OSAC 2022-N-0039 Standard for the Collection and Preservation of Entomological Evidence from a Terrestrial Environment

Crime Scene Investigation & Reconstruction Subcommittee
Scene Examination Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed Standard

OSAC 2022-N-0039
Standard for the Collection and Preservation of Entomological Evidence from a Terrestrial Environment

Prepared by
Crime Scene Investigation & Reconstruction Subcommittee
Forensic Entomology Task Group
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Disclaimer:

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Foreword

This document was developed to provide standards for collecting and preserving entomological evidence from terrestrial scenes. Entomological evidence may be collected by either a properly trained entomologist, medicolegal death investigator, crime scene investigator, or law enforcement officer. This document is intended to assist individuals engaging in collecting and preserving entomological evidence from a scene or victim. Portions of this document are adapted from Sanford et al. [9] and Byrd and Tomberlin [5]. Appreciation is expressed to Taylor and Francis Publishing for allowing the use of these materials in this document.

This document has been drafted by the Crime Scene Investigation and Reconstruction Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science through a consensus process.

Version No.	Issue Date	Section	Reason
2.0	May 7, 2024	--	Added to the OSAC Registry and publicly announced.
2.1	January 23, 2025	4.3.1	Removed text (ethanol concentration for relevant samples) from paragraph 3.

Keywords: *forensic entomology, insect evidence collecting, evidence preservation*

Table of Contents

Collecting and Preserving Entomological Evidence from a Terrestrial Environment

1. Scope

This document provides the standards for collecting entomological evidence for both preservation and rearing within the course of an investigation. This document is applicable to entomologists, medicolegal death investigators, scene investigators, and law enforcement officers who encounter entomological evidence.

2. Normative References

The following references are indispensable for the application of the Standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

- OSAC 2021-S-0013, *Standard Guide for Post-Mortem Examination Photography* (OSAC proposed standard)
- ANSI/ASB Standard 159, *Standard for Scene Investigation and Reconstruction-Foundational Principles*, 2024, 1st Ed
- OSAC 2021-N-0018, *Standard for On-Scene Collection and Preservation of Physical Evidence* (OSAC proposed standard)
- OSAC 2022-S-0037, *Standard for DNA-Based Taxonomic Identification in Forensic Entomology* (under development)

See Annex A, (informative) Bibliography, for other references.

3. Terms and Definitions

For the purposes of this document, the following definitions and acronyms apply.

3.1
cognitive bias
The class of effects by which an individual's preexisting beliefs, expectations, motives, and the situational context may influence their collection, perception, or interpretation of information, or their resulting judgments, decisions, or confidence.

3.2
exuvium
(plural: exuvia)
The cast skin of immature insects as they complete immature stages [8].

3.3
larva
(plural: larvae)
An immature stage of an insect, between egg and pupa, that undergoes complete metamorphosis [8].

3.4
minimum postmortem interval
minPMI
The minimum estimated amount of time that has elapsed since death, i.e., death could have been earlier.

3.5
myiasis
The colonization or infestation of healthy or decaying tissues of living humans, pets, or other vertebrate animals by the larvae of flies (Diptera) [3].

3.6
nymph
An immature stage of an insect, between egg and adult, with incomplete metamorphosis [8].

3.7
postmortem interval
PMI
The time between death and discovery of the decedent or remains; also known as time since death (OSAC Lexicon).

3.8
preserved samples
Samples that are killed and placed in a fixative.

3.9
pupa
(plural: pupae)
The insect life stage during which complete metamorphosis from larva to adult occurs [8].

3.10
puparium
(plural: puparia)
The sclerotized exuvium of the third larva of higher Diptera in which the pupa is formed [8].

3.11
rearing samples
Samples that are collected and maintained alive for maturation.

3.12
scene
A place or object that is subject to and/or requires forensic examination (ISO/FDIS 21043-1:2018[E]).

NOTE: In a situation that involves a potentially criminal act, definition would be preceded by "crime" (e.g., crime scene).

3.13
time of colonization
TOC
The time when insect offspring (i.e., eggs or larvae) were deposited on a human, pet, or other animal [3] or other decomposing organic matter (e.g., food, feces).

4. Entomological Evidence

Arthropods typically colonize humans and other animals while alive or soon after death. A common objective of forensic entomology is to estimate the time of insect colonization. Information determined from an assessment of forensic entomology evidence could generate investigative leads (e.g., when and where a suspected crime took place) in cases involving death, abuse, neglect or contamination, where insects have colonized or become associated with humans or other vertebrates.

