Protocol for Sentinel Apiary Program

(4 Colonies)

Overview:

By monitoring disease levels over time, you, the beekeeper, will be able to make better decisions about when to treat colonies and whether treatments are effective. Participating beekeepers will be asked to collect samples from 4 colonies once a month over a 6-month sampling season. These samples will be sent to the University of Maryland and processed to determine *Varroa* and *Nosema* levels. Each sampling involves opening the 4 colonies (the same 4 colonies are sampled each period) and removing one frame that contains young, developing brood. Adult bees from this frame are then collected following the standardized protocol (see attached) and placed into sample bottles containing a saltwater solution. You will collect two, ¼ cup scoops of bees from each hive. You will pour these two scoops of bees into the provided sample bottle and cap them tightly. As you spend time in each colony, you will take guided inspection notes within our app, which will be sent directly to our database after you upload them. You will then repeat this procedure for the remaining 3 colonies. In summary, you should leave the apiary with 4 sample bottles full of bees (one from each colony), and inspection notes uploaded to the app. As soon as possible after sampling, you will send the 4 samples to the University of Maryland Diagnostic Lab for analysis. Within two weeks of the samples' arrival to the lab, a report will be made available on the dashboard with your lab results and inspection notes.

More details about Sentinel are available here:
https://beeinformed.org/programs/sentinel/

You can view historical Sentinel data here:
https://bip2.beeinformed.org/sentinel/
https://bip2.beeinformed.org/hive-scales-org/bip/public

Please read this protocol carefully prior to initiating sampling. If you have any questions, please email Rachel Kuipers at rkuipers@umd.edu.

Materials:

In this kit, you received 6 months (6 sampling periods) worth of sampling materials. This kit includes:

Material (Figure 1):	**Quantity:**	**Checklist:**
Hive tags	6	☐
¼ cup measuring scoop	1	☐
Funnel	1	☐
Shipping boxes	6	☐
Mailing labels pre-addressed to UMD Bee Lab	6	☐
125 mL bottles with saltwater	24	☐
Quart zip top bags	6	☐

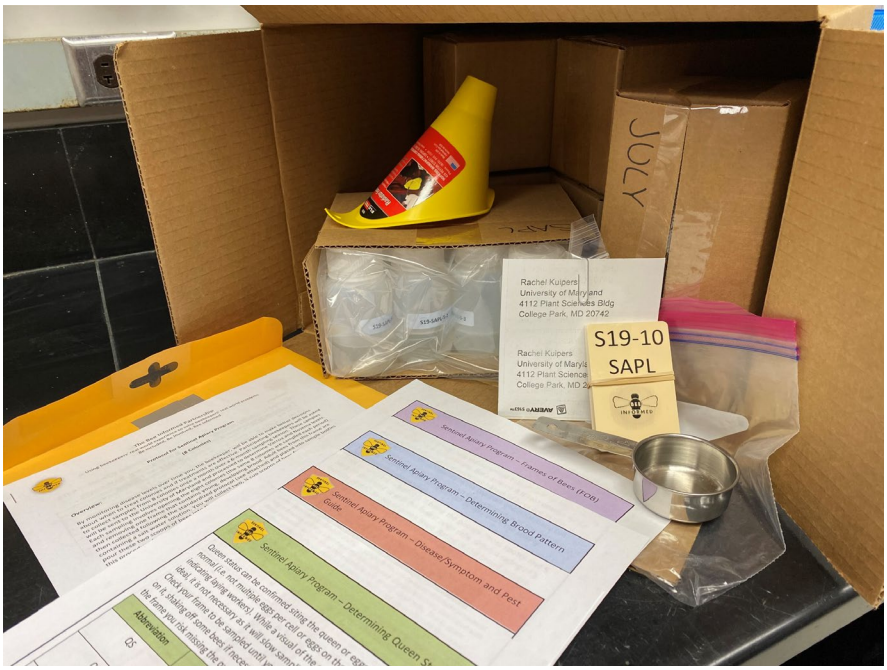


Figure 1: Sampling supplies that will be mailed to you. Kit shown is for 8 colonies; bottle number varies based on kit size.

