ARTICLES

SOME PROCEDURES AND RELATED EQUIPMENT FOR DISEASE-TRANSMISSION RESEARCH WITH *CULICOIDES*

ROBERT HENRY JONES [1]

Animal Disease Research Laboratory, Agricultural Research Service, U.S.D.A., Denver, Colorado

This paper describes some techniques for disease-transmission research, as modified for use with the biting midge *Culicoides variipennis* (Coquillett) in studies with bluetongue virus (Jones and Foster, 1966; and Jochim and Jones, 1966). The equipment used is covered only briefly, since both the specialized apparatus and the operations chamber containing it are still in the developmental stage.

EQUIPMENT. The equipment is designed so that all operations with live flies are performed within a single isolation chamber in which flies can be maintained from the time they come into contact with the virus until they die or are used in research. Because bluetongue disease is apparently noncontagious, and also noninfectious to human beings, full provision for virus security has not been necessary.

Construction of Chamber. The chamber, made of plexiglass, measures 5 by 3 feet by 2½ feet tall, and is supported on a table. The shell of the chamber is bolted together so that any side can be removed with relative ease. Eight small changeable panels (see photographs and parts list, nos. 5–12) slide into grooved openings in the chamber walls. These panels are fitted for different uses, and each one can be replaced by several others so that the same area can be used for different purposes.

To avoid giving a detailed description of the chamber and the apparatus within it, four photographs are presented, along with a listing of the important parts. Figure A is an oblique view of the front and left end of the chamber, and Figure B is a direct view of the left end. Figure C is an interior view of the left end of the chamber (front wall of the chamber removed), showing the area for microscope operations. Figure D is a view of the incubator (dismounted from the chamber) and the attached portion of the fly-release enclosure. The number assigned each part in the accompanying list is used throughout all the photographs. See p. 183.

The chamber is located in an insect-proofed room maintained at constant conditions of light, temperature, and relative humidity; air from the room is filtered and circulated through the chamber.

So that flies will not be lost when materials are introduced or removed, the chamber is fitted with a transfer box. At the front and left end of the chamber two pairs of openings for sleeves (or rubber gloves if desired) provide access to the inside for manual operations; when not in use these openings are closed by interior sliding doors. A shelf in the front portion of the chamber provides an area for the daily handling of flies that are being incubated. The left end of the chamber is modified for the stereoscopic microscope; this area also contains the equipment for artificially infecting the flies by inoculation and for conducting transmission experiments with embryonating chicken eggs.

PROCEDURES. *Inoculation of Flies.* Flies are inoculated, while under microscopic observation, with control or virus-containing fluid by means of a lateral, intrathoracic puncture into the hemocoel. For

[1] The author is a member of the Entomology Research Division, and is associated with the Denver Laboratory of the Animal Disease and Parasite Research Division.

