

# A TECHNIQUE FOR DRYING EGGS OF *ANOPHELES STEPHENSI* AND ITS EFFECT ON THEIR VIABILITY AFTER STORAGE AT 4°C<sup>1</sup>

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**ABSTRACT.** A simple apparatus is described for drying mosquito ova. Eggs of *Anopheles stephensi* Liston dried by this method and maintained at 4°C retained 50% viability

The U. S. Navy Malaria Vaccine Program is presently supported by an insectary of limited size. In order to provide maximum mosquito production while maintaining operating flexibility we have developed or adapted several techniques of possible interest. This paper presents a modification of the egg drying method of

following storage for 5 days, permitting increased flexibility in mosquito colony operations.

Dame et al. (1978) which permits short-term storage of *Anopheles stephensi* Liston eggs prior to their utilization.

## MATERIALS AND METHODS

A drying box (Fig. 1) was constructed using 4 pieces of 1.6 x 15.25 x 15.25 cm particle board for the sides and 2 pieces of 0.6 x 18.45 x 15.25 Masonite® for the top and bottom. A 3.5 cm diameter hole was drilled in the center of the top to accommodate the base of a 15 cm diameter plastic funnel. A similar hole was drilled in one side to fit the hose of a vacuum cleaner, in our case a Pullman Model JB 365 Industrial Tank Vacuum.

Eggs from the stock colony of *An.*

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*stephensi* were washed with demineralized water through 16 mesh screening to remove debris. They were then transferred by washing into a cone formed from a 380 mm diameter circle of standard qualitative filter paper and supported by a cone of 16 mesh screening resting in the plastic funnel. A slightly greater angle of the screen cone allowed full air flow between it and the inner surface of the funnel. The funnel, with filter paper and eggs, was then placed in the top hole of the

drying box and subjected to approximately 15 min of vacuum pressure. Dried eggs were brushed from the filter paper with a stiff plastic-bristled brush and either used immediately or stored in stoppered glass vials at 4°C. This temperature was chosen following a preliminary observation that ova kept at room temperature rapidly lost viability.

Our colony is routinely maintained on guinea pigs, and the drying experiments were initially restricted to this blood

