

AN IMPROVED TECHNIQUE FOR MOUNTING MOSQUITO LARVAE

JAMES F. BUCKNER

From the Gorgas Memorial Laboratory, Panama, R. de P.

The common method of killing mosquito larvae with hot water followed by the usual steps in dehydration causes so much distortion and the loss of hairs that it interferes with the identification and complete description of the larvae. It occurred to the writer that the method of killing was too drastic. The technique to be described should be carried out in a series of small preparation dishes or watch glasses. The steps are itemized as follows:

1. Place the larvae in 1.5 per cent magnesium sulphate solution for fifteen to twenty minutes. This will clear the specimen of foreign matter.
2. Place in clear water for two or three minutes.
3. Transfer to 2 per cent cocaine hydrochloride solution (1) until they are dead. The siphonate larvae require four hours the asiphonate larvae two hours in this solution before it kills them. The results, however, more than repay for the time consumed.
4. Pass the specimen for ten minute intervals through each of the graded alcohols: 30, 50, 70 and 95 per cent.
5. Transfer to absolute alcohol for fifteen minutes.
6. Clear in cedar wood oil (water white).
7. Pass rapidly through xylol and orient in balsam in cells made as indicated below.

CELLS FOR MOUNTING

To be of value ring-cells must be permanent and withstand the action of xylol and changes in temperature. They must be composed of materials that are easily obtained and that are not prohibitive in price. Cells made from tin or glass rings are ideal

but the process is time consuming, expensive and the materials are not always readily obtainable. There is a dental wax (2) on the market that meets the above requirements but it must be melted at a low temperature and that temperature maintained. This wax is marketed under the trade name of Tru-Wax and sold by the Dentist's Supply Co., New York, N. Y. If this wax is used the hard grade is recommended. A wax made of the following ingredients is very satisfactory and is not injured even when permitted to boil:

	<i>grams</i>
Beeswax.....	25
Paraffin (melting point 60° to 62°C.).....	10
Gum mastic.....	6
Prepared chalk.....	2
Vermillion (for color).....	4

Powder the gum mastic and mix it with the chalk and vermilion and then add this mixture to the melted beeswax and paraffin. Permit this entire mixture to maintain a degree of heat that will make it simmer, but not vigorously boil, for fifteen minutes. Stir with a glass rod during this period.

PREPARATION OF THE CELLS

The materials required are as follows: micro slides 3 x 1 inch; turn table; camel's hair brushes; needles, small, for orienting the specimen; micro cover glasses, circles of $\frac{7}{8}$ -inch diameter and $\frac{1}{2}$ -inch diameter; and wax as described above.

Melt the wax. Place a clean, grease free slide on the turntable and with the aid of a camel's hair brush that has been trimmed to a small point and saturated with the melted wax, proceed in the usual manner to build the rings. They can be made as thick or as thin as one desires. Rings of one layer, the thickness of a hair, are as durable as those made of many layers.

Next place a small amount of balsam in the new cell and orient the specimen then fill the cell with whatever mounting medium is to be employed and cover it with a micro cover glass of the correct diameter. A small amount of the mounting medium will probably ooze over the edges but this can be disregarded. Place

the micro slide on an even, level surface and let the mounting medium set. In a few hours the slide is again placed on the turntable and sealed with a layer of the same wax used to build the cell.

These cells have been in use for the mounting of mosquito larvae, ticks and fleas. The specimens have been exposed to temperatures of 100°F., to the freezing compartment of an electric refrigerator, to tropical sun rays and they are in the same excellent condition as when first prepared.

The writer extends his thanks to Mr. L. H. Dunn, Medical Entomologist and Assistant Director of Gorgas Memorial Laboratory, for his help and suggestions during the development of this technique.

REFERENCES

- (1) WARD, H. B. AND WHIPPEL, G. C.: *Rousselet's Method*. Described in *Fresh Water Biology*, p. 583. Published 1918. Sold by John Wiley and Sons, Inc., New York City.
- (2) JAMES. Quoted by BOYD, M. F.: *Introduction to Malariology*, p. 375. Published in 1930 by the Harvard University Press, Cambridge, Mass.