Facilities and Equipment for Aseptic and Safe Handling of Blood Products

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This teaching editorial was written by members of the Canadian Advisory Committee on Radiopharmaceutical Quality Assurance in Nuclear Medicine. The committee is an advisor to the Health Protection Branch, Health and Welfare Canada, on radiopharmaceutical quality assurance. At its annual meeting, the Committee recognized the need for a two-part reference article that would provide the nuclear medicine community with a basic discussion on facility and equipment requirements and concepts and techniques of universal precautions, aseptic technique, and radiolabeling for those departments currently performing or planning to start cell radiolabeling procedures.

Many nuclear medicine procedures involve the use of "closed system" sterile products with relatively simple aseptic manipulations of vials, syringes, disinfectants, and injections.

When dealing with the isolation and radiolabeling of blood cell components, the degree of sophistication needed to maintain sterility becomes much greater. Isolation of a particular cell fraction is often necessary, since most of the lipophilic radiolabeling agents are nonspecific and will label all cell types to some extent in a blood sample (*1*). Several papers have been published on methods to isolate and radiolabel various cell fractions such as red blood cells (*2*), white blood cells (*3*), granulocytes (*1*), and platelets (*4*).

In general, while isolating particular cell fractions, access into an open container is often required, and the duration and complexity of the manipulation increases the risk of contamination. It must be ensured that there is no introduction of microorganisms, particulate material, or pyrogens.

The purpose of this two-part teaching editorial is to discuss the theoretical and practical aspects of aseptic manipulation of blood for radiolabeling purposes, with emphasis on procedures requiring access into open sterile tubes. The facility design and equipment will be discussed in this overview paper with special emphasis on biological safety cabinets. The second paper will discuss personnel and product protection, with emphasis on the importance of aseptic technique and universal precautions, as well as procedures for dealing with open sources of radioactivity.

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FACILITY DESIGN AND EQUIPMENT

There are generally opposing requirements for air flow patterns when working with both radioactive and sterile products. In areas for handling radioactivity, a negative air pressure in relation to the environment is normally established, in order to avoid release of radioactivity. Meanwhile, in a sterile working environment, a positive pressure is desirable in order to exclude particles and microorganisms.

A major consideration for cell labeling procedures, which cannot be performed in closed systems, is the environment for performing critical operations. Under normal circumstances, air is in a turbulent state and contains thousands of suspended particles per cubic meter of air (*6*). The level of activity and the number of personnel present in a room may have a marked effect on the number of particles shed into a clean environment. Some of the suspended particles carry microorganisms. It is therefore important to filter the air to remove the particles and to optimize air flow patterns to prevent nonfiltered air from entering the work area.

Unidirectional air movement (parallel to the opening of the cabinet and at a velocity greater than either side) is important in order to provide the air curtain which will prevent transverse movement of airborne particulates through the opening (*7*).

The appropriate biological safety cabinet will provide the necessary air flow patterns for protection of product and personnel, during the critical operations in the cabinet.

Facility Design and Layout

The biological safety cabinet should be located in an area free from drafts and heavy personnel traffic. Simple things like the opening of a door, a person walking, or use of a room air conditioner produce air velocities much higher than that of the biological safety cabinet and may overcome the air

barrier of the unit (*7*). Figure 1 places the air flow velocity in the laminar air biohazard cabinet into perspective. All windows should be permanently sealed shut to prevent ingress of microbial and particulate contamination. Window air conditioners should not be installed in the room. The face of the cabinet should not be obstructed as turbulent air flow will interrupt the air curtain.

Sufficient space is necessary around the unit for access to prefilters and for maintenance and inspection procedures. Fluctuations in power supply will affect the air dynamics of the cabinet so the electrical supply must be adequate for all requirements of the cabinet, including the blower, the lights, and receptacles.

The air supply to the room in question must be considered in relation to the exhaust requirements of the cabinet. Biological safety cabinets with a low percentage recirculation and a high percentage exhaust will eliminate large volumes of room air, which need to be replaced with an adequate air supply.

The unit itself and the room must be designed and constructed to maintain cleanliness and to prevent shedding and accumulation of dust and other particulate matter. Surfaces should be smooth and impervious to repeated application of cleaning and disinfecting agents. An absence of sharp corners, cracks and open joints is desirable.

