

# Forensic Investigation Research Station

## Technical Manual Series



## Entomology Protocols

FIRS Technical Manual 5  
Second Edition

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This technical manual is designed to act as a guide to collection, rearing, and preservation of blow flies and blow fly larva. For forensic casework, verify that any collections are done to the standard requested by the forensic entomologist that will examine the specimens.

### **Larval Collection, Preservation, and Care Protocol**

#### **Stage 1: Collection of blowfly larvae**

Collect samples from all locations where larvae are present and accessible on the body. It is possible that there may be larvae of different species or in different stages of development in different parts of the body. Collect enough samples to accurately represent the larvae present on and around the body.

Supplies:

thermometer	gloves
container with a lid	aluminum foil
wet cat or dog food (not fish based)	sawdust
spoon or similar scooping tool	collection form

#### **Set up**

If the resources are available to kill and preserve larva immediately after collection, all that is needed is an empty container with a lid (such as a petri dish). If the larvae must be transported to another location or cannot be processed immediately, follow the steps below:

1. Fold the foil in to a bowl shape that can be pinched shut.
2. Line the bottom of the container with sawdust and place foil enclosure inside leaving the top of the foil and the container open.
3. Steps 1 and 2 can be done in advance away from remains for easy access. At the time of collection, open the cat food and put a few tablespoons of food into the foil enclosure.
4. Repeat this process for the number of collections desired.

#### **Collection of Environmental Data**

Blow fly development schedules are highly temperature dependent. Temperature data from the scene, in combination with larval stage and length, can help the entomologist estimate the postmortem interval of the decedent with greater accuracy. Document temperature data at the time of collection to provide to the forensic entomologist. Basic weather data to document before insect collection includes:

1. Ambient temperature: position the sensor of the thermometer about four feet above the decedent, shielded from direct sunlight.
2. Ground temperature: Place the sensor of the thermometer away from the body and any decomposition fluids on the surface of the ground. Again, keep the sensor shielded from direct sunlight.

3. Ground-body interface: Place the sensor between the body and the ground (or whatever surface is directly under the body). Ideally, the sensor is positioned centrally under the torso, make note of the actual location if this is not possible or practical.
4. Maggot mass: place the sensor in the center of a maggot mass. Make note of which larval mass was measured on the collection form.
5. General observations: Finally, make note of the general conditions. This includes location, presence of clothing, evidence of scavenging, weather observations, observed trauma.

### Larval Collection

1. Search the body and surrounding areas for larval activity. Common areas to find larva include the mouth, nose, underneath the torso, and between the legs.
2. Prior to collection document the date, time, environmental data, location and stage of blowflies observed.



**Figure 1:** A large maggot mass.

3. Wearing gloves, use a spoon or similar scooping tool to collect 50-100 maggots from the body and place them directly into the food in the prepared container (or empty container for immediate processing). NOTE: All the maggots in a container should be collected from the same location on the body at approximately the same time.
4. If there are maggots of varying sizes, collect a larger sample that is representative of the population present in the area.
5. Pinch the foil together to keep the maggots contained and close the container.
6. Label the container with the case number, sample location, time, and date. Make sure that this information is documented on the collection form as well.
7. If larvae will not be processed immediately, store in a secure location until processing. Document the storage conditions (temperature) on the collection form.
8. Repeat this process for maggot masses throughout the body if needed, keeping maggots from different sample areas in separate labeled containers.



**Figure 2:** Left: Collection using a plastic spoon. Right: Collected maggots in a petri dish.

## Stage 2: Killing and preserving blowfly larvae

After collection, the larvae can be separated into a group to kill and preserve and a group to rear to adulthood. The preserved group can be used by the entomologist to identify the development stage and the genus of the larvae. While larvae can be used for DNA analysis to provide a species identification, this level of identification can also be done at lower cost though visual identification from adult flies. Before sending specimens to a forensic entomologist, verify the type of specimens (alive or dead, development stage) they will accept and analyze.

Supplies:

gloves	live larvae
tweezers	heat resistant container (such as a glass measuring cup)
screen	vial and lid
70% isopropyl alcohol	Paper and pencil
additional larva container (such as a petri dish) if there is variation in larval size	
electric hot water kettle (or hot water produced by other means)	



**Figure 3:** Supplies needed to par boil maggots for preservation.

1. Heat water to slightly below boiling.
2. If there is significant variation in the size of the collected larvae, separate them by general size. This will increase the likelihood that the live larvae will pupate at a similar time. If the larvae need to be separated, use the tweezers to transfer maggots of similar size from the original

collection container to the additional container. Work quickly as the larvae will attempt to leave the containers as soon as they are opened.

