PROTOCOL	Insect Shipping Methods for the ETAIN Project
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1. Purpose

This Standard Operating Procedure (SOP) describes the standardized methods for shipping insect samples within the ETAIN project consortium. Four distinct preservation and shipping methods have been validated and are documented here to ensure sample integrity during transport between partner institutions.

2. Background

The aim of the ETAIN project is to study the effect of radio-frequency electromagnetic field exposure on insects. This research requires the collection, preservation, and inter-laboratory shipping of various insect species including bees and other pollinators. Different partner institutions have developed and validated specific methods optimized for the final use of the insect samples. This protocol consolidates four proven methods used successfully within the consortium. Three methods involce the shipping of whole, intact insect specimen for imaging and experimental purposes. One method was aimed as shipping insect materials with the aim of preserving material properties rather than preserving insect morphology. The different methods are described below.

3. Shipping Methods

3.1. Method for Insect Shipping and Preservation in Ethanol - Cyprus University of Technology

Materials Required:

- Small falcon tubes
- 99% absolute ethanol
- Parafilm
- Small plastic storage box
- Bubble wrap
- Waterproof labels

Procedure:

1. Place collected bees/insects directly into small falcon tubes
2. Fill tubes almost completely with 99% absolute ethanol, ensuring specimens are fully covered
3. Seal tubes tightly and wrap the closure with parafilm for additional security
4. Label each falcon tube with collection date and sampling area (full name or location code)
5. Place sealed tubes in a small plastic storage box (Figure 1)
6. Fill empty spaces in the box with bubble wrap to prevent tube movement during transport
7. Seal the storage box and label with shipping information

Figure 1: Illustrations of shipping method used by Cyprus University of Technology. (Left) Plastic storage box with falcon tubes. (Right) Detail view showing a falcon tube with wid bee specimen.

3.2. Method for Insect Shipping and Preservation in Ethanol – Agricultural University of Athens

Materials Required:

- Collection containers
- 95% ethanol
- Appropriate labeling materials

Procedure:

1. Collect insects while alive
2. Immediately preserve specimens in 95% ethanol

3. Ensure complete submersion of specimens in preservation medium
4. Label containers with appropriate collection metadata
5. Package for shipping according to hazardous materials regulations

3.3. Method for Individual Specimen Preservation and Shipping using ethanol or hand gel sanitizer (ELGO)

Materials Required:

- Eppendorf tubes (1.5 ml or 2 ml)
- 80% ethanol OR hand gel sanitizer (ETAPROBEN or equivalent)
- Parafilm
- Zip-lock bags
- Permanent markers for labeling

Hand Sanitizer Composition (ETAPROBEN):

- Ethanol: 52%
- Isopropanol: 10%
- Benzyl alcohol: 1%
- Glycerol: 3%
- Water: remainder (34%)

Procedure:

1. Place each individual bee/insect in a separate Eppendorf tube
2. Fill tube almost completely with preservation medium (80% ethanol or hand sanitizer)
3. Secure each tube with parafilm around the cap
4. Group tubes containing the same insect species in separate zip-lock bags
5. Label bags clearly with species and collection information
6. Store in appropriate shipping container

Note: Hand gel sanitizer has been observed to preserve specimens better than 80% ethanol under field conditions.

3.4. Method for Homogenized Sample Preparation and Shipping (ELGO-UGENT)

Materials Required:

- Porcelain mortar and pestle
- Plastic pestle
- Eppendorf tubes
- Falcon tubes
- Vortex mixer
- Fresh larvae (3-4 days old)

Procedure for Larvae/Pupae:

1. Collect approximately 50-60 larvae of 3-4 days age
2. Place larvae in porcelain mortar

3. Crush specimens using porcelain pestle for 5-6 minutes until homogeneous
4. Transfer homogenized mixture to an Eppendorf tube
5. Further homogenize using plastic pestle and vortex mixer
6. Transfer final homogenized mixture to falcon tube, filling completely
7. Label with collection data, species information, and preparation date
8. Store appropriately for shipping

4. General Guidelines

Labeling Requirements:

- Collection date (DD/MM/YYYY format)
- Sampling location (full name or standardized location code)
- Species identification (when known)
- Preservation method used
- Collector name/initials and affiliation
- Project code: ETAIN

Packaging Requirements:

- Use leak-proof primary containers
- Employ secondary containment (plastic boxes, zip-lock bags)
- Include absorbent material to minimize collisions between vials
- Cushion containers to prevent breakage during transport
- Include chain of custody documentation
- Comply with international shipping regulations for biological samples and ethanol

5. Quality Control

Each participating institution should maintain detailed records of sample collection, preservation, and shipping. Visual inspection of samples should be performed before shipping to ensure adequate preservation and proper labeling. Photographic documentation is recommended for valuable or rare specimens.

This Standard Operating Procedure was compiled from validated methods used by ETAIN project consortium partners.

Visual documentation shows actual field implementation of the described methods.

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