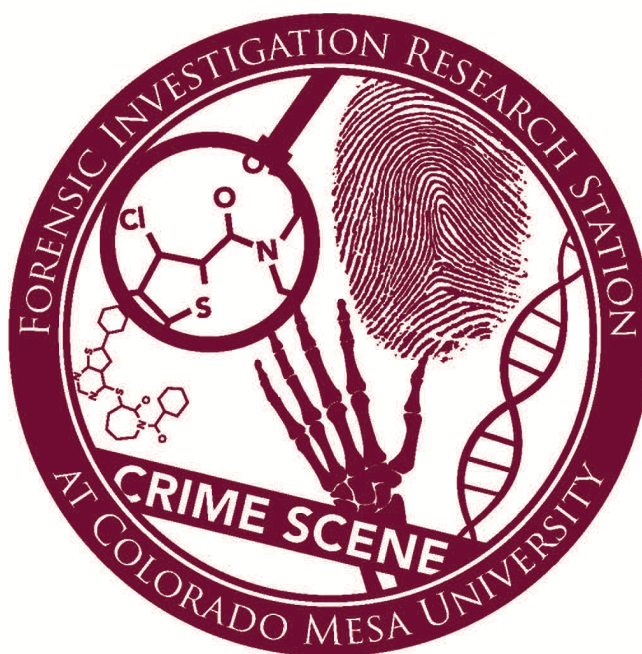


Forensic Investigation Research Station

Technical Manual Series



Entomology Protocols

FIRS Technical Manual 5
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Introduction

Blow flies can be extremely valuable in the estimation of postmortem interval. This is because blow flies commonly colonize remains within seconds of death and have very reliable development schedules. With the species, location, temperature, life stage, and scene information it is usually possible to accurately estimate when the remains were colonized (Time of Colonization), which is often very close to the time of death.

To make these estimations accurately, forensic entomologists need properly collected and preserved specimens and certain information about the scene. This technical manual is designed to act as a guide to collection, rearing, and preservation of blow flies and blow fly larvae. While these methods should be sufficient for forensic casework, verify that any collections are done to the standard requested by the forensic entomologist that will examine the specimens before collecting and preparing specimens.

For a list of certified forensic entomologists and entomology technicians, see the American Board of Forensic Entomology: <https://forensicentomologist.org/members/>

Larval Collection, Preservation, and Care Protocol

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 a representative sample from each area; the goal is to capture the variation present, not to collect every larva present.

Supplies

thermometer	camera
container with lid	gloves
wet cat or dog food (not fish based)	sawdust
spoon or similar scooping tool	collection form
hot water (in a thermos)	paper towel and rubber band
vials with 70-80% ethanol	vials with 95% ethanol
open heat resistant container	forceps
labels	pencil

Set up

Some materials can be prepared before departing for a scene. It is best to kill and preserve larvae as soon as possible after collection to preserve their life stage. To facilitate this, prepare a thermos of hot water (near boiling) and fill a few small vials with 70-80% ethanol and a few small vials with 95% ethanol (if DNA testing is desired). For preservation, put a heavy layer of sawdust inside the sealable container. Cat or dog food can be added just before or after collection.

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 with greater accuracy. Basic 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).
4. Maggot mass: place the sensor in the center of a maggot mass. To do this, hold the thermometer in loosely with the palm flat and gently place the tip of the probe just inside the

mass. The mass will pull the probe farther in allowing for measurement of the center of the 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, unique scene conditions, and unique insect activity (see collection form).

Larval Collection

Whenever possible collect both a sample for preservation and a sample to be reared. The preserved samples can be used for identification and life stage; however, identification is far easier and more precise when adults are used. So, always preserve a representative sample, but rear an additional sample when practical and when sufficient larvae are present.

