



# DIVISION of PLANT INDUSTRY

## Honey Bee Diagnostics Laboratory: Sampling Guide

The FDACS Division of Plant Industry's Honey Bee Diagnostics Laboratory serves to support Florida beekeepers and the apiary industry. The laboratory uses traditional and molecular techniques to identify pathogens ranging from bacterial, viral, fungal to other diseases and pests affecting honey bee health.

This sampling guide serves as a basis for sending samples to the lab for successful and reliable pathogen testing. All samples must be shipped to the address listed below. For questions or concerns regarding a sample or this sampling guide, contact your apiary inspector or the Honey Bee Diagnostics Laboratory directly.

### Apiary Inspection Contact:

Phone: 1-888-397-1517

Email: [DPIHelpline@FDACS.gov](mailto:DPIHelpline@FDACS.gov)

### HBDL Contact:

Phone: 1-888-397-1517

Email: [DPIBeeDiagnostics@FDACS.gov](mailto:DPIBeeDiagnostics@FDACS.gov)

### Pricing:

\$55 per sample if subsampling is required, additional charges may accrue. Contact the HBDL before shipping samples to discuss pricing.

### Ship samples to:

Division of Plant Industry  
c/o Honey Bee Diagnostics Laboratory  
1911 SW 34<sup>th</sup> ST  
Gainesville, FL 32608



### DIAGNOSTIC SERVICE CAPABILITIES

- Africanized honey bee mitochondrial testing
- *Nosema* spp. identification and spore count
- Bacteria and fungal disease diagnosis
- Antibiotic resistance screening
- *Varroa* counting
- Varroacide resistance testing
- Virus detection and identification
- *Tropilaelaps* monitoring
- Coleoptera (beetle) diversity screening



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Below is a list of clinical signs that may be observed in your honey bee colonies. If one or more of the following clinical signs are examined, proceed to the associated sample collection page for further instructions.

Adult Bee and Whole Insect collections should be a complete, intact specimen not crushed or split into multiple segments.

	Observation Within Colony:	Description of Clinical Signs:	Collection Method:
Example 1		<ul style="list-style-type: none"> <li>- Defensive bees.</li> <li>- Frequent swarming.</li> <li>- Frequent stinging.</li> </ul>	Whole Insect Page: 5  Photo from American Pest
Example 2		<ul style="list-style-type: none"> <li>- Melted or shrunken larvae.</li> <li>- Yellow or brown larvae.</li> <li>- Spotty brood pattern.</li> <li>- Cappings are sunken or perforated.</li> <li>- Scale at the bottom of the capping that is easily removed.</li> </ul>	Whole Insect Page: 5 OR Swab Page: 8
Example 3		<ul style="list-style-type: none"> <li>- Perforated and sunken caps that appear greasy.</li> <li>- Scale on the lower half of the cell that is not easily removed.</li> </ul>	Whole Insect Page: 5 OR Swab Page: 8  Photo by Rob Snyder, Bee Informed Partnership
Example 4		<ul style="list-style-type: none"> <li>- Deformed or twisted wings.</li> <li>- Bloated abdomen.</li> <li>- Smaller body size.</li> <li>- Odd foraging behavior – confused bees.</li> </ul>	Adult Bee See page: 4
Example 5		<ul style="list-style-type: none"> <li>- Wings shaped like a "K."</li> <li>- Bees are unable to clip wings together at rest.</li> <li>- Bees are unable to fly.</li> <li>- Disorganized bees.</li> <li>- Bees crawling on the ground near hive.</li> </ul>	Adult Bee See page: 4  Photo from Honey Bee Research and Extension Lab, University of Florida
Example 6		<ul style="list-style-type: none"> <li>- Brood appear to be in a fluid sac.</li> <li>- Larvae do not pupate.</li> <li>- Yellow, brown or black larvae.</li> <li>- Shrunken head appearing out of the cell.</li> </ul>	Whole Insect Page: 5  Photo by Rob Snyder, Bee Informed Partnership

Example 7		<ul style="list-style-type: none"> <li>- Trembling bees.</li> <li>- Flightless.</li> <li>- Shiny or hairless bees.</li> </ul>	<p>Adult Bee See page: 4</p> <p>Photo by Giles E. Budge, Newcastle University</p>
Example 8		<ul style="list-style-type: none"> <li>- Hungry bees.</li> <li>- Dysentery.</li> <li>- Dead bees accumulating at the entrance of a hive.</li> </ul>	<p>Adult Bee See page: 4</p> <p>Photo by David Broberg</p>
Example 9		<ul style="list-style-type: none"> <li>- Spotty brood pattern.</li> <li>- White, grey, or black larvae.</li> <li>- Chalk-like larvae.</li> <li>- Larvae with a mummified appearance.</li> </ul>	<p>Whole Insect Page: 5</p> <p>Photo from USDA ARS Pollinating Insect-Biology, Management, Systematics Research unit</p>
Example 10		<ul style="list-style-type: none"> <li>- Slimy and fermented honey.</li> <li>- Loss of brood.</li> </ul>	<p>Whole Insect Page: 5</p>
Example 11		<ul style="list-style-type: none"> <li>- Treatment resistance.</li> <li>- Disease increase in honey bees.</li> </ul>	<p>Whole Insect Page: 5</p>
Example 12		<ul style="list-style-type: none"> <li>- Wasps displaying "hawking" behavior in front of hives.</li> <li>- Decrease in colony production.</li> <li>- Loss of foragers.</li> </ul>	<p>Whole Insect Page: 5</p>
Example 13		<ul style="list-style-type: none"> <li>- Bees displaying odd or unusual behavior not associated with a virus.</li> <li>- Visible fungal or bacterial growth is seen on the hive.</li> </ul>	<p>Bacteria and Fungi Page: 7</p>

