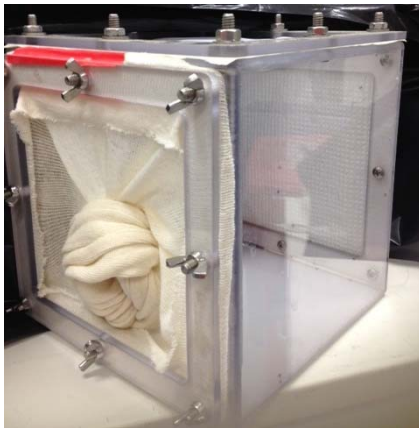


## Sand Fly Standard Operating Procedures

### Housing and Maintenance of Larval Pots

1. Check for physical damage to the pots when larval shipments arrive.
2. Larval pots should be maintained in an incubator set at 26°C, 80% relative humidity, and with a photoperiod of 12-hours light: 12-hours dark.
3. Pots will contain late 3<sup>rd</sup> or early 4<sup>th</sup> instar larvae and there is no need to add additional food.
4. Pots should be examined twice a week for adult emergence, mold growth, and the moisture content of the plaster.
5. When checking for adult emergence, pots should be opened inside a holding cage to release any emerged adults.



**Holding Cage**



**Larval Pot**

6. Pots not containing adults should be gently shaken to breakup any excessive mold growth (not performing this step may cause mold to overtake the larvae/pupae and no adults will emerge).
7. The plaster at the bottom of the pot should be slightly moist. Extremely dry plaster should be soaked in water for 5-10 minutes to keep the larvae/pupae from desiccating. This can be performed by filling a container with 1 inch of water at room temperature and placing the pot in the container for 5-10 minutes.

**Note:** It will take 30-60 days for all the adults to emerge from a single pot. This process of releasing the adults should be repeated twice a week until all adults have emerged. After there are no more larvae/pupae the pot can be discarded (recommend freezing for 24 hours).

### Housing and Maintenance of Adult Sand Flies

1. Adults should be housed in a cage that will allow for a sugar source to be accessed through a screen.
2. Adult cages should be maintained in an incubator set at 25°C, 80% relative humidity, and a photoperiod of 12-hours light: 12-hour dark.



**Cotton Pads on top of Holding Cage**

3. A 30% sucrose solution will provide the nutrition to sustain both adult males and females. Sugar purchased at a local supermarket will work. Cotton pads should be soaked in the solution and squeezed out just enough so that liquid does not drip onto the cage floor.
4. The cotton pads should be replaced daily to prevent mold growth; however, they can be left on for up to two days over the weekend.

**Note:** Please wait at least 48 hours post emergence if using the adults for an assay that will require the sand flies to bite or perform host seeking activities.

| <b>Sand Fly Weekly Task List</b>                     | <b>Week of:</b> |            |            |            |            |            |            |
|--|-----------------|------------|------------|------------|------------|------------|------------|
|  | <b>SUN</b>      | <b>MON</b> | <b>TUE</b> | <b>WED</b> | <b>THU</b> | <b>FRI</b> | <b>SAT</b> |
| Check incubator temperature and humidity             |                 |            |            |            |            |            |            |
| Change sugar cotton on adult cages                   |                 |            |            |            |            |            |            |
| Check larval pots for newly emerged adults (release) |                 |            |            |            |            |            |            |
| Check larval pots for moister (soak as needed)       |                 |            |            |            |            |            |            |
| Check larval pots for mold growth (gently shake)     |                 |            |            |            |            |            |            |

| <b>Sand Fly Weekly Task List</b>                     | <b>Week of:</b> |            |            |            |            |            |            |
|--|-----------------|------------|------------|------------|------------|------------|------------|
|  | <b>SUN</b>      | <b>MON</b> | <b>TUE</b> | <b>WED</b> | <b>THU</b> | <b>FRI</b> | <b>SAT</b> |
| Check incubator temperature and humidity             |                 |            |            |            |            |            |            |
| Change sugar cotton on adult cages                   |                 |            |            |            |            |            |            |
| Check larval pots for newly emerged adults (release) |                 |            |            |            |            |            |            |
| Check larval pots for moister (soak as needed)       |                 |            |            |            |            |            |            |
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| <b>Sand Fly Weekly Task List</b>                     | <b>Week of:</b> |            |            |            |            |            |            |
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| Check incubator temperature and humidity             |                 |            |            |            |            |            |            |
| Change sugar cotton on adult cages                   |                 |            |            |            |            |            |            |
| Check larval pots for newly emerged adults (release) |                 |            |            |            |            |            |            |
| Check larval pots for moister (soak as needed)       |                 |            |            |            |            |            |            |
| Check larval pots for mold growth (gently shake)     |                 |            |            |            |            |            |            |

**Please note:** Anyone receiving sand flies in the US must have an appropriate permit from the U.S. Department of Agriculture, Animal and Plant Health Inspection Service Veterinary Services (USDA APHIS VS permit) to receive sand flies.

**Acknowledging BEI Resources and WRAIR for sand fly stocks and protocols:**

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