

Project Monarch Health

Instructions: Testing Monarchs for OE

IMPORTANT HYGEINE PRACTICES:

To avoid spreading OE parasite spores, wear disposable exam gloves when handling monarch adults and larvae, or wash hands frequently with soap and water. (Hand sanitizer is not sufficient to kill OE spores.)

- After sampling each monarch: Wipe gloves with a disinfectant or baby wipe.
- After sampling every 10 monarchs: Change gloves. Wipe work area with 20% bleach.
- Once a month: Soak butterfly net in 20% bleach for 20 minutes and rinse with water.

Supplies you will need that are not included in kit (due to shipping weight):

- Disinfectant wipes
- Disposable gloves (any type)
- 20% bleach (80% water) mixture and spray bottle
- Pencil or pen and permanent marker
- *For capturing adults*: Butterfly net, glassine envelopes (These are useful if you want to temporarily hold adult monarchs before sampling). Envelopes between 3½ x 3½ inches and 4 x 4 inches will fit adult monarchs well.
- *For collecting larvae*: Milkweed stalks cut from collection site, small rearing containers with air holes or screened lids

STEP 1: COLLECT MONARCHS

To obtain monarchs for sampling you can either (1) catch adult butterflies or (2) collect late instar caterpillars to rear to the adult stage. Only adult butterflies can be tested for OE.

Catch Adult Butterflies or Collect and Rear Caterpillars

- **Catch a butterfly** using a butterfly net
- **Grasp the monarch** by holding the wings firmly between your thumb and forefinger.
- **Place the monarch in a clean envelope** until you are ready for Step 2. Crease the envelope shut. The monarch will move its legs around but will not be hurt. *To avoid contamination, do not reuse envelopes if you suspect that a monarch is infected.*
- **Change gloves, wipe your hands with a disinfectant wipe, or wash your hands** before handling the next monarch.

If you are interested in rearing caterpillars into adults, please collect 4th or 5th instar monarch caterpillars (“older larvae,” which are greater than 1.5 cm in length) from outdoor milkweed plants.



Life-size 4th instar



Life-size 5th instar

If you cannot find 4th or 5th instars caterpillars, you may rear “younger larvae” (less than 1.5 cm in length). However, we prefer samples from older larvae because they are less likely to be infected by contaminated rearing supplies and are more likely to reflect the natural amount of OE in their environment.

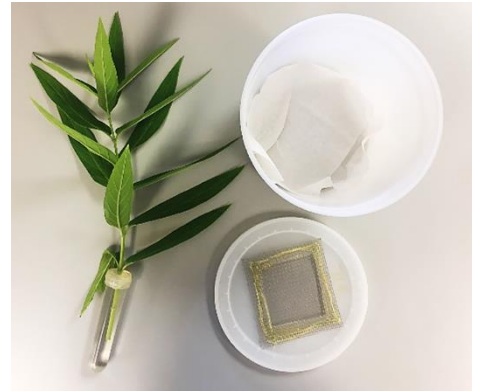
See page 2 for rearing instructions.

Rearing caterpillars

Wear gloves or wash hands when handling caterpillars and their containers

Prepare rearing containers:

- Containers should have air holes or screened lids.
- Add a moist paper towel (no standing water) to bottom of container.
- Add a sprig of milkweed **you collected from outdoors.**
DO NOT WASH OR BLEACH MILKWEED. This ensures that we are sampling the natural levels of OE present at your site.
- Add one caterpillar per container.
- Label container with collection date, collection location and stage ("young larva" or "old larva").



Daily care for caterpillars:

- Empty frass (feces) from container
- replace and moisten paper towel if needed
- add new milkweed stalks

We do not accept samples of monarchs reared from bleached eggs.

After the chrysalis forms, discard plant material and wait for the butterfly to emerge (6-12 days). **After the butterfly emerges.** Allow wings to harden for 4-6 hours after emergence before handling. Move to step 2.