1. Search the body and surrounding areas for larval activity. Common areas on the body to find larva include the mouth, nose, underneath the torso, and between the legs. Look for wandering larvae and any trails they may have left behind. If indoors, check all entry/exit points (doors, windows, vents, etc.), under carpet, within folds of fabric. While this sample doesn't need to be large, it should show the full diversity of the larvae present.
2. Prior to collection document the date, time, environmental data, location, and stage of blowflies observed. Make note on collection form and photograph overall scene and individual insects in place.



Figure 1: A large maggot mass.

3. Wearing gloves, use a spoon or similar scooping tool to collect a representative sample from the first mass and place appropriate container (see below). NOTE: All the maggots in a container should be collected from the same location on the body at approximately the same time.
 - a. For preserved sample: place in heat resistant container and skip to **Killing and preserving blowfly larvae**.
 - b. If DNA testing is desired: place larvae directly into vial of 95% ethanol.
 - c. For rearing sample: place in a container with sawdust and cat or dog food, cover the top of the container with a paper towel and seal with a rubber band.

4. 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. For the vial containing ethanol, place the label inside the vial and ensure the label is the full height of the vial and covers about half of the circumference. Write in pencil or micron pen.
5. Repeat this process for maggot masses throughout the body if needed, keeping maggots from different sample areas in separate labeled containers.



Figure 2: Collection using a plastic spoon.

Killing and preserving blowfly larvae

Properly preserved larvae are essential to accessing the life stage of the species present. When adults are not available, larvae can be identified through larval keys though these are not available for all species. Identification through DNA is also possible, though this is destructive.

1. Pour enough hot water into the heat resistant container to cover the larvae. If necessary, use forceps to carefully move larvae into the water. Let stand for 30-60 seconds. This amount of time allows for best preservation of larval morphology and length.
2. Use forceps to transfer larvae into a vial with 70-80% ethanol.
3. Create a label with the case number, sample location, time, and date. Make sure that this information is documented on the collection form as well. Place the label inside the vial and ensure the label is the full height of the vial and covers about half of the circumference. Write in pencil or micron pen.



Figure 3: Transferring larvae into storage vial.

Rearing larvae

Upon collection ensure there is sufficient food in the collection container to sustain the larvae. Add extra cat food as they grow if necessary. Check on the larvae daily to note their progress. NOTE: if sending live pupae, place in an empty vial with a slightly loose lid, label, and ship as fast as possible.

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 pupate, they do not need food (Figure 4).
2. If the food source looks insufficient or dry, add a tablespoon of additional food. Do not remove the old food as larvae may be inside.
3. Re-cover the container.
4. When pupae emerge and are developed enough to fly, place the container in a freezer.
5. Once all flies are dead, transfer into a vial with 70-80% ethanol.
6. Create a label with the case number, sample location, time, and date. Make sure that this information is documented on the collection form as well. Place the label inside the vial and ensure the label is the full height of the vial and covers about half of the circumference. Write in pencil or micron pen. Ensure the label specifies that this sample was collected as larvae and reared rather than collected as adults.



Figure 4: Stages of larval growth. Left: No wandering larvae, view from below. Center: Wandering larvae. Right: Pupae.

Pupal Collection Protocol

Pupae and exuviae (pupal casings) should be collected from the scene when observed. Pupal casings retain the impression of the larval form. This information can be useful in larval identification. Additionally, these casings can be used for toxicology if needed as any chemicals consumed by larvae will become part of the casing when it forms.

Supplies

gloves

forceps

vials (empty)

label and pencil

1. Photograph pupae or pupal casings in place before collection. Show both the overall context and a close-up of the pupa or casing.
2. Live pupae: collect live pupae using forceps and place in an empty vial with the lid left slightly loose.
3. Pupal casings: collect with forceps and place in an empty vial.
4. Create a label with the case number, sample location, time, and date. Make sure that this information is documented on the collection form as well. Place the label inside the vial and ensure the label is the full height of the vial and covers about half of the circumference. Write in pencil or micron pen.
5. Send any live pupae to a forensic entomologist as soon as possible as flies will emerge soon. Ship overnight.



Figure 5: Blow fly pupa.

