AN IMPROVED LABORATORY TEST CAGE FOR TESTING REPELLENTS ON HUMAN VOLUNTEERS[1,2]

RAJ K. GUPTA,[3] L. C. RUTLEDGE[3] AND W. J. LETOURNEAU[4]

Recent introduction of sustained-release technology in topical repellant formulations has resulted in a surge of activity to evaluate these new formulations against biting arthropods (e.g., Gupta and Rutledge 1989, Lillie et al. 1988, Sholdt et al. 1988). Many evaluations are conducted using the test cage described in the American Society for Testing and Materials (ASTM) standard E951-83 "Laboratory testing of noncommercial mosquito repellent formulations on the skin" (Anonymous 1983). The test cage described in the standard is made of Plexiglas® and is handcrafted, which makes it expensive ($25–$34/cage) and nonexpendable. Also, this cage cannot be autoclaved, hence there are cleaning and drying problems precluding immediate reuse. This paper describes an inexpensive (about $4 or less) disposable test cage that can be autoclaved and reused immediately for evaluating repellent formulations on the skin.

The new test cage is made of Lexan®, a polycarbonate plastic offering unique properties including clarity, stability, toughness, autoclavability and nontoxicity to test organisms. The body of the test cage with a groove for the slide was injection molded at the U.S. Army Natick Research, Development and Engineering Center, Natick, MA. Five holes (29 mm) were punched in line in the bottom and a 20 mesh screen was heat-pressed on the top (Fig. 1). This cage provides condensate-free visibility for observation of biting mosquitoes. The overall test cage dimensions are those described in ASTM standard E951-83. The cage is rectangular in shape; with length, width and height approximately 19.8 × 5.3 × 3 cm (Fig. 2). The cost of the cage is about $4 (cheaper if made in quantities of 50 or more at one time).

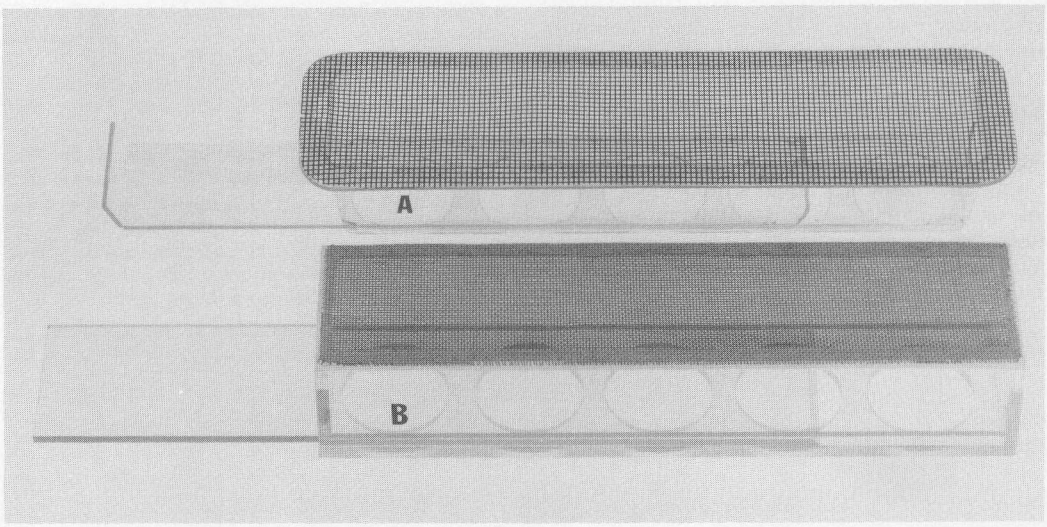

Fig. 1. Plastic test cage used for repellent testing (A) new test cage; (B) old test cage.

We conducted a series of experiments using *Aedes aegypti* (Linn.) to determine if there was a significant difference in efficacy data comparing the old and new test cages. Six volunteers participated in this study. The test procedure was similar to that described by Buescher et al. (1982). We outlined five circular, 29-mm diam

[1] Opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Department of the Army. Use of trade names does not constitute an official approval of the products mentioned.

[2] Human subjects participating in this study gave free and informed voluntary consent.

[3] Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800.