3. Once the larvae are separated, transfer 1/4 – 1/2 of the larvae from each group to the heat resistant container.



**Figure 4:** Larvae separated into two groups for preservation and rearing.

4. When the water is just below boiling temperature, pour the hot water over the larvae in the heat resistant container. Leave the larvae in the water for at least 30 seconds.
5. Carefully pour the water and larvae through a fine strainer or mesh. Pour slowly to prevent losing any larvae in the process.
6. Using the tweezers, transfer the dead larvae from the screen and container into the vial.



**Figure 5:** Left: Pouring water over screen to catch maggots. Right: Maggots on the screen after pouring, ready for transfer into storage vial.

7. Fill the vial with 70% isopropyl alcohol and clean all equipment and the exterior of the vial.
8. Note the case number, date of collection, time of collection, and sample number. This can be done in pencil on a paper placed in the vial, on a label on the outside of the vial, or in felt tip pen on the outside of the vial.



**Figure 6:** Left: Transferring larvae into storage vial. Right: Larvae in storage vial ready for shipment with correct labeling in pencil.

### Stage 3: Setting up the rearing cage

If larvae were placed in food upon collection, the collection container may be used for rearing if a rearing cage is not available. Ensure that the food source is loosely covered to keep moisture in the food source. If there are separate size groups from one collection, set up separate rearing cages for each group. If rearing space is limited, keep the number of rearing groups limited to whatever space is available.

#### Supplies:

rearing cage (or other suitable container)	wood shavings
wet cat or dog food	container of maggots
aluminum foil	label and pen

1. Fill the bottom of the cage (or other container that can be sealed but allows air space for larvae to breathe) with wood shavings. Make sure the layer is thick enough for the larvae to wander and pupate (about half an inch). If the layer is too thick it will be difficult to pack for shipping later.
2. Open the larval collection container and transfer the folded foil into the rearing cage. Empty the remaining contents of the container into the rearing cage, be careful to ensure that all larvae in the container are transferred into the cage. Open the foil and lay flat in the sawdust to allow for larvae to wander.
3. Add fresh cat or dog food to the collection foil. This can be added on top of the larvae and any pre-existing food source.
4. Place a tented piece of aluminum foil so that it covers the foil but leaves room for the larvae to wander. This will help maintain moisture in the food source.
5. Close the rearing cage.
6. Label the rearing cage with the case number, collection date, collection time, and sample number.



**Figure 7:** Left: Open petri dish with larvae and food source. Right: Aluminum foil tent over petri dishes.

#### Stage 4: Rearing larvae

Check on the larvae daily to note their progress and ensure they have sufficient food.

Daily check:

1. Check the larval development. This means looking not only at the food pile, but through the sawdust as well. If the larvae have not pupated, continue with the steps below. When the larvae molt into pupae, they are ready to send to an entomologist (Figure 8).
2. If the food source looks insufficient or dry, add a couple table spoons of additional food. Do not remove the old food as larvae may be inside.
3. Re-cover the food and larvae with foil and close the cage.

NOTE: If pupae are not sent in time they will become flies. Pay close attention to wandering larvae (larvae that leave the food source and are in the saw dust) as this is a sign that they will pupate soon.



**Figure 8:** Stages of larval growth. Left: Bottom of the rearing cage before larvae begin to wander. Center: Wandering larvae. Right: Pupae that are ready for shipment.

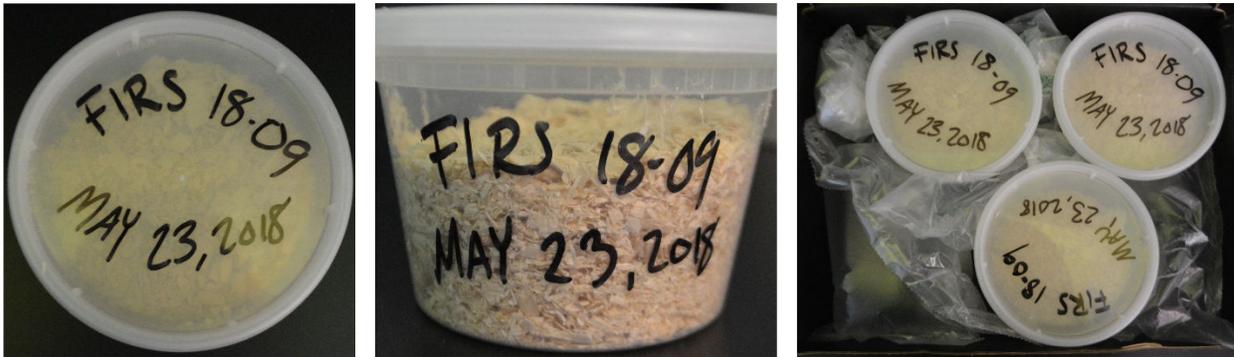
### Stage 5: Preparing for shipment

Once the larvae pupate, they are suitable for shipment to an entomologist.