OSAC 2021-S-0013, *Standard Guide for Post Mortem Examination Photography*, OSAC 2021-N-0015, *Guiding Principles for Scene Investigation and Reconstruction*, OSAC 2021-N-0018, *Standard for On-Scene Collection and Preservation of Physical Evidence*, and OSAC 2022-S-0037 *Standard for DNA-Based Taxonomic Identification in Forensic Entomology* shall be used in conjunction with this document because they provide the foundational principles upon which additional specific requirements, such as this document, will be based.

4.1 General Considerations

Recommendations in this document shall be followed to ensure proper collection and preservation of entomological evidence.

Collection and preservation shall be done with care to avoid cognitive biases. One form of bias is undue influence by task-irrelevant information, which may come from internal sources (e.g., a scene investigator's pre-existing motivations) or external sources (e.g., irrelevant features of the scene or learning other investigators' hypotheses). An example of such contextual biasing information in a death investigation would be learning when the victim was last seen alive [4]. Exposure to task-irrelevant information may cause other biases, including the failure to generate plausible alternative hypotheses or the premature rejection of alternative hypotheses.

Annex A provides a list of suggested collection and preservation materials that can be utilized on scene and compiled prior to a scene response involving entomological evidence.

4.2 Documentation

4.2.1 Photography

All photographs specific to entomological evidence shall be taken of the following, with and without a scale:

a) if present, the body *in situ*
b) the scene after removal of the body
c) close-up images of the body and any wounds
d) insects on and off the body

4.2.2 Additional Notations

In addition to the documentation standards and recommendations provided by OSAC 2021-S-0013, *Standard Guide for Post-Mortem Examination Photography* (OSAC Proposed Standard), ANSI/ASB Standard 159, *Standard for Scene Investigation and Reconstruction- Foundational Principles.* 2024. 1st. Ed, OSAC 2021-N-0018, *Standard for On-Scene Collection and Preservation of Physical Evidence* (OSAC Proposed Standard), and OSAC 2022-S-0037, *Standard for DNA-Based Taxonomic Identification in Forensic Entomology* (under development) notes specific to entomological evidence shall be taken, including:

a) body diagrams to indicate locations of insect colonization
b) the environmental conditions of the scene (e.g., in sun or shade, indoor or outdoor), including changes made to the environment after the discovery of remains (e.g., opening of windows, changing of thermostat settings)
c) time/date the body was discovered
d) any times and details relating to specimen collection and further actions to preserve or kill specimen(s)
e) if specimens are collected at autopsy, the time the body is placed in refrigeration, the time removed for autopsy, and the temperature of both refrigeration unit and morgue rooms shall be recorded
f) where possible, provide:
    i. hourly air temperature at the scene using temperature data loggers [7]
    ii. dependent upon scene/circumstances:
        1) body surface temperature
        2) maggot mass temperature(s) (if present)
        3) when specimens are collected from the soil, take the temperature of the soil

4.3 Specimen Collection

4.3.1 Collecting Specimens

Representative specimens (approximately 50 individuals if available) of all life stages (Annex B) observed at a scene shall be collected if possible [6], including visually different insect species

and from different sites of infestation or off-body scene locations (e.g., under body, surrounding vegetation).

During collection, each sample shall be placed in appropriate containers with labels. Do not mix samples as it may result in material being destroyed or inaccurate estimates of insect age at the time of collection being made. Different locations on a body could be colonized at different times and by different species. Therefore, live samples collected for rearing shall not be commingled (e.g., from different life stages - eggs, larvae, and intact puparia; species; or collected from different locations on the body). The same approach shall be applied for samples from off-body locations.

For each preservation sample collected (4.3.2), a comparable live sample for rearing (4.3.3) should also be collected. Include case number, item number, collector name, time and date, geographical location, site of the collection - on or around the body. Most immature insects (i.e., eggs, larvae/nymphs, pupae) can be collected using forceps or gloved fingers.

For collecting adults, a sweep net or sticky trap can be used. Sticky traps can be placed near the body. Once the scene has been processed, the trap can be labeled, inverted into a cylinder with the sticky side on the inside ensuring not to overlap the sticky portion, and placed in a sealable plastic bag. Avoid crushing the cylinder as it will prevent the removal and identification of the insects collected.

a) Appropriate life stages (Annex B) shall be collected from on and around the body, bearing in mind that:
   i. certain stages might not be present
   ii. early stages can be small
   iii. later stages (mature larvae, puparia) can be dispersed several meters around the body and, depending on the environment, may have become hidden (e.g., burrowed into soil and under carpet)
   iv. specimens can vary in size and appearance: examples of the following are presented in Annex B:
      1) eggs
      2) larvae
      3) pupae or puparia
      4) adults
      5) empty puparia and larval skins (exuvia)
b) For larval samples, collect specimens representing a full range of sizes [2].
c) Use tandem labeling (i.e., place a label in the container - using a pencil or alcohol-resistant archival ink - and a duplicate label on the outside of the container).
d) Labels shall include case number, item number, collector name, time and date, geographical location, site of the collection - on or around the body, ethanol concentration for relevant samples.