You will also need (not provided):

- ☐ A staple gun (to affix the hive tag) or nails
- ☐ Postage to return the sample kits (estimated cost: $12/month)
- ☐ Washtub (strongly recommended)

STEP 1: Select 4 colonies

1. Select 4 random colonies located in the same apiary to start the sampling survey. It is important you select colonies of differing strengths, so we can obtain an accurate representation of disease levels in your apiary.

2. Affix a colony tag to each hive (see Figure 2). It is vital to sample the same 4 colonies throughout the duration of the season. Note that you have received 6 tags. Use only 4 initially and save the spare 2. These will be used if a colony dies and you need to tag another.

NOTE: IF A COLONY DIES DURING THE SAMPLING PERIOD, REMOVE THE SAMPLE TAG AND USE ONE OF THE SPARE TAGS FOR A NEW COLONY IN THE SAME APIARY. If you use the last sample tag, request more. Please do not reuse tags.

Figure 2: Colony ID Tag

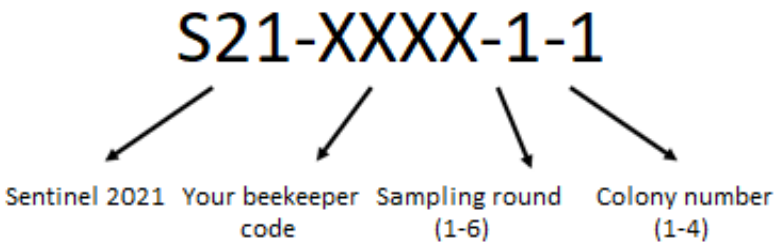
3. Fill out the required hive provenance information in the app. You only need to do this for month 1, unless a participating colony dies and a new one is chosen.

STEP 2: Sampling in the apiary

You will need to repeat the following steps every month, around the 15th of the month ± 1 week. Try to sample around the same time each month.

1. When you arrive in the apiary, place each sample bottle on the colony with its corresponding hive tag number (e.g., colony S21-XXXX-1 will get bottle # S21-AAAA-1-1; see below for a description of what your bottle labels mean).

Anatomy of A Label:



2. As you normally would, open the selected colony to the brood nest and examine for disease and queen status/condition. Grade queen status, frames of bees, and brood pattern using the provided info sheets as references. Record these numbers and any other observations (e.g., disease, honey production, management, etc.) in the app. Information recorded during the inspection will be available in your report for your records.
3. Remove the lid from the sample bottle with the matching colony number and place the funnel in the opening.

Figure 3: Sample bottle with funnel

4. Find a frame containing some uncapped brood.
5. Carefully examine the frame to ensure the queen is not on this frame. You don't want to collect her!
6. Firmly shake the frame over the washtub to dislodge the bees. Gently knock the corner of the tub on the ground, so the bees collect in the corner. Gently scoop two, ¼ cup scoops of adult bees (1/2 cup total = about 300 bees) from the tub and place them into the funnel (Figure 4) one at a time. Gently knock the bottle and funnel to get the bees to fall through the funnel and into the solution. 300 bees should fill about 2/3 of the bottle (Figure 5). If you do not have a washtub, you can gently scoop bees directly from the frame by lightly dragging an open bottle upward across the frame so the bees fall in. Be careful not to harm wax cappings or sample the queen, and make sure the bottle is about 2/3rd full.

Figure 4. Scooping bees from a tub into the funnel.

Figure 5. A sample bottle 2/3rd full with ~300 bees.

7. Close the bottle tightly, shaking it to make sure the bees are fully dampened with solution.
8. Repeat steps one through six until 4 colonies have been sampled into individual bottles.

STEP 3: Mailing the samples to the lab

1. Double check that all the lids on the bottles are tightly in place and all bottles are labeled.
2. Place the 4 sample bottles (containing bees) into the provided zip top bag to contain any leaks from the solution before placing them into the shipping box (Figure 7)**.**

Figure 7: Packaging the 4 sample bottles for return shipment to UMD

3. Ensure that your inspection notes are uploaded into the app.

4. Place the mailing label on the shipping box. Write FROM and your return address on the upper left corner of the box. If you lose the mailing labels, use this address:

   Rachel Kuipers
   4291 Fieldhouse Drive
   4112 Plant Sciences Building #036
   College Park, MD 20742

Your report should be available within 2 weeks of when the UMD lab receives your samples. We will upload your lab results and inspection notes into the dashboard as soon as possible, where you will be able to access your report.