Biological Containment Facility for Studying Infectious Disease

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To effectively characterize newly recognized viruses (Marburg, Lassa, etc.) and to study other highly virulent infections for which no effective prophylaxis or therapy exists, special containment facilities must be utilized and conventional techniques modified to minimize risk to laboratory personnel. This paper describes a laboratory facility for such studies, contained within a larger research facility; two separate biological safety cabinet systems, animal rooms support laboratories, change room facilities, shower, air lock, and other safety features are contained in the area. Details of design, construction, airflow, and equipment are described in addition to a discussion of operation, techniques, and modification of laboratory equipment utilized in actual studies.

A number of severe infectious diseases of proven or presumed viral etiology have been identified during the past decade. Examples are Marburg fever, Lassa fevers, Argentine and Bolivian hemorrhagic (BHF), fevers, Kyasanur Forest disease, alastrim, and Korean hemorrhagic fever. To characterize these infections, as well as to study other highly infectious pathogens such as Herpesvirus simiae, Coccidioides immitis, Brucella, Bartonella, and Actinobacillus mallei and the diseases they produce, it is imperative that facilities be available which provide maximal safety to laboratory personnel. During the planning stages that preceded construction of the research facility of the U.S. Army Medical Research Institute of Infectious Diseases, it was anticipated that additional research might be required for diseases for which effective prophylaxis or therapy did not exist.

DESCRIPTION OF FACILITY

A special laboratory suite was included within the larger facility for the study of these diseases; a 36 \( \times \) 36-foot space was designated for this purpose (Fig. 1). Contained within the area are two separate, gas-tight, biological safety cabinet systems, animal housing rooms, support laboratories, change room facilities, water shower, disinfectant shower, autoclaves, and an air lock for passage of materials.

Concrete block construction was used for all walls. Floors and ceiling are of poured concrete. All wall and ceiling joints were sealed with a polysulfide caulking compound before being finished with epoxy paint. The floors were treated with a monolithic epoxy finish. Lighting fixtures, pipes, conduit, and other services were installed through the secondary barrier wall to maintain absolute physical separation between the research area and surrounding corridors. Electrical conduits were internally sealed. Fluorescent lighting fixtures were installed flush against the ceiling to prevent dust accumulation. The liberal use of viewing windows and speaking diaphragms in walls of laboratories, animal rooms, and outside corridors permits photographs to be taken without the necessity for camera decontamination. Air balance within the area maintains the animal rooms at a pressure negative to other portions of the suite; in turn, the suite itself is maintained at a lower pressure than that of the surrounding corridors. A pressure-sensing device triggers a visible and audible alarm if the suite pressure is not at least 0.5-cm \( H_2O \) below that of the rest of the building. Exhaust air (15 air changes per hour) from each room of the suite passes through a dust filter and two fiber glass, Hi-Flo Aerosolve filters (total retention efficiency of 95% of 0.3-\( \mu \)m particles; Cambridge Filter Corp., Syracuse, N.Y.) before being exhausted into the atmosphere. Ultraviolet lights are located in the air lock, animal room anteroom, passbox, laundry bag, support rack, and around the shower exit doorway; these are periodically checked for radiation levels. A standby generator is automatically
switched on in case of power outage within the building. Interlocking air lock doors preserve the integrity of the pressure differential and prevent direct contact of personnel entering the air lock with those within the suite. An intercom system permits audible contact across the air lock itself, and an ultraviolet light-sterilized passbox permits removal of small items, single sheets of paper, etc., from the suite after 10 to 15 min of irradiation.

The main equipment feature of the suite is the existence of two independent systems of abso-
lute-barrier biological cabinets (S. Blickman Corp., Weehawken, N.J.), which are shown as a shaded area in Fig. 1. Each gas-tight cabinet system provides working space, animal housing, an incubator, and a refrigerator and is furnished with compressed air, vacuum, 110-V electrical outlets, water, and drains. (Gas is not provided because of explosive hazard.) One system contains an inverted microscope with eyepieces sealed by airtight gaskets (rubber O-rings mounted in flexible rubber sheeting, which permit adjustment of the interpupillary distance of the microscope eyepieces). The other system contains a modified Henderson apparatus for aerosol studies (1). Entry and egress to the cabinets are provided by an autoclave, an ultraviolet light- or disinfectant-sterilized transfer box, or an immersion tank filled with a suitable disinfectant with an internal baffle to provide separation of room and cabinet air.