## Adult Bee Collection:

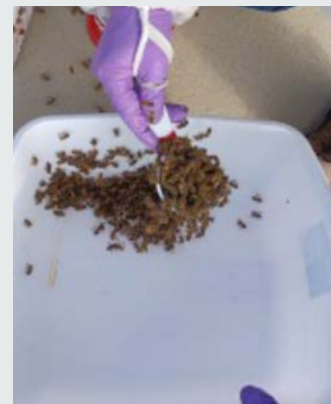
### Collection Materials:

- Plastic leakproof sample container (i.e. 50 mL tube or specimen collection containers)
- Nitrile gloves
- 70% ethyl alcohol, 70% isopropyl alcohol, or rubbing alcohol
- Labels
- ½ cup measuring cup
- Collection container
- Funnel
- Packing tape



### Procedures:

1. Select hive(s) or bees for testing.
2. Prepare the number of sample containers needed for use.
  - a. If testing multiple hives, a clean separate sample container must be used for each hive.
  - b. Multiple hives **cannot** be combined into one sample container.
  - c. Add enough 70% alcohol so samples are completely submerged when added to the leakproof sample container. **Ethyl alcohol must be used for disease panels.**
  - d. Label each sample with a unique identifier. This will allow the submitter to trace laboratory results to each individual sampled colony. This can be done with pre-printed labels or clearly written white paper labels with **pencil**.
    - i. Florida residents should also list their apiary registration number.
3. While wearing a pair of nitrile gloves, shake a frame full of bees into a collection container.
4. Using a ½ cup measuring cup, scoop approximately 100-150 bees into the sample container filled with alcohol. Use a funnel on the sample container to easily put the bees into the sample container.
5. If collecting from more than one hive, separate each of the hive samples into separate sample containers.
  - a. **Do not** reuse the alcohol from different hive containers.
  - b. Change gloves after each hive collection.
6. Clean collection container, measuring cup and funnel with alcohol after each hive if collecting more than one sample. Additionally, clean all tools after completing collection to decrease the spread of disease.
7. Once finished collecting, seal the sample containers completely with the lid. Use packing tape around the sample lid to complete the seal.
8. Proceed to **Page 9** for shipping instructions.



## Whole Insect Collection

### Collection Materials:

- Forceps or tweezers
- Small glass or plastic vials with secure lids
- 70% ethyl alcohol or 70% isopropyl alcohol or rubbing alcohol
- Labels
- Packing tape



### Procedures:

1. Select hive(s) or insects to be tested.
2. Prepare the number of vials needed for use.
  - a. If testing multiple hives, a clean separate vial must be used for each hive.
  - b. Add enough 70% alcohol so sample is completely submerged when added to the vial.
  - c. Label each sample with a unique identifier. This will allow the submitter to trace laboratory results to each individually sampled colony. This can be done with pre-printed labels or clearly written white paper labels with **pencil**.
    - i. Florida residents should also list their apiary registration number.
3. **Brood Collection:**
  - a. Randomly select at least 8 brood chambers per hive with clean forceps (or tweezers) and collect approximately 8 larvae/pupae from each of the cells and place into the vials with alcohol.
    - i. Brood should be complete and intact.
  - b. Carefully remove the cap from the brood chamber and remove the brood with the forceps. Do not break or crush the brood.
    - i. **Note:** preferably collect drones but workers can be accepted.

#### Collection for an Insect:

- c. With clean forceps, place the insect in a sample container filled with alcohol.
    - i. One insect is sufficient for testing, but three or more is best.
  - d. If collecting more than one type of insect, separate each insect type into different containers.
    - i. Insects should be complete and intact.
  - e. Use different forceps for each insect or thoroughly clean the forceps with alcohol between each use.
4. Place the samples from one hive or one insect type into a sample container with alcohol and seal with lid.
  5. Once finished collecting, seal the sample containers completely with the lid. Use packing tape around the sample lid to complete the seal.
  6. Proceed to **Page 9** for shipping instructions.

## Frame/Comb Collection:

Please email the lab before submitting a frame or comb for sampling.

