Wax Sampling Protocol for the National Honey Bee Disease Survey

Please read this protocol carefully and watch the training video (https://www.youtube.com/watch?v=Znv2Bs9ZrjM) prior to sampling. For additional information or questions concerning sampling methods call the University of Maryland Honey Bee Lab at 301-405-7947, or email the UMD Survey Coordinator Rachel Fahey at faheybrl@umd.edu.

List of equipment:

- ✓ Latex gloves
- ✓ Bee brush
- ✓ Clean hive tool
- ✓ Permanent marker
- ✓ 50-mL Falcon tube
- ✓ Bleach solution

Overview:

Acute and sub-lethal poisoning from pesticides can be a problem for beekeepers. Honey bees come into contact with pesticides outside the hive while foraging for nectar and pollen, and inside the hive while feeding, contacting beekeeper applied miticides, or contaminated comb wax. In previous years, the National Honey Bee Disease Survey collected pollen from select beekeepers for pesticide analysis; however, pollen only provided a glimpse of the pesticide exposure of a colony over a short time. Current studies indicate that wax may provide a more comprehensive measure of the total number of pesticide residues in the hive. Wax is also shown to be a better predictor of colony and queen mortality.

For the 2018-2019 National Honey Bee Disease Survey, wax samples will be collected for pesticides analysis from 5 beekeepers participating in the **longitudinal monitoring study**. State Apiary Inspectors will select and sample 5 apiaries (1 apiary from each of the 5 beekeepers) twice for pesticides–once in the spring (before or at the start of the honey flow), and again in the fall after honey flow.

Important: Inspectors should sample the same 8 colonies in the fall that were sampled previously in the spring. To achieve this, inspectors need to locate the colonies marked with the USDA APHIS survey sticker. If any of the original 8 colonies are dead outs, you may take a sample from other colonies in the apiary to bring the total back up to 8 colonies sampled.

Procedure:

1. Put on a new pair of latex gloves prior to sampling each set of 8 colonies as this prevents cross-contamination between samples.
2. Select a frame from the brood chamber that has drawn wax but little to no brood, nectar, or pollen. Sampling from frames that have dislodged the bees for the National Honey Bee Disease Survey is preferred. Find an area of the frame that has drawn wax but little to no brood, nectar, or pollen (Figure 1).

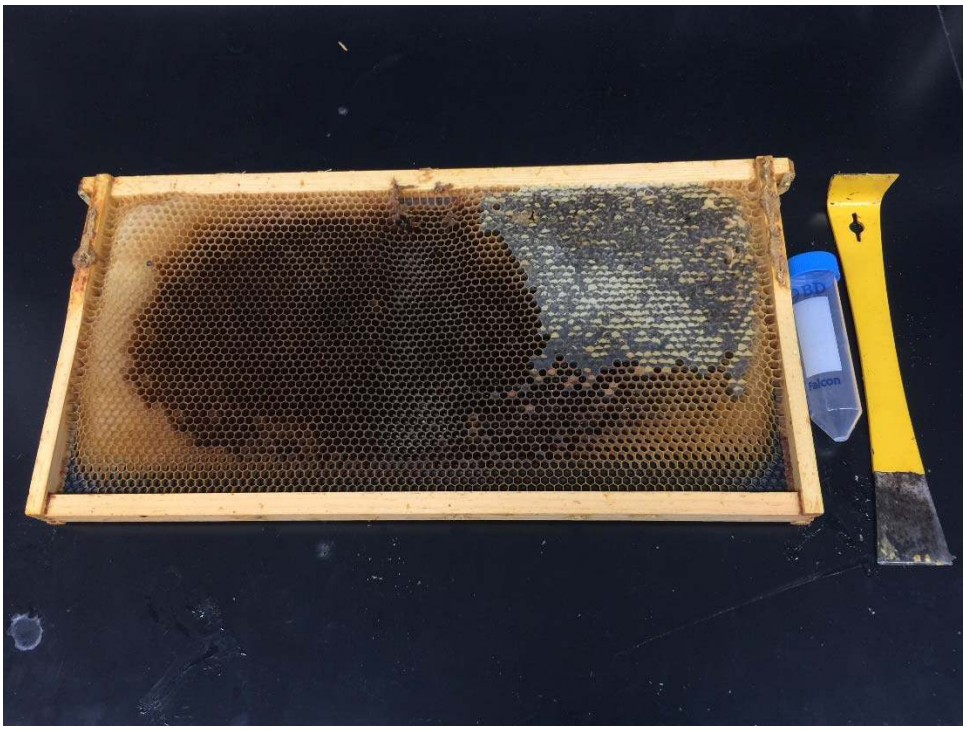

Figure 1

3. Use the flat end of a clean hive tool to cut into the wax. Then, leaving one corner of the tool in place, pivot 90° (clockwise or counterclockwise) to cut out a quarter of a circle's worth of wax (Figures 2 and 3).

