

(iii) The publication by the American Committee on Arthropod-Borne Viruses Subcommittee on Arbovirus Laboratory Safety (SALS) entitled Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates in the American Journal of Tropical Medicine and Hygiene, 29(6), 1980, pp. 1359-1381.

(iv) The Department of Health and Human Services Publication No. (NIH) 76-1165 by the National Cancer Institute (NCI) entitled Biological Safety Manual for Research Involving Oncogenic Viruses.

(2) When samples with unidentified viable agents are obtained, a knowledgeable and qualified scientist will evaluate the risks and make recommendations to the safety officer, who will add recommendations for review and approval by the commander or institute director. When guidelines for a specific organism are not established, in addition to these steps, the CDC or SALS or both will be consulted. Their recommendations will be documented and provided to the commander or institute director before approval.

(c) *Selection of facilities.* The facility requirements identified by the risk assessment will be adhered to. Any variations and compensatory measures will be approved by the IBC (when recombinant DNA molecules are involved), the safety officer, and the commander or institute director before a request for an exception or waiver is submitted as stated in AR 385-69.

(d) *Policies and procedures.* Policies in the form of a laboratory safety manual, regulations, memorandums, or SOPs are required for work with etiologic agents in the BDP. Before beginning a new procedure, the policies and procedures will be reviewed to ascertain that the intended operations are described and to determine the requirements that apply to the operation. If procedures exist for the intended operation, personnel will be trained to follow them; if procedures do not exist, then a detailed SOP will be written, reviewed, and approved before beginning the operation. SOPs will conform to the requirements stated in § 627.7(d), and be signed by all personnel who are required to follow the procedures, thus acknowledging that they have read and

understood the contents. All SOPs that pertain to a specific area (room, laboratory, or suite) will be available at the worksite.

§ 627.12 General laboratory techniques.

The general requirements for use of etiologic agents are composed of two sets of requirements, with the requirements for toxins being a subset of the requirements for handling viable etiologic agents. These requirements are as follows—

(a) *General techniques applicable to etiologic agents.*

(1) A fully fastened long-sleeved laboratory coat, gown, uniform, or coveralls will be worn in laboratories or animal rooms.

(2) Eating, drinking, smoking, and applying cosmetics are not permitted in the work areas.

(3) Personnel must wash their hands after they handle etiologic agents or animals, and before leaving the laboratory area.

(4) Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used.

(5) Gloves—(i) Will be worn when manipulating etiologic agents and handling containers of etiologic agents. Gloves are not required when materials are packaged appropriately for shipment.

(ii) Will be selected based on the hazards.

(iii) Will be changed frequently (or decontaminated frequently), and will be decontaminated or discarded into a labeled biohazard container after each use and immediately upon observable direct contact with an etiologic agent.

(iv) Will be removed at the workspace (workbench or hood) after handling etiologic agents to ensure that doorknobs and other surfaces are not contaminated.

(6) Good housekeeping will be maintained. This includes—

(i) Work areas free of clutter.

(ii) Work environment free of tripping hazards, with adequate access to exits, emergency equipment, controls, and such.

(iii) Benches and general work areas will be cleaned regularly using a wet

sponge or similar method with disinfectant as appropriate. Methods that stir up dust such as sweeping or using vacuum cleaners, (except for HEPA-filtered vacuum cleaners) are unacceptable.

(iv) Specific work areas will be cleaned and decontaminated immediately following each use of an etiologic agent (at least once a day) and after any spill of viable material.

(v) Hallways and stairways will not be used for storage.

(7) All solutions, reagents, and chemicals will be labeled.

(8) All contaminated liquid or solid wastes will be inactivated before disposal.

(9) Work will be conducted over spill trays or plastic-backed absorbent paper. The paper will be removed, decontaminated, or disinfected, and the general area wiped with decontaminant at the end of each day or at the end of the experiment, whichever occurs first.

(10) Etiologic agents will be kept in closed containers when not in use. Cultures, solutions, or dried etiologic agents in glass vessels transported or incubated within a room or suite will be handled in nonbreakable, leak-proof pans, trays, pails, carboys, or other secondary containers large enough to contain all the material, if the glass vessel leaks or breaks. Etiologic agents removed from a room or suite for transport to another approved area within the same building will be placed in a closed unbreakable secondary container before removal from the laboratory. The secondary container will be labeled on the exterior with a biohazard symbol and identification of the contents, including the required biosafety level, the scientific name, the concentration (if applicable), and the responsible individual. The secondary containers will be wiped with suitable disinfectant before removal from the laboratory or area.

