Processing of larval samples

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I- Preservation of mosquito immatures

- Preservation of larvae and pupae in the field

Formula A

-Alcohol 70%, 95ml

-Glycerol 5ml

Formula B

-Water 94 ml,

-Formalin 6 ml,

-Borax 0.5 gm

Preservation of mosquito immatures

- For detailed microscopic examination the egg, larvae and pupae can be preserved on slides with mounting medium.
- For making permanent preparation of the larvae can be mounted in Euparol or Bhatia's media, dehydrating it with 90% methanol or may be mounted in Canada balsam for which the material has to be passed through various grades of Ethyl Alcohol viz:30%,50%,70%, 90% and 100% and is finally treated in xylol.
- For temporary mounting the larvae can be directly mounted in Hoyer's medium.

Preservation of mosquito immatures & Mounting

- For permanent mounting of fourth instar larvae, drop the larvae in chloral hydrate solution (80gms of chloral hydrate powder in 20 cc of 30 per cent Acetic acid) and retain for 24 hours.
- Larvae should not be kept in the above clearing agent for a long time to avoid any damage to the delicate setae.
- The larvae should be lifted out of the chloral hydrate solution with the help of a fine needle loop (larvae should not be handled with brush or straight pointed needle as they might cause destruction of the delicate setae), by passing the loop over the tail.
- Each larva should then be mounted in Hoyer's medium on a clean glass slide.

Preservation of mosquito immatures & Mounting

- The refractive index of Hoyer's Medium is lowest (1.45) among all the mounting media in use, viz: Canada balsam (1.524), Phenol balsam (around 1.5), Creosote balsam and Chloral gum (little less than 1.5) and Bhatia's Medium (1.497). This method is rapid and simple and requires a minimum handling of specimen.
- The mount is transparent and colourless which sets hard by itself in 12 hours without being kept in an incubator.
- The preparation is permanent and there is no need to seal the edges of the mount with any cement. Remounting, if need be, can be done by flooding the mounted area with 45 per cent.
- Acetic acid which will loosen the coverslip within a short time facilitating its removal with ease.

II. Preservation of mosquito larvae

For the Identification of the mosquito species., the larva collected during the entomological surveys can be send. Therefore, the larvae must be preserved and mounted.

Materials required

Wide mouth pipette, 70% Ethanol, 95% Ethanol, Hot water, Beakers, Glass vials, Cotton wool, Microslides & Coverslip, Lactophenol, Nail varnish & Euparal

II. Preservation of mosquito larvae

Procedure:	Pickup the 4th Instar with the pipette and transfer it to the clear water in a container
	If the larvae is of early instars keep them until they become 4" Instar
	Transfer the larvae to hot water (70 C) to kill the then without any deformity
	Transfer the killed larvae to 70% alcohol in a container and leave them for 24 Hours
	Then transfer the larvae to small containing 95% alcohol and Plug the vial with cottonwool
	Label the tube with Locality.
	Type of habitat
	Date of collection
	Name of collector

Mounting of Mosquito Larvae

Mounting media such as Lactophenol (Lactic acid 10m1, Phenol 10 gm, Glycerol20ml &Distilled water 10 ml and store in a dark bottle) and Polyvinyl Lactophenol can be used for quick mounting and can be kept of several years.

For a more permanent mount Euparal is used.

Procedure:

At the Center of a clean labelled slide put one drop of clean water and draw a circle for the placement of the larva

Transfer one larva using a dropper over the water drop and adjust the specimen facing dorsal side up

Remove the water with a filter paper strip

Cover the specimen with a small drop of mounting medium

And a small drop of Mounting medium to the Center of the cover slip

Mounting of Mosquito Larvae

- Invert he cover slip and gently lower onto the specimen so that the mounting medium join together
- Leave the side to dry and while drying if any space appears between the slide and coverslip ill it with more mounting medium
- Apply clear nail varnish to the edge of the cover slip to form á sea
- Label the slide with
- a) Locality b) Type of habitat c) Date of collection d) Name of collector e) Species
- Examine the specimen under the microscope
- For Euparal mounting place the larvae in 70% alcohol for 24 Hrs
- Transfer the larvae to 95% Ethanol and keep for 24 Hrs
- Transfer the larvae to clove oil the larvae sink on to the bottom

Mounting of Larvae

- Place the larva
- Examine the specimen under the microscope
- For Euparal mounting place the larvae in 70% alcohol for 24 Hrs
- Transfer the larvae to 95%
- Ethanol and keep for 24 Hrs
- Transfer the larvae to clove oil the larvae sink on to the bottom
- Place the larvae in a drop of Euparal at the Center of a clean-labelled slide and mount it as before
- Allow the slide to dry (This will take several days and require no sealing
- Ready for microscopical examination and can be store for any number of years.

Conservation of specimens

Larvae

- **For immediate morphological or genetic identification**:
- collection in vials with 70–80% ethanol.
- **For morphological identification only after further development:**
- collection in vials/small containers together with water from breeding place for rearing L1-L3 larvae to L4 larvae (which can be identified with higher reliability) or for keeping the larvae until adult emergence.

Conservation of specimens

- *MALDI[matrix-assisted laser desorption ionization]-TOF-MSA tool for rapid accurate and cost effective identification of cultured bacteria & fungi in clinical microbiology

- **Eggs**
  - Oviposition supports can be stored in a closed plastic bag, at room temperature or in a fridge (5–15 °C). The bag should not contain free water, but should still be humid (around 55% relative humidity).
  - For genetic identification (PCR, DNA sequencing), the eggs can be put in 70–80% ethanol.
  - For MALDI-TOF MS*, eggs are best kept on the oviposition support (but should not dry out) and transferred to (dry) microtubes before sending them out for analysis.