[4] U.S. Army Natick Research, Development and Engineering Center, Natick, MA 01760-5019.

test areas on the flexor regions of the test subjects' forearms using a plastic template and a felt-tipped pen. The marked areas were randomly treated with 0.025 ml of the diluent and four serial dilutions (0.002–0.016 mg/cm^2) of diethyl methylbenzamide (deet) in absolute alcohol. The plastic cages (old and new) were then applied to different arms of each participant and secured with Velcro® strips. The numbers of bites received over 90 sec on each arm were recorded. The test was replicated at the same range of doses until a statistically reliable estimate of the median effective dosage (ED_{50}) was obtained.

Table 1. Mean number of bites received per test using old and new cages against *Aedes aegypti* (n = 20).

		Concentration of deet (mg/cm^2)			
Test cage	Control	0.02	0.04	0.08	0.16
Old cage	3.8	1.7	1.0	0.2	0.1
New cage	3.8	2.3	1.2	0.5	0.2

Test results indicated no significant difference in the median effective doses of deet obtained with new and old test cages (Tables 1 and 2). The test results were also analyzed sequentially; 20 replications were sufficient to obtain reliable estimates with both cages. The evidence indicates that the new cage provides results comparable to those provided by the old cage; thus, future evaluations can be compared with older data. Furthermore, the new cage has all the advantages described in the introductory paragraphs.

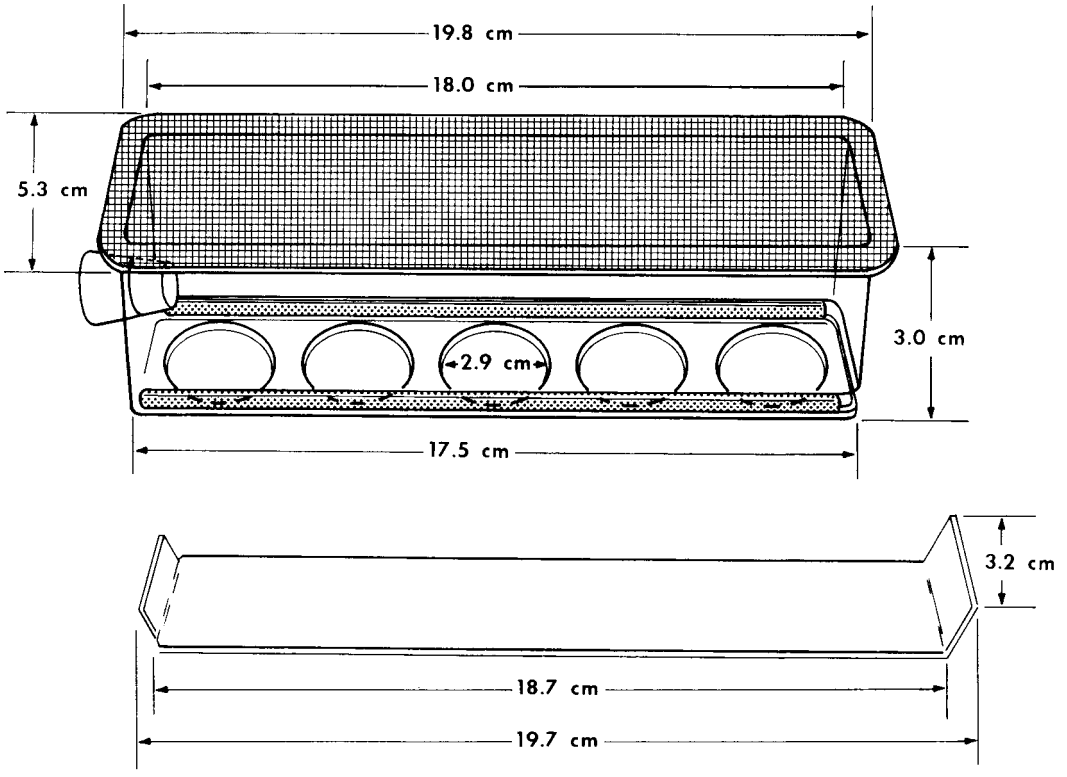

Fig. 2. Repellent test cage.

Table 2. Median effective dosage of deet against *Aedes aegypti* (20 replications).

Test cage	ED_{50} (mg/cm^2)	95% confidence limits	ED_{95} (mg/cm^2)	95% confidence limits
Old cage	0.0018	0.0006–0.0030	0.0086	0.0059–0.0163
New cage	0.0026	0.0020–0.0031	0.0137	0.0113–0.0176

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