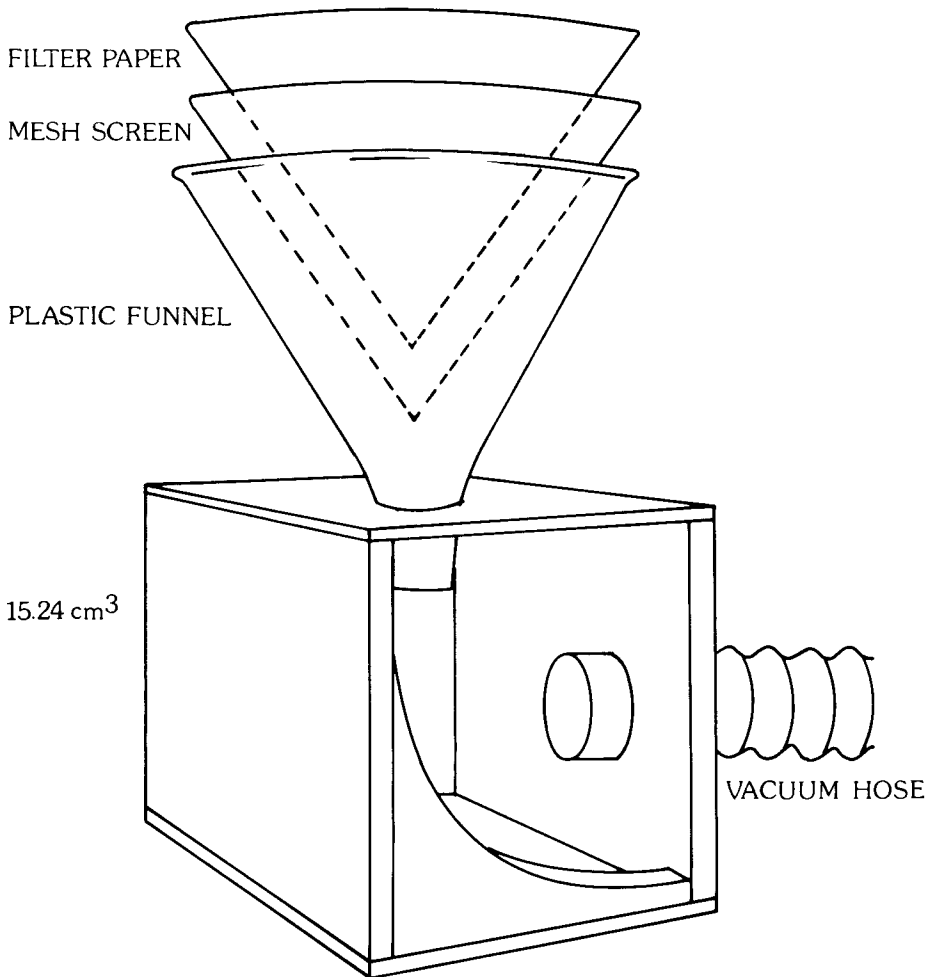


Figure 1. Egg drying box.

source. As other blood sources became available, however, they were included to determine the effect, if any, of the origin of the blood meal on the ability of the resultant eggs to withstand drying and storage.

Mosquitoes were fed directly on guinea pigs and mice. Bovine and human blood, and sheep red blood cells, suspended in Alsever's solution, were presented in animal membranes (Young Rubber Corp., Atlanta, Ga.).

To determine the effect of drying on the viability of ova, samples of 100 eggs each were removed from the drying box and placed in glass beakers containing demineralized water at rearing room temperature (28°C). When hatching was completed, the percent success was determined by counting the free-swimming larvae. In each instance a similar sample of undried eggs from the same source served as a control.

The following number of replicates were tested from each blood source: Guinea pig, 8; mouse 6; sheep RBC's, 4; human, 2; and bovine, 2. A paired *t* test was used for statistical analysis of the data.

The effect of storage at 4.0° C on egg viability was determined by removing samples of 100 eggs each at intervals from 2 to 9 days following drying and allowing them to hatch as described above. Percent hatch following each storage interval was recorded and plotted graphically.

## RESULTS AND DISCUSSION

The dried eggs from each blood source displayed increased viability relative to undried eggs from the same source. The differences were statistically significant, however, only for mouse blood and sheep RBC's. Although there was no significant difference in viability between dried and undried ova derived from guinea pig blood, the normal source of food for our colony, the results clearly establish that drying of *An. stephensi* ova by this method has no deleterious effect on their viability.

Following storage at 4°C the viability of dried ova derived from guinea pig blood dropped below the 50% level by day 5, and to zero by day 9. Interestingly, ova from blood sources not employed for routine maintenance of the colony were less affected by storage than those derived from guinea pig blood. Of these, ova from bovine blood and sheep RBC's displayed the greatest resistance, withstanding c. 8 and 7 days storage, respectively, before viability dropped to the 50% level. It was observed that dried eggs from all sources stored for more than 3 or 4 days exhibited a 12 to 24 hr delay in hatching which must be taken into consideration when utilizing dried ova in routine procedures.

The results from this study have several practical applications within our insectary operation. Dried eggs are easily handled and can be measured volumetrically for optimal and reproducible seeding of larval rearing pans. In conjunction with cold storage, eggs may be kept for 5 or 6 days with an anticipated hatch of about 50%. This permits flexibility in accommodating to holiday routine or absence of personnel, although the lowered viability of the ova must be allowed for when seeding rearing pans.

We have observed no deleterious effects on developing larvae or adult mosquitoes raised from eggs dried and stored by this method, and the technique is routinely employed in our insectary operation.

## ACKNOWLEDGMENTS

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### *Literature Cited*

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