### Collection Materials:

- Labels
- Packing tape
- Nitrile gloves
- Scissors
- Forceps or tweezers
- Heavy duty trash bags (Frame sample)
- Sealable bags (Comb sample)



### Procedures:

1. Identify the hive(s) for testing.
  - a. If testing multiple hives, a clean separate bag must be used for each hive.
  - b. Label each sample with a unique identifier. This will allow the submitter to trace laboratory results to each individually sampled colony. This can be done with pre-printed labels or clearly written white paper labels with **pencil**.
    - i. Florida residents should also list their apiary registration number.
2. While wearing nitrile gloves, select 1-2 frames from a hive and shake all the bees off over the hive. Ensure there are no bees on the frame when preparing to send a frame.
3. **If sending the whole frame:**
  - a. Carefully place the frame into the bottom of a heavy duty black trash bag. Wrap the excess bag around the frame.
  - b. With packing tape, wrap around the bag to seal it closed.
  - c. Make sure there are no openings in the bag and the frame is not visible.**If sending pieces of comb:**
  - d. With clean forceps and scissors, carefully cut off the section of comb for testing and place into a clean, dry sealable bag.
4. Do not freeze, refrigerate, or store in alcohol as this can degrade the sample.
5. Proceed to **Page 9** for shipping instructions.

## Fungal/Bacterial Growth:

### Collection Materials:

- Small glass or plastic vials with secure lids
- Labels
- Sealable bags
- Nitrile gloves
- Hive tool
- Packing tape



### Procedures:

1. Identify the hive(s) for testing.
2. Prepare the number of sample containers needed for use.
  - a. If testing multiple hives, a clean separate vial or bag must be used for each hive.
  - b. Label each sample with a unique identifier. This will allow the submitter to trace laboratory results to each individually sampled colony. This can be done with pre-printed labels or clearly written white paper labels with **pencil**.
    - i. Florida residents should also list their apiary registration number.
3. While wearing nitrile gloves, use a hive tool to carefully scrape off the growth.
4. Place this growth into a sample container or sealable bag.
5. Once finished collecting, seal the sample containers completely. If using a container with a lid, use packing tape around the sample lid to complete the seal.
6. Do not freeze or place samples in alcohol as this will degrade the sample.
7. Proceed to **Page 9** for shipping instructions.

## Swab Collection:

### Collection Materials:

- Sealable envelope or bag
- Labels
- Toothpicks or cotton swabs
- Wax paper
- Packing tape



### Procedures:

1. Identify the hive(s) for testing.
2. Prepare the number of sample containers needed for use.
  - a. If testing multiple hives, a clean separate envelope or sealable bag must be used for each hive.
  - b. Label each sample with a unique identifier. This will allow the submitter to trace laboratory results to each individually sampled colony. This can be done with pre-printed labels or clearly written white paper labels with **pencil**.
    - i. Florida residents should also list their apiary registration number.
3. With a sterile toothpick or cotton swab, insert the pick or swab into the brood chamber and rotate in the cell, enough to thoroughly coat it. Repeat this step on three different brood chambers for each frame using different toothpicks or cotton swabs.
  - Do not use the same toothpick or swab for different hives to decrease the spread of disease.
4. Wrap the individual samples in wax paper and store in an envelope or sealable bag. Secure the envelope or bag closed with tape.
5. Proceed to **Page 9** for shipping instructions.



## Shipping Instructions:

### Shipping Materials:

- Packing tape
- Shipping box
- Bubble wrap
- Sealable bag
- Freezer packs (optional if samples are not stored in ethyl alcohol)
- Insulated cooler (optional if samples are not stored in ethyl alcohol)



### FOR ADULT BEE AND WHOLE INSECT SAMPLES, BEGIN AT STEP #1. FOR ALL OTHER SAMPLE TYPES, SKIP TO STEP #4.

1. Wrap all sample containers individually with bubble wrap and secure with packing tape.
2. Place all individually-wrapped containers into a large sealable bag.  
For samples intended for a disease panel (Adult Bee) or samples NOT shipped in ethyl alcohol (Whole Insect), use an insulated cooler with multiple freezer packs to ship samples to avoid sample degradation.
3. Place sealable bag inside insulated container with multiple freezer packs and secure with packing tape.
4. Place samples in a box suitable for shipping and mail to the Honey Bee Diagnostics Lab (address on Page 1). The shipping container must be sent via Overnight or 2-day delivery. The package should be received by the lab within 48 hours after collection.
  - **Note:** It is best to ship samples in the beginning of the week (Monday or Tuesday) to ensure delivery during normal business hours and the samples are not degraded during shipping. Normal business hours of the laboratory are Monday to Friday, 8:00am -5:00pm.
  - **Do not** ship over a weekend as the laboratory is closed and unable to receive samples.
    - If the samples are collected at the end of a week, they may be stored at 39° F (refrigerator temperature) for approximately 48 hours prior to shipping on ice. If the shipping delay will be more than 48 hours, store at -4° F (freezer temperature) until shipped on ice.