Supplies:

gloves	rearing cage/container full of pupae
plastic tubs	one small box
packing material	preserved larvae
packing tape	shipping information

1. Open the cage, remove the tented aluminum foil, and set aside. Check the food and foil (both sides) for larvae. If any live larvae or pupa are recovered, place them in a plastic tub. Once all larvae and pupae are removed, discard the foil and old food.
2. Transfer the wood shavings and pupae from the rearing cage into the tubs. Leave about three quarters of an inch of air at the top of each container.
3. After removing all pupae and shavings from the rearing cage, close all the tubs. Check work surface for pupae. Remove and dispose of gloves.
4. Label each of the containers with the case number, date of collection, time of collection, and sample number.
5. Put the tubs in the box making sure to surround them on all sides with packing material. Make sure they are secure inside the box.
6. Add any vials of killed larvae from the collection to the box. All vials should be rolled up in packing material for protection. Secure these in the box just before the final layers of packing material. Add the collection form to the box. Seal the box and send overnight to a forensic entomologist.



**Figure 9:** Left and center: Proper labeling of plastic tub. Right: Inside of a box that is ready for shipment.

## Blow Fly Collection and Preservation Protocol

### General Process for Fly Collection and Preservation

Below is an overview of all the steps and supplies involved in collection and preservation of blow flies. Following this overview, there are detailed descriptions of the more involved steps in the process. Refer to the detailed sections for photographs relevant to those steps.

Supplies:

kill jar	ethyl acetate
collection net	gloves
paper towel	tweezers
size 0 pins	storage box
labels	

1. Charge the kill jar by adding ethyl acetate to the insert inside the lid of the jar.
2. Wearing gloves, use the net to catch flies from on or around the body. Transfer the flies into the kill jar. Catch about 10-15 flies, aiming to get as much variety in the sample as is present in the field (see sections below on "[How to Catch Flies](#)" and "[How to Transfer Flies into the Kill Jar](#)").
3. If the flies survive for more than a few minutes in the kill jar, place the entire jar of flies into a freezer. Loosen the lid slightly to prevent damage to the jar during freezing. Label the jar with the case number, collection date, collection time, and sample number. Leave the jar in the freezer overnight. If all collected flies are killed in the kill jar, there is no need to freeze them before pinning.
4. Pin the flies and put them into the storage box (see "[How to Pin Flies](#)" below).
5. Create labels for each of the flies collected. They should have the case number, location, and date of collection.
6. Once the labels are printed and cut, pin the flies to them (see photographs in "[How to Pin Flies](#)" below). Place them in the box in an organized fashion, all facing the same direction.

## How to Catch Flies

Depending on where flies are relative to the body and how they are behaving, different strategies are useful to catch them. Below are a few recommended techniques for different situations. Be cautious to control the net and avoid disrupting the scene or the body while collecting.

### General tips for catching flies:

- Be mindful of moving shadows near the body and flies, the movement may cause flies to leave the area.
- If there are not many flies present, avoid large motions as this will cause flies to leave the area.
- After attempting to catch a fly, wait a few moments before trying again. This brief pause will allow flies to return to the area.

### How to catch flies that are on the ground near a body:

1. Approach the fly slowly with the net. Be mindful of any shadows relative to the fly.
2. Once the net is within about 6 inches of the fly, quickly cover the fly with the net.
3. Move closer to the net and grab the end of the net holding it up in the air. Leave the ring and handle of the net on the ground. This will encourage the flies to move to the end of the net, making them easier to capture.



**Figure 1:** Holding the tip of the net up allowing flies to move to the top (Step 3).

4. Proceed to "[How to Transfer Flies into the Kill Jar.](#)"

### How to catch flies that are on a body:

1. Hold the net in one hand toward the base of the handle. With the other hand hold higher on the handle and loosely holding the end of the net.
2. Approach the fly slowly with the net. Be mindful of shadows.

