding tape.

27. Ensure the data sheet is completely filled out and legible. Please make a scan (there are many free phone apps that use a phone camera and can produce a pdf scan) of this sheet for your records and email it to nhbs@umd.edu, and then place it into a Ziplock bag (with any other data sheets you have already filled out). The Ziplock bag containing the data sheets should be placed in the mailing box containing sample bottles (Fig 23).

Pro tip: Cut a piece of transparent shipping tape and have it ready to cover the hole in the live bee box BEFORE you take the funnel out. Bees will try to fly out as soon as the hole is opened.

28. Remove the funnel from the live bee shipping container and cover the hole with the cardboard flap (Fig 24).
29. Secure the hole and the lid of the live bee shipping box to the bottom of the box with the transparent shipping tape (Fig 25).
30. Place the mailing label with the address of your assigned molecular lab (see Shipping Instructions document) over the now sealed hole on the top of the live bee shipping box and secure it with tape (Fig 26). Write "FROM" and your return address on the upper left corner of the box.

Note: Please ensure that the sample label is clearly visible on the live bee box and is not covered by tape or postage.



Figure 24: Secure the hole in the lid of the live bee shipping box with clear binding tape.

Figure 25: Secure the lid of the live bee shipping box to the bottom of the box using the clear tape.

Figure 26: Place pre-addressed shipping label on the box. Clearly print "FROM" and your return address on the top left corner of the box. Make sure the identification number label is legible and securely attached to box. Mail the live bee shipping box, containing collected live bees, at the nearest Post Office as soon as possible.

31. **Mail the live bee shipping box, containing collected samples, at the nearest Post Office or other shipping facility as soon as possible.**
32. Collect and clean sampling equipment (pans, hive tools, funnels, and ¼ cup scoop) with 10% bleach and water solution. You may also sanitize hive tools by scorching them in your smoker.
33. Email the UMD Survey Coordinator (nhbs@umd.edu) within 24 hours of shipment to notify the UMD lab that a live bee shipment is expected. Please also email Rachel Fahey within 24 hours after shipping alcohol and filtered samples.

Important: We request that all *Tropilaelaps* frame bump samples be sent in as soon as possible for us to perform our screening diagnostic. If you are sampling multiple apiaries over the course of a week, sending all the *Tropilaelaps* samples together would be optimal.

Post Sampling Checklist

- ✓ Scan data sheets and email them to the UMD Survey Coordinator (nhbs@umd.edu)
- ✓ Put paper copies of data sheets into a Ziploc bag. Please do not put them in the Ziploc bag with the ethanol preserved samples as the samples may leak while in shipment and destroy the writing on the sheets.
- ✓ Take the live bee sample to a United States Post Office or other shipping facility within 24 hours and mail it to your assigned molecular lab (see Shipping Instructions document). Email the molecular lab (UMD: nhbs@umd.edu or WSDA: Plantpathlab@agr.wa.gov) to notify the lab that a live sample is expected.
- ✓ If you are not planning on shipping the ethanol preserved samples right away, store them in a refrigerator until your sampling trip has concluded. Please do not hold them for an extended time period because it delays the lab's examination for invasive threats to honey bees.
- ✓ When ready to ship alcohol preserved samples, double check all lids are securely fastened, put the sample bottles in Ziplock bags and add packing material (e.g., newspaper), if necessary, so bottles are not damaged during shipment. Add sealed Ziplock bag with data sheets and seal box with binding tape. Again, notify nhbs@umd.edu when you are mailing samples to the lab.