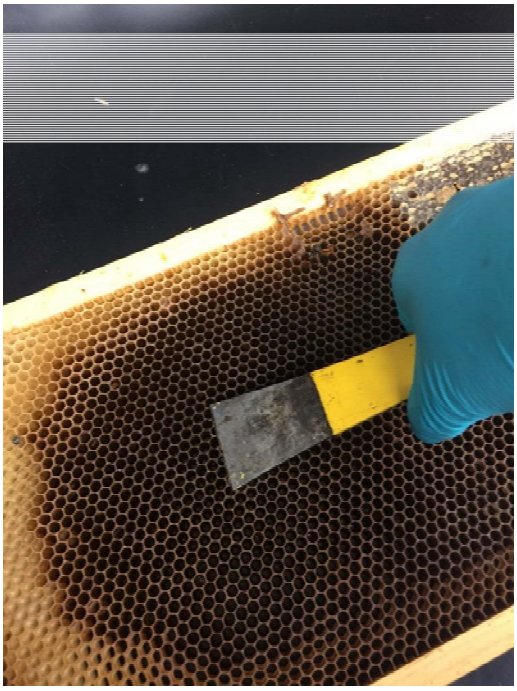

Figure 2

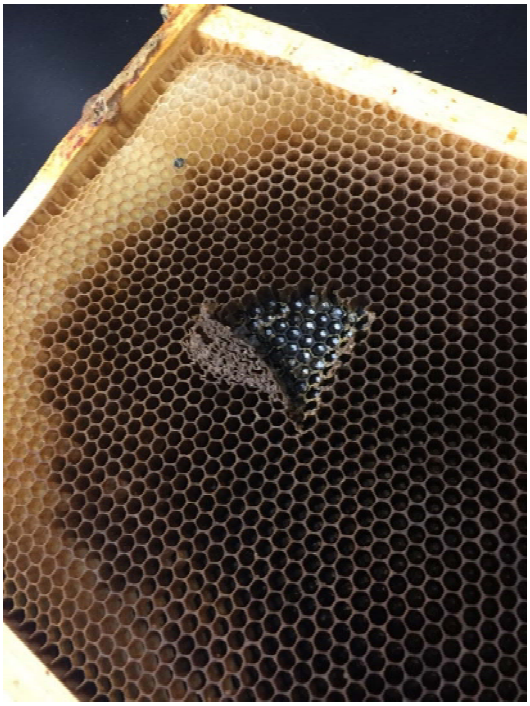

Figure 2

4. Using the hive tool and a gloved finger, pick up the scraped piece of wax and place it into the 50-mL Falcon tube (Figures 4, 5 and 6).