STEP 2: SAMPLE ADULT MONARCHS FOR OE PARASITES

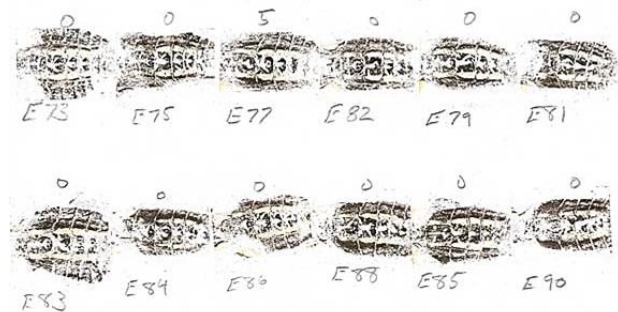
- **Wipe work area** with 20% bleach-water and put on **new gloves.**
- **Grasp the monarch** using one gloved hand. If possible, do not touch the monarch with your second hand, so you can keep your second hand free of OE spores. Otherwise, wear and replace two gloves.
- **Pick up a clear sticker** with your "clean" hand.
- **Firmly apply the sticker to the abdomen** of the monarch, so that it comes into firm contact with at least the front and sides of the abdomen. *Monarchs are more durable than they look. Sampling them with moderate to firm pressure does not harm them.*
- **Place the sticker onto the index card.** It should have **one black patterned imprint.** These are the monarch's body scales, which are naturally lost over time.
- **Label the sticker on the index card** with the number that corresponds to the data sheet. You can fit 8-10 stickers per card.
- Go to www.monarchparasites.org/videos to watch "How To Sample Monarchs For OE" for a video tutorial of this step.



Apply sticker to monarch and then place on index card.



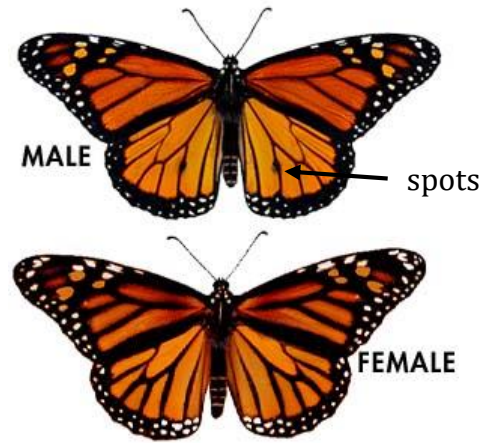
If you run out of stickers, please email us to get more or use Scotch® Transparent tape. Please avoid frosted or Scotch® Magic tape, which is too cloudy to observe under the microscope.



Index card with **labeled** sticker samples

STEP 3: RECORD DATA, MARK, AND RELEASE MONARCH

- **Record the sex of the monarch on the data sheet:**
- Males have “claspers” on the end of their abdomens and two black alar glands (spots) on their hindwings.
- Females have neither of these, but they have a notch in the underside of their abdomen that is clearly visible from the side.
- Completely fill out the datasheet with all locations, dates, stages.
- **Mark the monarch** lightly with an ultra fine point permanent marker (optional). Make a small dot or write a number to indicate it was already sampled. This will not harm the monarch.
- **Release** the monarch back into the wild, and celebrate your participation in monarch health research!



Sanitize all materials that came into contact with monarchs to remove OE spores, which can remain infectious for up to a year.

Soak rearing containers in 20% bleach (80% water) for several hours. Spray or wipe 20% bleach on surfaces that came into contact with monarchs. *Alcohol and Lysol are not sufficient to kill spores.*

SKIPPING THIS STEP COULD CONTAMINATE FUTURE MONARCHS.

Once you are done sampling butterflies for the season, please send your index cards of samples and your datasheet in the pre-addressed envelope to our lab:

Project Monarch Health
c/o Sonia Altizer
Odum School of Ecology
University of Georgia
Athens, GA 30602

Thank you for monitoring the health of monarchs!

We will send you the results of your samples via email. Your data will help us understand where and how often monarchs get sick with the OE parasite, if this is changing over time, and may help us identify factors that contribute to the spread of OE.

If you have any questions or comments, please contact us at monarchhealth@gmail.com or visit our website monarchparasites.org for more information and results from previous years.