(11) Working stocks of etiologic agents will be stored in double containers. The primary and secondary containers will provide a positive seal and the secondary container will be unbreakable. The secondary container will be labeled as stated in § 627.12 (a)(10) and with the date stored.

(12) Storage units (for example, freezers, refrigerators, cabinets, and hoods) will be labeled with the universal biohazard sign and indicate the classes of etiologic agents contained in them. Storage units will be secured when not in use.

(13) All contaminated materials, containers, spills, and solutions will be decontaminated or disinfected by approved methods before disposal.

(14) After injection of an etiologic agent into animals, the site of injection will be swabbed with a decontaminant.

(15) Syringes. (i) Reusable or disposable syringes will be of the fixed needle or LUER-LOK type (or equivalent) to assure that the needle cannot separate during use.

(ii) After use, nondisposable glass syringes with attached needles contaminated with etiologic agents will be submerged in a container of decontaminant. Disposable syringes will be discarded with needles attached in puncture-proof rigid containers. Needles will not be recapped after use.

(iii) Sterilized or decontaminated containers marked "Syringes and/or Needles" may be deposited in appropriate refuse containers after proper packaging and destruction of the contents.

[NOTE: Many States, especially those on the Eastern seaboard, have implemented strict requirements for the disposal of medical wastes. For example, Maryland has designated all waste from a microbiological laboratory as hazardous waste with licensing requirements for generators of 50 kilograms per month or more of waste, while all medical waste released for transport off-site must be manifested to a State licensed medical waste hauler with the destination specified. Additionally, in some cases, the local government (for example, a city) regulates the disposal of these wastes. These requirements will be identified and followed.]

Needles or syringes may not be destroyed by clipping. A mechanical shear may be used to smash or sheer needles after or concurrently with sterilization or decontamination.

(16) Refrigerators, deep freezers, and dry ice chests should be checked, cleaned out, and defrosted periodically to remove any ampules, tubes, and so forth, containing etiologic agents that may have broken during storage. Rubber gloves and respiratory protection

appropriate to the materials in storage should be worn during cleaning. Do not store flammable solutions in nonexplosion proof refrigerators.

(b) *Additional techniques applicable to work with viable etiologic agents.* The major objective of these techniques is to assist in protection against laboratory acquired infections. Air sampling studies have shown that aerosols are generated from most of the manipulations of bacterial and viral cultures common to research laboratories. The generation of aerosols during routine laboratory manipulations must be considered when evaluating the individual degree of risk, keeping in mind the four main factors governing infection: dosage, virulence of the organism, route of infection (for example, skin, eyes, mouth, lungs), and host susceptibility (for example, state of health, natural resistance, previous infection, response to vaccines and toxoids). The requirements stated below are minimum handling requirements to prevent accidental infection created by incidental aerosols.

(1) All procedures are performed carefully to minimize the creation of aerosols.

(2) No infectious mixtures will be prepared by bubbling air through a liquid.

(3) Pipettes.

(i) No infectious material will be forcibly ejected from pipettes. Only to deliver (TD) pipettes will be used.

(ii) Pipettes used with infectious or toxic materials will be plugged with cotton unless they are used exclusively in a gas-tight cabinet system.

(iii) Contaminated pipettes will be placed horizontally in a rigid container containing enough disinfectant for complete immersion. Cylinders used for vertical discard are not recommended. The container and pipettes must be autoclaved as a unit and replaced by a clean container containing fresh disinfectant.

(iv) Pipetting devices must be used. Under no circumstances is mouth pipetting permitted.

(4) Syringes. (i) Using syringes and needles for making dilutions of etiologic agents is not recommended.

(ii) When removing a syringe and needle from a rubber stopper bottle containing viable etiologic agents, an

alcohol soaked pledget around the stopper and needle will be used.

(iii) Excess fluid and bubbles should be expelled from syringes vertically into a cotton pledget soaked with disinfectant or into a small bottle containing disinfectant-soaked cotton.

(iv) The site of injection of an animal will be swabbed with a disinfectant before and after injection.

