

# Bee Informed Partnership

## Emergency Response/Diagnostic Kit



### Sampling Protocol

This sampling project is based on the sampling procedures for the National Honey Bee Disease survey. While the sampling procedure is largely the same it does differ in some important ways; however, it may be good to watch the watch the training video ([http://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/honey\\_bees/downloads/National%20Bee%20Survey%20Video\\_small.wmv](http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/National%20Bee%20Survey%20Video_small.wmv)) prior to initiating sampling. BE SURE TO READ THESE INSTRUCTIONS CAREFULLY BECAUSE THERE ARE SOME IMPORTANT DIFFERENCES BETWEEN THE NATIONAL HONEY BEE DISEASE SURVEY AND THIS ONE. If you have any questions or need additional information, email [heversol@umd.edu](mailto:heversol@umd.edu), 301-405-3799.

This Emergency Response/Diagnostic kit is optimally designed for sampling 16 colonies total, 8 from healthy colonies and 8 from weak or crashing colonies. ***If you do not have 16 colonies, you may sample fewer but should still sample from two groups: assumed healthy colonies and those that you suspect are weak.*** You should not expect results to be a definitive diagnosis as to the causes of honey bee mortality in your operation from the processing of these kits. Rather, we should be able to rule out factors that have not contributed to your losses.

***Please note: It is best to ship live bees between Monday and Wednesday so they do not arrive at the University of Maryland Lab on the weekend when there is no one available to receive them. Please do not sample and ship live bees on Thursday.***

**Overview:** The goal is to collect 2 samples of adult bees, 1 sample from 8 strong colonies (or less if you don't have 8 in this group) in each apiary and 1 sample from 8 weak or crashing colonies (or less if you don't have 8 in this group). You will need to open 16 colonies (or fewer, depending on your sampling number) and remove 1 frame that contains young, developing brood (if available) and shake the adult bees into the box used for the emergency response/diagnostic kit. Scoop the bees into the measuring cup. You will collect three,  $\frac{1}{4}$  cup scoops of bees from each colony. Two,  $\frac{1}{4}$  cup scoops of bees will go into salt water bottle and then sealed for an individual salt water sample, and the third,  $\frac{1}{4}$  cup scoop of bees will be placed in the live bee box making a combined live bee sample from 8 colonies. This procedure will be repeated a second time for the 8 unhealthy hives. You will leave the apiary with 2 combined samples of live bees (one box containing the assumed healthy colonies and one box containing the assumed sick colonies), 16 samples of bees in salt water solution in the small salt water bottles. You will want to provide each colony with an identification to label the hives that

you sampled. Please also label each salt water solution bottle to match your colony ID and include on that label whether the colony was 'healthy' or 'crashing'.

**YOU WILL NEED TO PROVIDE:**

- large Priority Mail flat rate boxes (to mail salt water samples)
- postage for above boxes
- transparent shipping tape
- writing pen
- marker or sharpie
- 1 pair of scissors

**PROVIDED ITEMS (See Figure 1):**

Included in Emergency Response Kit and Diagnostic Test kit for all colonies are the following items:

- 2 live bee shipping boxes
- 3 mailing labels for live bee shipping boxes & concentrated salt water solution samples
- 2 data sheets
- 16 small (125 mL) bottles with concentrated salt water solution
- queen candy in petri dish on bottom of the live bee shipping box (food supply)
- 2 capped 15 mL tube filled with water and a sponge in live bee box (water supply)
- zip lock bags (place sample bottles and data sheets in bags prior to shipping)
- 1 funnel
- ¼ cup measuring cup



Figure 1: Sampling Kit

## Steps:

1. Open box and remove wax paper covering queen candy (in petri dish glued to floor of box). See Figure 2. Label 125ml sample bottles with a sharpie according to its corresponding colony.
2. Remove cap from 15 mL tube containing the wet sponge to allow the bees access to a water source (Figure 3). Place tube back in the live bee box (Figure 4) and secure it with tape to the inside of the box. Close the box making sure the two sides of the box with metal screens match up to ensure proper ventilation.



Figure 2: removing wax paper from fondant



Figure 3: Uncapping water source



Figure 4: Live bee box ready for use

3. Be sure to keep the live shipping box in the shade and out of direct sunlight and wind while collecting remaining samples.
4. Open colony to the brood nest and examine for disease and queen status/condition. Record any disease/queen status, or unusual conditions present on the data information sheet. Remember to record only healthy colony data on the same sheet and crashing colony data on the second sheet.
5. Find a frame containing uncapped brood. If uncapped brood is not available, find a frame from the center of the cluster.
6. Shake bees from frame into the original shipping box and scoop one,  $\frac{1}{4}$  cup of adult bees (about 150) (Figure 5) into the funnel inserted into the lid of the live bee shipping box. In order to prevent bees from escaping during sampling between hives cover the hole of the funnel. You can set the saltwater cap over the hole in the funnel if necessary.
7. Scoop two more  $\frac{1}{4}$  cups of bees from the box into the funnel inserted in the small bottle containing salt water, tap the bottle and funnel to force bees into the bottle for the individual salt water sample. This amount of bees should fill the bottle halfway. Close the bottle tightly, shaking it to make sure bees are fully dampened with salt water and

place to the side. Label each sample bottle to match your colony and whether the colony was 'healthy' or 'crashing'.



Figure 5: Collecting the live bee sample



Figure 6: Collecting the salt water

8. Repeat steps 3 through 6 until 8 strong colonies have been sampled. Then begin at step 1 with second provided live bee box for weak colonies until a total of 16, 8 strong and 8 weak colonies have been sampled.
  - a. **Note** we are sampling at the apiary level for the live bee sample, so samples from first 8 strong colonies are being placed in the same live shipping box and 8 samples for weak colonies will be placed in a second, separate box. You will be collecting 3 scoops of live bees from each hive and placing them in either the live bee shipping box or the individual salt water bottles.
  - b. **You will fill out 2 data sheets. One for the healthy colonies and a second one for the weak or crashing colonies.**
9. Place the small salt water bottles (containing bees) into large Ziploc bags to contain any leaks from the salt water before placing them into the large flat rate shipping box. Please make sure lids are tightly sealed to prevent leakage. Group healthy samples in separate bags as weak or crashing samples, labeling bags with either 'healthy' or 'weak' and **make sure each sample bottle is labeled with your name, date and hive number with a marker or sharpie** before placing in return shipping box. Please also include a listing of the hives sampled on a separate sheet of paper with pencil in the event of bottle leakage during shipping. Package should be shipped ground. Example: **Jane Bee, 10/15/12, #346**
10. Ensure **both** data collection sheets are completely filled out and legible and place in return shipping box.
11. Place the mailing label (Attn: The vanEngelsdorp Lab, 4112 Plant Science Bldg., College Park, MD 20742) over the now sealed hole on the top of live bee shipping box and secure it with tape. Write your return address on the upper left corner of the box. Write the date on the front of the box. Please **also write healthy colonies or weak/crashing colonies on the outside of the box.**

12. Email: Research Specialist Heather Eversole (heversol@umd.edu) within 24 hours of shipment or call 301-405-3799 to notify personnel that a live bee shipment is expected. Please also email Heather within 24 hours after shipping salt water and filtered samples to notify the Bee Research Lab that a shipment is to be expected.