Figure 3

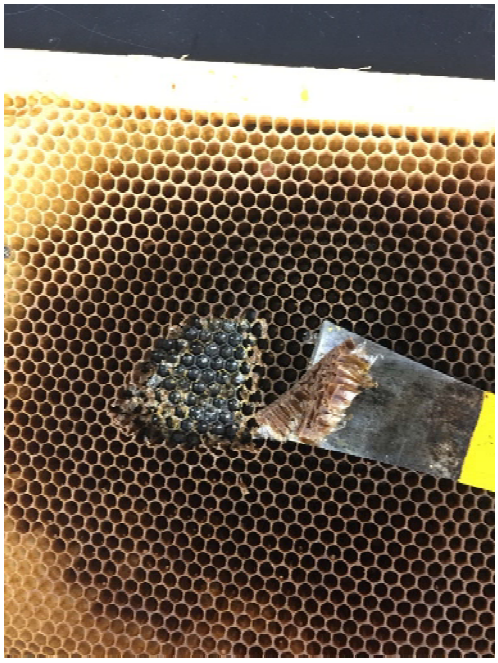

Figure 4

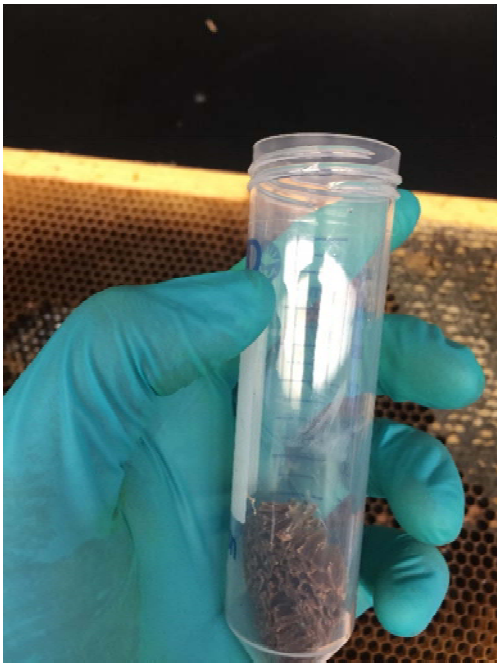

Figure 5

5. Repeat steps 2-4 for all 8 colonies, adding each piece to the same 50-mL tube. Use your gloved finger, if necessary, to push down the wax so all 8 pieces will fit (Figures 7 and 8). Once wax samples are taken from all 8 colonies, label the tube appropriately with the state, sample number and survey year (Ex. MD-01, 2016-2017) and cap it.

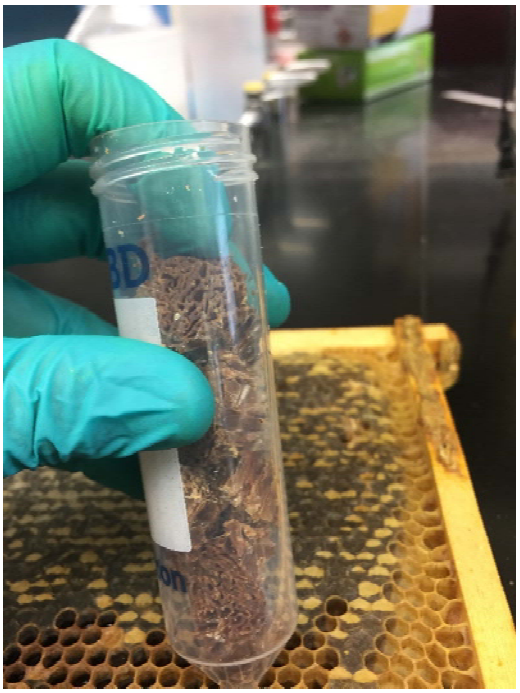

Figure 7

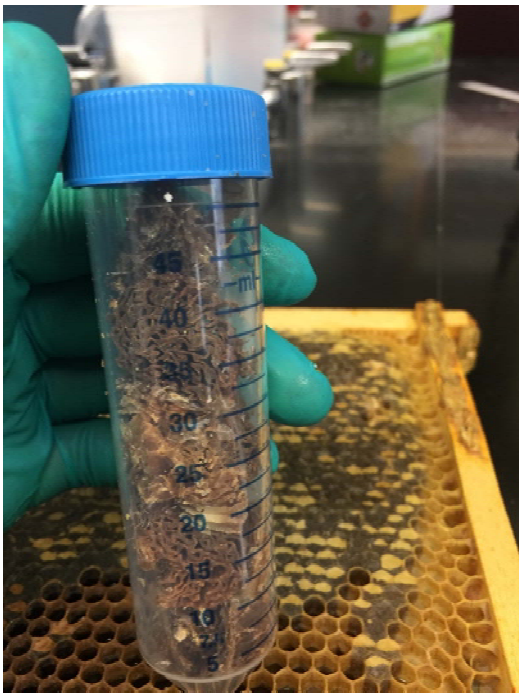

Figure 8

6. After each set of 8 colonies, discard the latex gloves and clean the hive tool with bleach solution to prevent cross-contamination.
7. Cover the tube with aluminum foil to prevent sunlight from degrading any pesticides.

Shipping:

Once all of the hives in the yard have been sampled for pests and disease, place the labeled tube of wax into a refrigerator or freezer for storage. Once all 10 samples have been collected, ship them to:

University of Maryland Honey Bee Lab
4291 Fieldhouse Drive
Plant Sciences Building Rm. 4112
College Park, MD 20742

It is preferred that these samples be shipped on dry ice, but if not possible, ship as overnight.