4.3.2. For Preservation Samples

a) To prevent sample decomposition in the early stages of preservation, larval or pupal samples should be killed by immersion in hot water (at least 80°C / 176°F) for at least 30 seconds and then transferred to a container with 70%+ ethanol. Ideally, hot water could be brought to, or prepared, on site. However, if resources are unavailable for a hot water kill within two hours of sample collection, the specimens shall be directly preserved by placing alive into a container with 70%+ ethanol [1]. Time and date of preservation and method shall be noted.
b) For samples collected for DNA analysis, see OSAC-S-0037-STRP *Standard for DNA-Based Taxonomic Identification in Forensic Entomology*.
c) Egg specimens shall be placed directly into a container of 70%+ ethanol and time and date of preservation shall be noted.
d) Adults caught with a sweep net shall be placed directly into a container with a liquid preservative, frozen, or stored dry. Time and date of preservation shall be noted. If adults are collected with a sticky trap, the trap shall be stored in a dry place or freezer, while avoiding flattening the trap which could result in specimen damage.

4.3.3 Temporary Storage of Immature Samples for Rearing

Samples to be reared to adults shall be placed in a ventilated (breathable) container that prevents escape and stored in a room with known temperature. Within 24 hours of collection, the samples should be processed for rearing following approved procedures (OSAC *Rearing Immature Flies to Adult Stage,* under development).

4.4 Other Information Required

Forensic entomology can be used to estimate the period of neglect for a living person or other animal suffering from myiasis. In such cases, it is usually advised that the collection is made by medical personnel under the direction of a forensic entomologist. When collecting insect evidence from a dead body, it is important to note other potential sources of insects near the decedent, as they might be either a source of the colonizing insects or a source of unrelated but confounding insects. These may include other carrion, decomposing food, garbage, or dirty diapers.

Temperature is vital to the calculation of the TOC as insect development is temperature dependent. Temperature data from government weather stations are most commonly utilized. It is important to locate the nearest weather station.

4.5. Scenes

4.5.1 Indoor

a) Bodies discovered indoors involve different challenges to those at outdoor scenes, including questions concerning insect access and potential modification of indoor temperatures.

For insect samples taken indoors, it is important to note:

- if windows/doors are open and with or without insect screens
- whether the sample was collected from a site in direct sunlight
- thermostat temperature and time settings
- ambient air temperature at the scene, prior to thermostat alteration if possible
- presence of decomposing food or feces
- any existing potential points of entry for insects, such as broken windows, missing doorknobs, gaps in the ceiling

b) In indoor scenes, it is important to note when first responders entered and whether they left doors open or opened windows to ventilate the scene, which can then allow both ingress and egress of adult insects. Flies that may have emerged from the scene may leave, whereas outdoor flies may enter to lay eggs, attracted by decomposition fluids and blood. Also, if doors and windows are opened, this changes the inside conditions, potentially increasing or decreasing the temperature.

c) After a period of time, fly larvae usually leave the remains and conceal themselves. In outdoor scenes, a suitable site may be found by burrowing into the nearby soil, but in indoor scenes with hard floor surfaces, the larvae may crawl much farther and may be located under rugs, in other rooms, within furniture, and under appliances. Therefore, a more extensive search may be required. Fly puparia may also be confused with rodent feces. When carpet/hide beetles have colonized the decedent, large accumulations of frass or insect feces and larval exuvia may be present, and the fragile pupae or exuvia may be easy to overlook.

4.5.2 Outdoor

Outdoor scenes often have many more unknowns than indoor scenes, particularly as regards temperature. However, there is usually no impediment to insect access unless the body is buried or wrapped.

a) Whether the body is in the sun or shade and for how long should be noted as this can impact temperature.

b) Once the body has been removed, the soil beneath shall be searched for insect activity (e.g., insect larvae or pupae).

   i. Depending on soil compaction, the search can be as deep as 10 cm.

   ii. Soil samples collected from beneath the body to about two meters distant laterally.