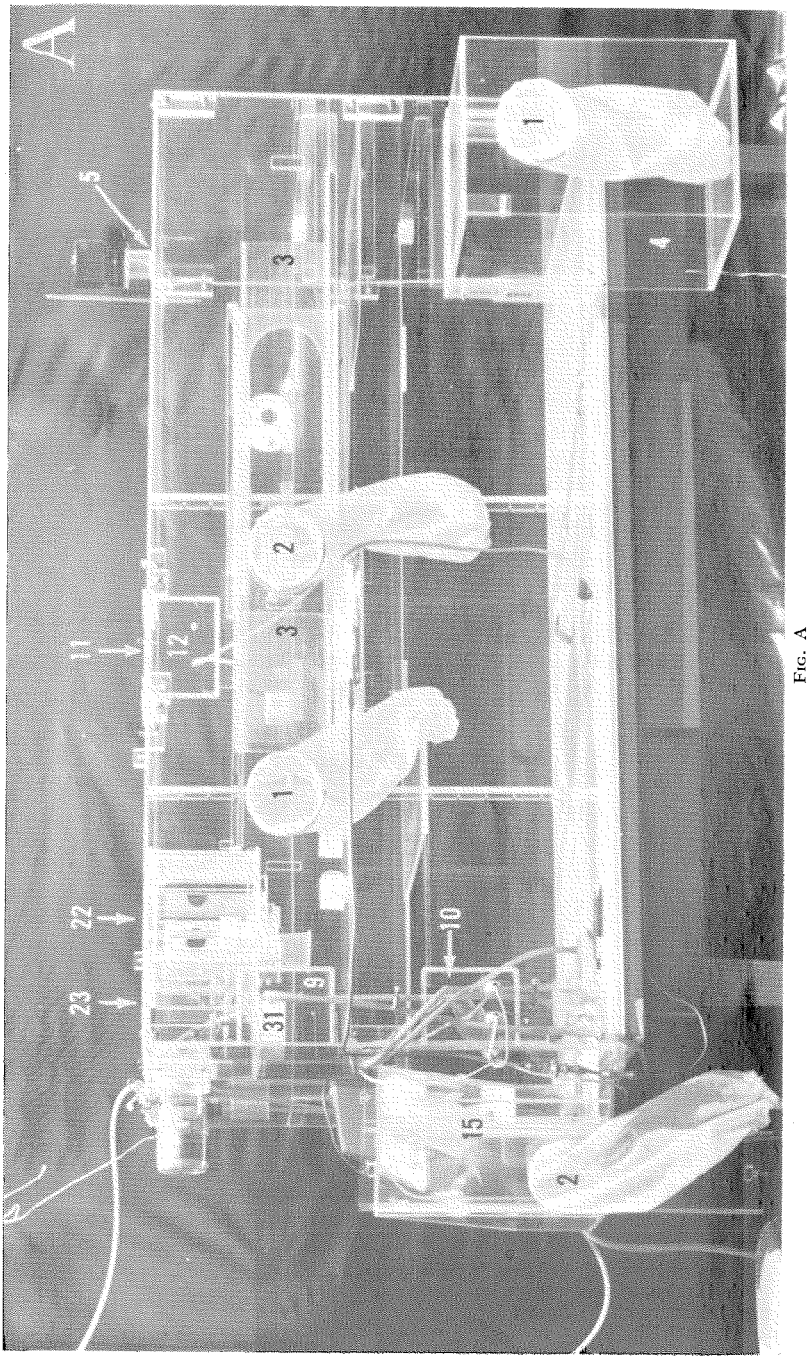

FIG. A

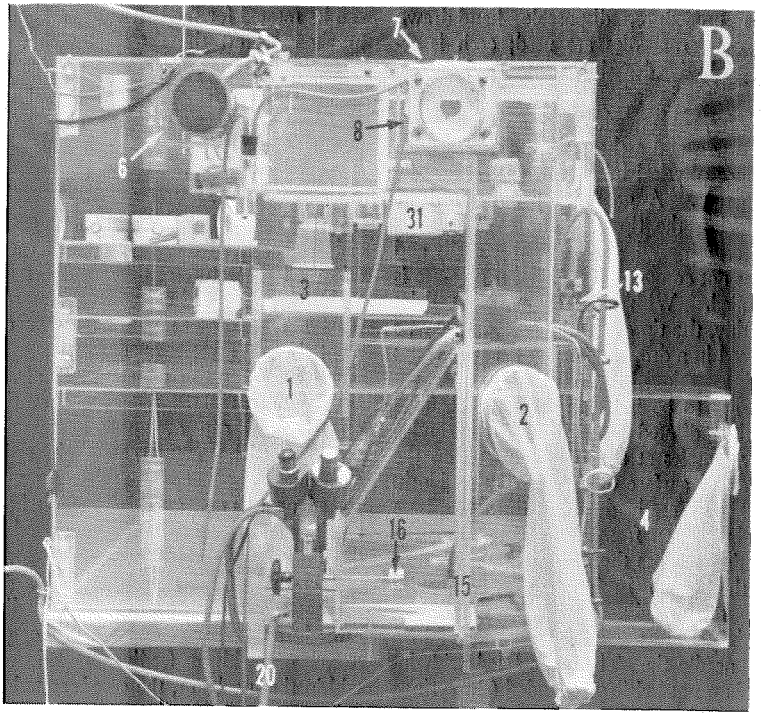

FIG. B

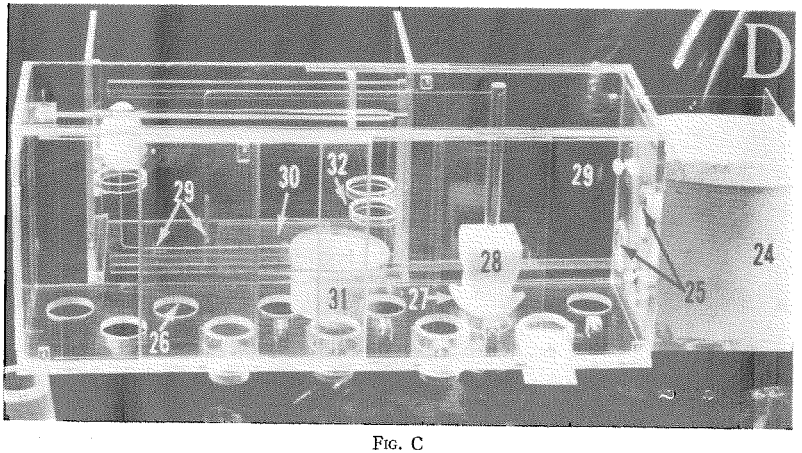

FIG. C

this procedure, disposable hand-drawn glass microneedles are used; each is cemented with dental wax into a 27½-gauge disposable hypodermic needle. With *Culicoides,* microneedles with a diameter less than 55μ and a sharp tip are necessary to reduce physical injury to the fly from the puncture. A good needle, obtained by breaking the tip until the edge is satisfactorily jagged, penetrates very easily and does not compress the fly when pressure is applied. With an inoculum that dries readily, the tip of the microneedle may be placed in a droplet of water between series of inoculations.

Flies to be inoculated are anesthetized with undiluted carbon dioxide gas, and a few females at a time are placed on the screened surface of the inoculation block. (The flies are held against the screen by a slight suction from a vacuum pump; the suction reaches the screen through a hole in the block.) Each fly is punctured with the microneedle and then withdrawn slightly from the stage while impaled on the needle tip. The fly is filled with inoculum until visibly distended (the syringe can be rotated for the best view) and then brushed against the thread stretched in front of the screen to remove it from the needle tip. An experienced operator can handle 5 anesthetized flies at a time, and takes less than 8 minutes to inoculate 20 flies.

Feeding of Flies on Embryonating Chicken Eggs. For this procedure, a special incubator (see figures) is used to maintain the temperature of the eggs. A small enclosure at one end of the incubator contains a transformer-regulated heater element in an asbestos-lined compartment and a 6-volt fan to circulate warm air.

Eggs are prepared for use by cutting off the shell cap over the air-cell and dampening the membrane with saline solution. These eggs, which may be placed up to 11 at a time in 1-inch holes in the incubator floor, are positioned so that the opened end is exposed to the lower temperature of the chamber proper. To prevent the escape of flies during feeding procedures, the open end of each egg is closed off with a feeding cage below; within the incubator, the egg is seated on