(v) After use, syringes contaminated with residual infectious fluid will be submerged in a container of disinfectant in a safety cabinet prior to removal for autoclaving. To minimize accidental injection of infectious material, the removable needles should remain on such syringes until after autoclaving. When possible, syringes with attached needles should be placed in a pan separate from that holding other discarded materials.

(vi) Caps will not be placed over needles until after disinfection. During recapping, procedures to prevent personal injuries will be used.

(5) Centrifuges and shakers. (i) Before centrifuging, tubes, rotors, seals, and gaskets will be checked for cleanliness and integrity. In low speed clinical-type centrifuges, a germicidal solution may be added between the tube and trunnion cup to disinfect the outer surfaces of both and to cushion against shocks that might break the tube. Metal or plastic tubes (other than nitro-cellulose) will be used.

(ii) Decanting from centrifuge tubes will be avoided. If decanting is necessary, the outer rim will be wiped with a disinfectant after decanting so that material on the lip cannot spin off as an aerosol. Centrifuge tubes will not be filled beyond the level the manufacturer recommends.

(iii) Broth cultures will be shaken in a manner that avoids wetting the plug or cap.

(6) Water baths in which viable etiologic agents are incubated must contain a disinfectant. For cold water baths, 70 percent propylene glycol is recommended. The disinfectant should be changed frequently.

(7) When a laboratory vacuum is used to manipulate viable etiologic agents, a secondary reservoir containing disinfectant and a HEPA filter must be employed to ensure that the laboratory

vacuum lines do not become contaminated.

(8) Test tubes. (i) Tubes containing viable etiologic agents should be manipulated with extreme care. Studies have shown that simple procedures, such as removing a tube cap or transferring an inoculum, can create a potentially hazardous aerosol.

(ii) Manipulation of biohazardous test tubes will be conducted in biological safety cabinets. Tubes and racks of tubes containing biohazardous material should be clearly marked. The individual employee must ensure that tubes containing biohazardous material are properly sterilized prior to disposal or glassware washing. Safety test tube trays should be used in place of conventional test tube racks to minimize spillage from broken tubes. When safety test tube trays are not used, the conventional test tube racks will be placed in a tray large enough to contain any potential spill. A safety test tube tray is one having a solid bottom and sides deep enough to hold all liquids, should a test tube break.

(9) Care should be exercised when using membrane filters to obtain sterile filtrates of viable etiologic agents. Due to the fragility of the membranes and other factors, such filtrates cannot be considered noninfectious until laboratory culture or other tests have proven their sterility.

(10) The preparation, handling, and use of dry powders of viable etiologic agents in open containers presents unusual hazards. The slightest manipulation of such powders can cause the generation of aerosols containing a high concentration of etiologic agents. Therefore, work with dry powders of etiologic agents in open containers should be carried out in gas-tight biological safety cabinets.

§ 627.13 Biosafety level 1.

(a) *Requirements beyond those for all etiologic agents.* BL-1 operations follow the general techniques described in §§ 627.12(a) and 617.12(b).

(b) *Additional laboratory requirement.* Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the labora-

tory. Examples of suitable containers are metal tubs with lids or plastic bags that are sealed and then placed inside a rigid container for transport.

(c) *Additional animal requirements.* (1) Bedding materials from animal cages will be removed in such a manner as to minimize the creation of aerosols and disposed of in compliance with applicable institutional or local requirements.

(2) Cages are washed manually or in a cagewasher. Temperature of final rinse water will be a minimum of 180 ° F.

(3) Laboratory coats, gowns, or uniforms worn in animal rooms shall not be worn in other areas.

§ 627.14 Biosafety level 2.

(a) Additional requirements. In addition to the general microbiological techniques stated in § 627.13, BL-2 operations include the following requirements:

(1) When etiologic agents are in use, a hazard warning sign incorporating the universal biohazard symbol is posted on the access door of the work area. The hazard warning sign identifies the etiologic agent, lists the name and telephone number of the institute director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

(2) Animals not involved in the work being performed are not permitted in the laboratory.

(3) Special care is taken to avoid skin contamination with the etiologic agents; gloves will be worn when handling etiologic agents or infected animals.

(4) All wastes from laboratories and animal rooms are decontaminated before disposal.

(5) Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles.

(6) Spills and accidents which result in a potential exposure to etiologic agents will be reported immediately to the safety officer, the project leader, and the institute director.

(7) Biological safety cabinets (Class I or II) will be used when:

(i) Procedures with a high potential for creating infectious aerosols are conducted.