Manipulations within the cabinet are performed through ports fitted with arm-length, 15-mil-thick, neoprene gloves in the working areas and with 30-mil-thick gloves covered with a leather glove in the animal-holding section of the cabinet. The cabinet is maintained at 1.9-cm H₂O negative air pressure to that of the suite. Cabinet transfer boxes are kept at 1.1-cm H₂O, preventing excessive aerosol contamination from the test of the system. Valved inlet air to the cabinets is filtered through a size B Airpure HEPA filter (99.99% bacterial filtering efficiency, 99.9% retention of 0.3-μm particles; Flanders Filters, Inc., Riverhead, N.Y.). Cabinet exhaust air passes first through a HEPA filter, then through a laboratory-developed electric incinerator (500 to 600°F), and then through two more deep-bed filters (described above) before entering the atmosphere. The refrigerators and incubators are bottom-mounted, raised hydraulically, and contain rotating shelves. There are no freezers within the cabinets, but materials sealed in appropriate leakproof containers can be removed after a 5-min immersion in the disinfectant dunk tank, or they can be washed with disinfectant, allowed to remain under ultraviolet light for 10 min in the transfer box, and stored in mechanical freezers located elsewhere within the suite. In-line autoclaves (American Sterilizer Co., Erie, Pa.) are used for removal of all waste materials. These autoclaves have interlocking doors that prevent both from being unlocked at the same time; once the inner door is opened, a cycle must be completed before the outer door can be unlocked. Condensate return from the autoclaves drains into the liquid waste system, and vented air is filtered and incinerated. Each cabinet section is equipped with overhead fluorescent and ultraviolet lights. A transfer box with ultraviolet lights also is provided to permit direct passage of animals into holding rooms. Liquid drain waste from the system is sterilized with live steam and held for 25 min at 280°F before leaving the vicinity of the Research Institute. One cabinet system is depicted in Fig. 2, which shows glove ports, autoclave, and transfer box.

The safety cabinet suite became operational in September 1970. In June 1971, work was begun with certain members of the Tacaribe group of arenaviruses: Machupo virus, the causative agent of BHF, and an isolate from Cochabamba, Bolivia, the causative agent of a recent BHF-like outbreak in an area of Bolivia outside the normal endemic area of BHF (C. J. Peters et al., manuscript in preparation).

Admission to the area has been strictly limited. Individuals who work in the area are required to wear special clothing and shower with germicidal soap upon completion of work. Lysol (5%) is used as the disinfectant of choice for material removal, dunk tank, etc. Initial studies were conducted with a nonpathogenic arenavirus to simulate procedures which were to be employed subsequently with material from the Cochabamba outbreak. It soon became apparent that when standard techniques and equipment were utilized inside the cabinet system, personnel still faced a major hazard from penetrating wounds by sharp objects. All procedures were revised to substitute plastic for glass materials and to eliminate use of pointed instruments within the cabinets. For example, animal necropsies are now performed with blunted scissors and toothless forceps using a plastic necropsy board with clamps, rather than the conventional wooden board with pins. Organs are transferred to leakproof plastic containers.

![Fig. 2. Biological cabinet system within the research area.](image-url)
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nectors from Philadelphia, Larger animals, such as rhesus monkeys, are housed in metal cages or plastic chairs in the adjacent animals housing rooms. Recent studies with chaired monkeys were conducted in which wires from rectal thermometers were attached to connectors mounted on the outer-corridor windowpane, permitting the recording of individual animal temperatures on a constant recorder located outside the area. All bedding, dead animals, and refuse from the contaminated animal rooms are autoclaved (121°C for 90 min) in the immediate area before they are brought into the central large room.

When infected animals are housed in either animal room, personnel protection is afforded by means of ventilated suits (Snyder Mfg. Co., New Philadelphia, Ohio). These suits are made of flexible, nylon-reinforced vinyl and are connected to compressed air lines (15 to 18 cubic feet at standard temperature and pressure) located at various sites throughout the suite. When leaving the animal area, suited personnel enter the disinfectant shower (Fig. 3). Although this shower was designed for use with more rigorous disinfectants, such as peracetic acid, 5% Lysol has been used during the Machupo studies. The shower is constructed of stainless steel, has interlocking doors, and gives a timed sequence of water, disinfectant, and water rinse.

Because all refuse is autoclaved for 90 min at 121°C before leaving the suite, material from the contaminated animal area or the cabinet systems receives a double autoclaving. Temperature recorders and sterilization indicators are used with all autoclaves, and STERILE-CONTAMINATED signs are attached to all autoclaves and transfer boxes. Nonautoclavable materials leaving the suite are decontaminated with formaldehyde vapor or ethylene oxide.

Decontamination of animal rooms, safety cabinets, and the entire suite can be accomplished with heat-depolymerized paraformaldehyde powder at greater than 60% relative humidity (2). Efficacy of decontamination is determined by seeding patches with Bacillus subtilis var. niger and by placing them in random locations. After a 1-h contact time with the formaldehyde vapor, the patches are placed in thioglycolate broth and incubated for 48 h at 37°C to ascertain effectiveness of decontamination.

RESULTS AND DISCUSSION

During the 18-month period this facility has been in operation, a wide variety of techniques and manipulations (animal inoculations, serum processing, leukocyte and platelet counts, tissue culture plaque neutralization tests, necropsies, platings, tissue harvests, antigen production, complement-fixation tests, and preparation of fluorescent antibody-stained cover slips, etc.) have been performed, and no bacterial contamination has occurred. In addition, there has been no evidence of cross-contamination between the two systems.

Although only one accident occurred within the area during this period, it was a potentially disastrous one. During the course of performing hematological studies on Machupo virus-
infected rhesus monkeys, an investigator broke a glass microhematocrit tube when pushing the end into sealing wax. After puncturing both the neoprene and rubber gloves, the broken tube entered his finger. Prompt action was taken to encourage bleeding, and the investigator was placed in isolation in the Institute hospital facility, where the finger was infused with immune plasma, and four units of immune plasma were administered intravenously. Fortunately, no illness resulted from this accident, and the use of glass microhematocrit tubes has since been eliminated.

It is recommended that the use of facilities, techniques, and equipment similar to those described be considered minimal for the safety of personnel engaged in the study of highly infectious disease agents.

LITERATURE CITED

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