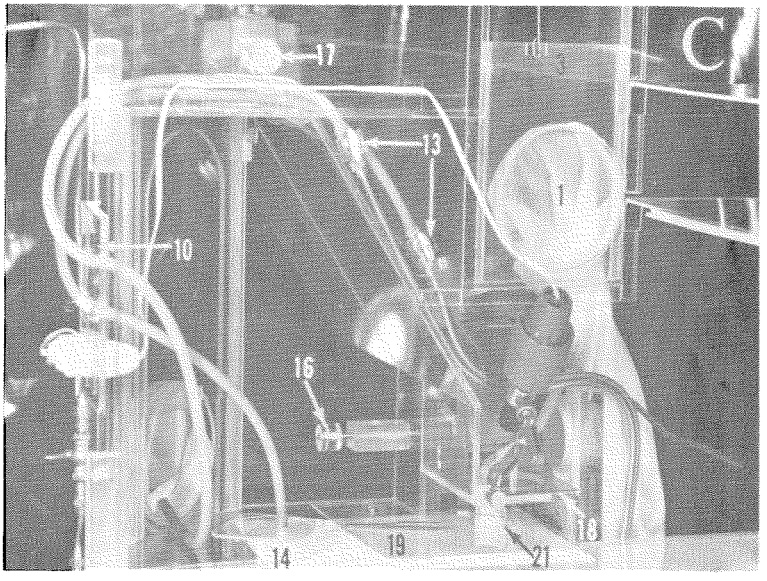

FIG. D

Part number	Best shown in figure:	Description
1	A, B	Sleeve, or the opening for it, into chamber—stationary.
2	A	Sleeve, or the opening for it, into chamber—movable.
3	A, C	Interior sliding door to close chamber opening at sleeve.
4	A	Transfer box with sliding door opening into chamber.
(5–12)		Changeable panels, in chamber wall or ceiling:
5	A	Ceiling panel with blower fan (at screened exit) for air circulation.
6	B	Side panel with filter-humidifier at air intake, and with electrical outlets of incubator to 6-volt battery and to heater transformer.
7	B	Ceiling panel (edge)—paired with side panel (8).
8	B	Side panel with external opening of fly-release enclosure (23) (opening shown here without sleeve, closed on inside by sliding door), and with aspirator tubing and two tubes for air circulation.
9	A	Side panel with tubing for distilled-water inlet.
10	A, C	Side panel with two electrical and two CO_2 tubing inlets.
11	A	Ceiling panel (edge)—paired with side panel (12).
12	A	Side panel with aspirator tubing.
13	B, C	Brass pin valve (handle on interior of chamber) for delivery of CO_2.
14	C	Anesthetizing cover.
15	A, B	Appendage to chamber—area for microscope operations.
16	B, C	Internal knob for microscope focus.
17	C	Internal knob for microscope illuminator.
18	C	Plate-glass cover over microscope observation field.
19	C	Removable plate to which inoculation block is attached.
20	B	Tubing to vacuum pump (provides suction at screen of inoculation block).
21	C	Inoculation block with screen, showing thread stretched above screen surface.
22	A, D	Incubator for embryonating chicken eggs.
23	A, D	Fly-release enclosure.
24	D	Incubator heater compartment.
25	D	Hole in incubator for air circulation to heater.
26	D	One-inch holes in incubator floor.
27	D	Polyfoam cushion for egg.
28	D	Egg anchoring device.
29	D	Sliding doors in incubator.
30	D	Sliding door in floor of fly-release enclosure.
31	A, D	Cage supported under fly-release enclosure.
32	D	Four rings in fly-release enclosure to support eggs.

a cushion of polyfoam, and may also be held in place by an anchoring device. Holes in the incubator floor that are not in use are closed off from above by pieces of dental dam, or from below by any of the several types of small removable cages.

In transmission experiments, it is necessary that only one fly bite each egg. With *Culicoides,* a relatively fast and easy method for achieving this is made possible by the fly-release enclosure, an antechamber between the chamber wall and the incubator. Since these insects walk as well as fly (as compared to mosquitoes, which generally fly), it is possible to release them into this enclosure and entice them onto the membrane of the egg. The enclosure is provided with four rings to support eggs, a sliding door in the bottom to let flies in from a cage underneath, a sliding door into the incubator, and an opening for the operator's hand in one of the changeable panels (parts list, no. 8). An aspirator is provided for discarding flies that do not feed readily. In addition, two tubes are provided for forced air circulation to remove condensation caused by the operator's hand.

For transmission experiments, one or two prepared eggs are placed on the floor of the fly-release enclosure. The operator releases a single fly into the enclosure and maneuvers an egg so that the fly climbs up inside the edge of the shell. The egg is rested on one of the rings until the fly begins feeding, and then the egg with the attached fly is transferred to the incubator and rested in a hole with a cage underneath. After completion of feeding, flies are anesthetized and removed for microscopic examination to determine the extent and type of meal (blood or allantoic fluid) that each fly has taken.

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