3. Get close to the fly, trying to get it to fly off the body. It may be necessary to gently nudge the fly with the rim of the net. Again, be careful not to disrupt the body or the scene with the net.
4. As soon as the fly is airborne, sweep toward it with the net. Be sure to release the tip of the net as this is done. It may be necessary to sweep back and forth multiple times to catch the fly.
5. Once the fly is in the net, flip the end of the net over the rim trapping the fly.
6. Proceed to "[How to Transfer Flies into the Kill Jar.](#)"



**Figure 2:** Using the rim of the net to trap flies.

How to catch flies that are flying around a body:

NOTE: this is best used when there is a swarm of flies around or on the body.

1. Approach the swarm of flies (or body covered in flies).
2. Holding the handle of the net in both hands, sweep the net back and forth through the swarm. If the flies are mainly on the body, start closer to the body to get them flying, then swing the net higher to catch flies as they move away from the body.
3. Once one or more flies are in the net or the swarm dissipates, flip the end of the net over the rim to trap the flies inside (Figure 2).
4. Proceed to "[How to Transfer Flies into the Kill Jar.](#)"

### How to Transfer Flies into the Kill Jar

1. Once the fly is in the end of the net, grab the net to close the fly inside. Leave enough space at the end of the net to ensure the fly is trapped.
2. Use both hands to decrease the size of the enclosed area.
3. Keep the flies contained by pinching the end of the net (Figure 3).



**Figure 3:** Left: Trapping captured flies in the end of the net (Steps 1 and 2). Right: Changing hand placement in preparation to transfer flies into the kill jar (Step 3).

4. Open the kill jar and put the fly-containing hand partially inside the jar (Figure 4). If there are already live flies inside the jar, move quickly to keep them contained.
5. Ensure there are no gaps between the net and the rim of the jar, then release the fly inside the jar.
6. Pull the net tight over the rim of the jar (Figure 4).
7. Pick up the lid and bring it close to the top of the jar.
8. Tap the jar against the ground (avoid rocks) to disorient any live flies. This will cause flies to drop to the bottom of the jar. This step is particularly important (and may need to be repeated) if there were live flies in the jar prior the current addition.
9. While the flies are at the bottom of the jar, close the lid while pulling the net out quickly to seal the jar.
10. Collect more flies, repeating the collection and transfer processes until there are 10-15 flies in the jar.
11. Proceed to step 3 of the "[General Process for Fly Collection and Preservation.](#)"



**Figure 4:** Transferring flies from the net into the kill jar. Left: Releasing flies into the jar (Steps 4 and 5). Right: Closing the kill jar without releasing flies (Steps 6-9).

## How to Pin Flies

Supplies:

jar of dead flies	paper towel
tweezers	size 0 pins
storage box	

1. Lay the paper towel on a table.
2. Remove the flies from the kill jar placing them on the paper towel.
3. Use tweezers to position the fly so the dorsal side is visible.
4. Using a size 0 pin, stab the fly in the right side of the thorax.
5. Use the tweezers to carefully move the fly about two thirds of the way up the pin. A piece of foam may be used for this to ensure all flies are placed at the same height.
6. Stick the pinned fly into the storage box with the other flies from that collection group.
7. Proceed to step 5 of the "[General Process for Fly Collection and Preservation.](#)"



**Figure 5:** Left: Correct location to pin a fly. Center: Position of fly on both the pin and the label. Right: Flies from one collection labeled and pinned in two rows in storage box.

**FIRS Entomology Collection Form**

Date: \_\_\_\_\_ Case #: \_\_\_\_\_

Time of collection: \_\_\_\_\_ Collected by: \_\_\_\_\_

Scene location (address, GPS coordinates): \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Body Condition/microenvironment:**

- Indoor       Water       Scavenging: \_\_\_\_\_  
 Outdoor       Buried       Penetrating trauma: \_\_\_\_\_  
 Shade       Sun exposure       Clothing       Partial remains

Notes: \_\_\_\_\_  
\_\_\_\_\_

**Weather:**

Ambient: \_\_\_\_\_ Ground: \_\_\_\_\_ Under body: \_\_\_\_\_ Larval mass: \_\_\_\_\_

Weather conditions: \_\_\_\_\_  
\_\_\_\_\_

**Insect stages present:**  Larvae (maggots)     flies                       pupa     pupal casings

Locations of larval masses: \_\_\_\_\_  
\_\_\_\_\_

Insect stages collected:  Larvae (maggots)     pupa     pupal casings

Location maggots collected from: \_\_\_\_\_