4.5.3 Autopsy

Ideally, insect evidence should be collected at the scene. However, in some cases (e.g., limited time, lack of equipment), it may not be possible, or insects may not be noticed until the autopsy. In such cases, as previously noted, the time that the remains entered the morgue refrigerator and the time that they were removed from the refrigerator shall be noted as insect development may slow down or cease when refrigerated.

a) The temperature of the refrigerator and the morgue suite shall also be recorded.
b) As the ambient temperature of the morgue suite may allow some insect development, to mitigate this issue, insects shall be collected as soon as possible after the body is removed from refrigeration.
c) If noted during autopsy, ticks, lice, or fleas shall be collected and placed in 70%+ ethanol. Notes shall include whether such specimens were alive when collected as well as if any insect artifacts are present (e.g., bee, wasp, or ant stings as well as cockroach or other insect feeding-sites on the body).

4.5.4 Burials, Wrapped, Confined

a) Insects shall be collected from buried bodies in the same way as from any other bodies, but an increased search of the surrounding soil may require the use of coarse sieves.
b) When bodies are wrapped, insects may be found throughout the wrapping.
c) When dealing with confined spaces (e.g., trunk of car), use methods previously outlined for other locations (e.g., indoor) as specimens may be found in the seams or crevices of the confined area.

Annex A
(informative)

Suggested Equipment and Supplies List for Entomology-Based Collection in Forensic Investigations.

General Field Equipment

- **Case notebook** including body outline templates (see 4.2.2 and [2]) for recording information relevant to forensic entomology assessment (e.g., location of insect activity)
- **Camera** for photo-documenting remains at the scene and location and type of entomology evidence
- **Ruler** to be used as a scale of reference for images taken with a camera
- **Waterproof ink pen and ethanol-proof writing utensils** (e.g., pencil, permanent ink pen)
- **Precut paper or stickers** for preparing labels which are placed inside containers with live or preserved specimens (e.g., containing ethanol) as well as attached to the outside of the container
- **Evidence bags** (include an outer paper bag to protect specimens from direct sun and spectators from viewing contents)
- **Headlamp flashlight** (facilitates using both hands to collect at the scene) and **extra batteries**
- **Personal protective equipment (PPE)** (e.g., one-piece suit, gloves, shoe covers, mask, mosquito repellent)

Sampling Equipment for Insect Evidence

- **Container for hot water** kill fixation of soft-bodied specimens (e.g., a thermos of at least approximately 80°C / 176°F water, electric hot water heater that can plug into automobile or other external power source)
- **500 mL of 70%+ ethanol** (avoid isopropyl and denatured alcohol as these interfere with DNA extraction)
- **Forceps** for collecting entomological specimens
- **Positive closure screw cap containers** or other leak-proof containers (e.g., 5 mL containers)
- **Ambient air temperature thermometer** to record the temperature at the time the scene is processed
- **Probe thermometer** for measuring substrate (e.g., water, soil) temperatures or maggot mass temperatures (when a non-contact thermometer is unavailable)
- **Non-contact thermometer** (e.g., infrared, laser) for measuring maggot mass temperatures or other surfaces where contact will either disturb evidence or the body
- **Temperature data logger** for scene temperature collection on the hour every hour for five days
- **Packing material** (e.g., paper towels) to secure glass vials during transport
- **Flat trowel** to scrape and search the soil for insects associated with the decomposing remains
- **Hand-held net** for sampling adult entomological specimens

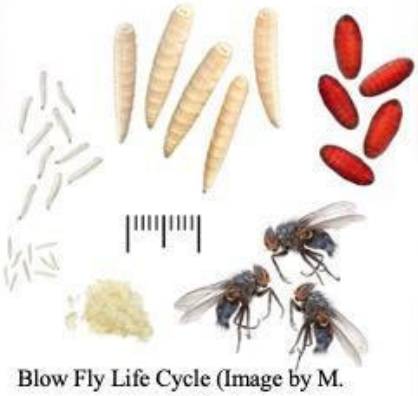
- **Sticky traps** for the passive collection of adult insect

Annex B
(informative)

Life Stages of a Blow Fly

Blow Fly Larvae (Image by M. Hall)

Blow Fly Eggs (cream color) in Cup (Image by G. Anderson)



Blow Fly Life Cycle (Image by M. Hall)

Blow Fly Puparia, which Contain Pupae (Image by G. Anderson)

Blow Fly Adult (Image by M. Hall)

Annex C
(informative)

Bibliography

This bibliography is not meant to be an all-inclusive list, as the group recognizes other publications on this subject exist. When this document was drafted, these were some of the publications available for reference. Also, any mention of a particular software tool or vendor as part of this bibliography is purely incidental, and any inclusion does not mean that the authors of this document endorse it.

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