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	forceps
vials with 70-80% ethanol	absorbent material (paper towel)
labels and pencil	glue trap

1. Place a paper towel soaked in ethyl acetate in the bottom of a jar and close the lid.
2. Wearing gloves, use the net to catch flies from on or around the body. Transfer the flies into the kill jar. Catch a representative sample of 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. Once flies are dead, remove from the jar with forceps and place in a vial with 70-80% ethanol.
4. Create a label with the case number, sample location, time, and date. Make sure that this information is documented on the collection form as well. Place the label inside the vial and ensure the label is the full height of the vial and covers about half of the circumference. Write in pencil or micron pen.
5. Alternatively, if there is a large swarm around the remains invert the glue trap and place near the body. Flies will land on the glue trap. Once sufficient flies are attached to the trap, carefully refold the trap and place in a plastic bag. Create a label with the case number, sample location, time, and date. Place a label inside and on the outside of the bag.

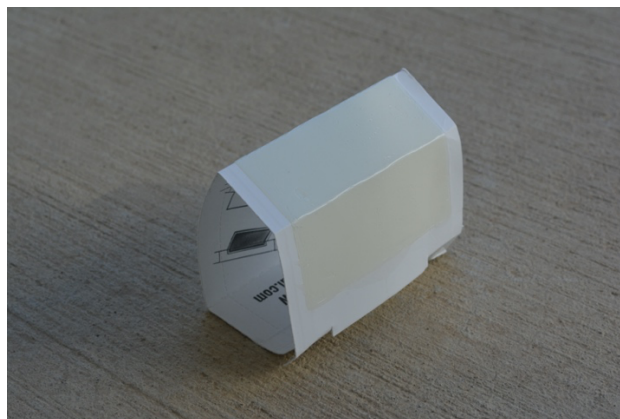


Figure 6: Inverted glue trap set up to capture flies.

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.
- If possible, use a glue trap as this is easier, less disruptive, and more efficient than using a net.

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 7: 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 8: 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. Again, a glue trap may be a better option in this situation.

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 flies are contained in the sweep net, confine them to the end of the net. This can be achieved by restricting the net with a hand and slowly moving toward the end.
2. Twist the end of the net and place inside the prepared kill jar. Seal the jar.
3. Keep the jar sealed until flies are no longer moving. Allow extra time to ensure flies are dead.
4. Open jar and transfer flies into vials with 70-80% ethanol.
5. Create a label with the case number, sample location, time, and date. Make sure that this information is documented on the collection form as well. Place the label inside the vial and ensure the label is the full height of the vial and covers about half of the circumference. Write in pencil or micron pen.



Figure 9: 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).



Figure 10: Transferring flies from the net into the kill jar.

Shipping Entomological Specimens

Once all specimens collected at the scene are ready for analysis, they can be sent to a forensic entomologist. Be sure to contact the desired entomologist before sending any samples or data to ensure they are willing to analyze the sample and the material sent is prepared in the manner they require.

Ethanol is a hazardous material, so special precautions must be taken when shipping specimens.

Supplies

Vials with collected specimens	Padding/absorbent material
Bags that can be heat sealed	Completed collection form
Shipping box	

1. Place each vial containing ethanol in its own heat-sealed bag. Each vial may contain a maximum of 30 mL of ethanol. A maximum of 1 L may be shipped in one box.
2. Place all bags together in another heat-sealed bag with absorbent material.
3. Ensure all material is appropriately padded within a firm cardboard box. Include scene collection form and any other relevant information.
4. Seal the box and label with "Scientific research specimens, not restricted. Special provision A180 applies."
5. Add shipping address and ship via air (not ground) to a forensic entomologist. Board certified forensic entomologists can be found on the American Board of Forensic Entomology website here: <https://forensicentomologist.org/members/>

FIRS Entomology Collection Form

Date: _____ Case #: _____

Time of collection: _____ Collected by: _____

Scene location (address, UTM or lat/long 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: _____

