H107 How Should Live Entomological Samples Be Stored?

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After attending this presentation, attendees will better understand the current state of the art in collecting and storing entomological samples from a body during autopsy or at the death scene.

This presentation will impact the forensic science community by providing guidelines for sampling and storing living samples of the forensically important blow flies, *Lucilia sericata* and *Calliphora vicina*.

Sampling and storing insect evidence alive is an important task in forensic entomology as different methods can influence survival and growth rates of the living samples. The best practices recommend that living fly larvae should kept under controlled and known conditions (2°C-6°C, preferably) and stored in vials with an air-permeable lid equipped with sawdust or a paper for absorbing excretion liquids. Samples should then be transferred to an expert within 24h. Although this time interval seems to be a realistic approach, cooling the samples appropriately or catering them during storage seems to be a serious logistical problem. Unfortunately, many of the above recommendations are mainly based on expert opinions rather than scientific evidence.

In order to look at the effect of the lack of cooling and/or inappropriate storing of entomological samples before their arrival in the laboratory, the following experiment has been performed. Samples of all larval stages (*L1*=24 hours after hatching at 25°C, *L2*=48h after hatching, and *L3*=72h after hatching) of the forensically relevant blow fly species *Lucilia sericata* and *Calliphora vicina* were divided in two main groups with 33 larvae for each group. The first group was stored at room temperature (~20°C) and the second group in a refrigerator (5°C) for 16h without air, a food supply, or sawdust. Next, they were kept at 2°C-6°C in a Styrofoam™ box for 8h, simulating a transport situation. After 16+8=24h, the storage Mortality Rate (MR) was calculated and 25% of the surviving larvae were killed in hot but not boiling water. Their length was measured and the remaining living specimens were reared (25 C) on their food substrate until adult eclosion. The results were then compared with a control group in which larvae were not sampled but left to feed in the rearing boxes with air-permeable lids on their food substrate at 25°C. All containers were checked for MR every 24h for the effect of 24h of hypoxia on adults; later, hatched flies were frozen and separated according to sex. The length of the left mesothoracic tibia and dc-um vein of the left wing were measured using a dissecting microscope and computer software.

Results revealed a high MR for L3 larvae stored both at Room Temperature (RT) and in a cool environment (100% for both species at RT; for chilled larvae 100% of *L. sericata* and 54.03% of *C. vicina* with an MR in the control 2.53% for *L. sericata* and 8,08% for *C. vicina*). For *L. sericata* L1, the MR was respectively 16.16% at RT, 4.55% for chilled samples, and 2.25% in the control group. For *L. sericata* L2, the MR was 24.73% at RT, 10.61% for chilled samples, and 7.24% in the control group. For *C. vicina* L1, the MR was 35.86% at RT, 10.6% for chilled samples, and 2.94% in the control group. For *C. vicina* L2, the MR was 12.65% at RT, 10.61% for chilled samples, and 9.05% in the control group. Results highlight that the 24h interval time of storage can stop the larval growth in comparison with the control group. The lack of growth was extremely significant in *L. sericata* samples: 0.39cm for L1 at RT and 0.38cm in chilled samples; 0.72cm and 0.71cm for L2 at RT and in chilled samples; 0.17cm and 0.14cm for L3 at RT and in chilled samples. The lack of growth was in *C. vicina* samples of 0.47cm for L1 at RT and 0.45cm in chilled samples, 0.68 cm for L2 at RT, and in chilled samples, 0.25 cm and 0.24 for L3 at RT and in chilled larvae.

The duration of storage needs to be considered when performing the age calculation of larvae to estimate the minimum Postmortem Interval (PMI_{min}). The living larval samples should be stored at least at cool temperatures (e.g., in a refrigerator) instead of room temperature. This is strongly recommended for larvae with a size <1cm (L1-L2 stages) based on the high MR for L3 samples. For larvae >1cm, such as L3 samples, the recommendation is to add paper to the storage vials with air-permeable lids for absorbing the liquids (excrements, enzymes) and to reduce the interval of storage and transport considerably, keeping the temperatures low at a refrigerator level.

Forensic Entomology, Sampling Methods, Storing Methods

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