The work area should be well marked, annotating restricted access and should be physically isolated from the rest of the department. An anteroom to act as a changing room for putting on gowns and washing hands is ideal. Handwashing must be performed before and after the procedures. Ideally, sinks should have foot or elbow operating controls for the taps. The changing/scrubbing room could be shared with an adjacent aseptic room used to prepare sterile radiopharmaceutical products. It will also generally be necessary to provide a centrifuge (with an RPM indicator so that g forces can be determined) and perhaps a waterbath in the room. Access to a dose calibrator is also needed to measure the radioactivity.

The biological safety cabinet should be designated as handling only blood products. Also, only one patient's blood should be handled at one time, to avoid any possibility of mix-up or cross-contamination (*8*).

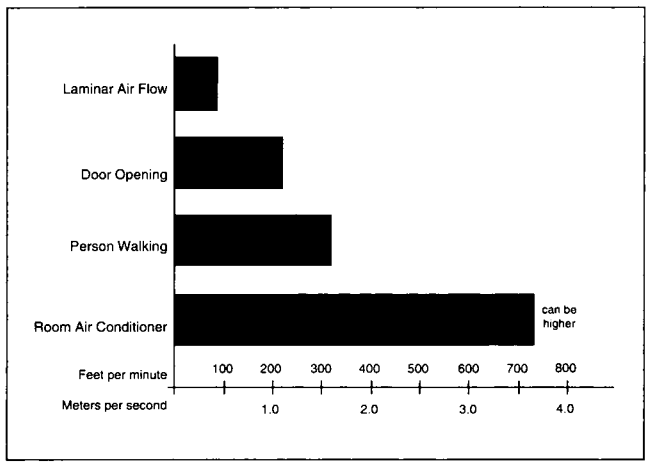

FIG. 1. Comparison of air velocities.

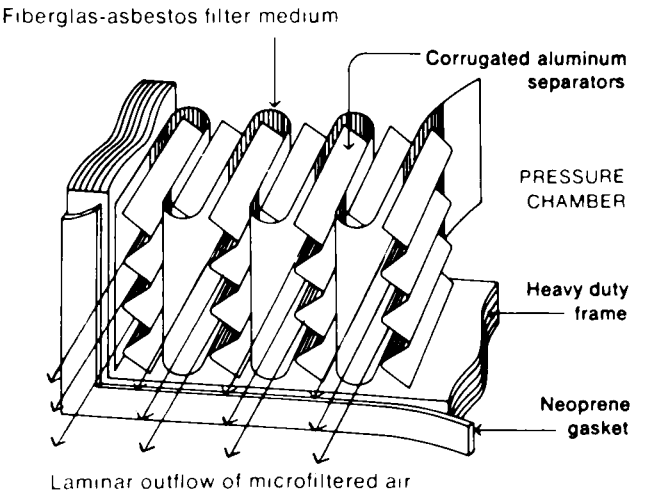

FIG. 2. Construction of high efficiency particulate air (HEPA) filter. (Courtesy of Abbott Laboratories, Abbott Park, IL.)

Adequate space is needed above the exhaust filter for proper functioning (*6*). Other considerations are the relative dimensions of doorways, rooms, and elevators, as well as electrical supply, air, gas, or vacuum service requirements (*8*).

Biological Safety Cabinets

Principles. A biological safety cabinet provides a clean air environment and prevents airborne particulate matter from entering the semi-enclosed work space, i.e., the clean air chamber. The clean air environment concept in a biological safety cabinet consists of two principles: high efficiency particulate air (HEPA) filtration and uniform parallel air flow.

The HEPA filter consists of a boron silicate or fiberglass microfiber membranous filter medium that is pleated back and forth across corrugated aluminum separators (Fig. 2). The separators on both sides of the filter act as baffles to direct the air in a laminar air flow (i.e., a uniform parallel flow without turbulence) (*6*). The filter removes a minimum of 99.97% of all particles that are 0.3 μm or larger (bacteria are typically 0.3 to 30 μm, human hair is 30–200 μm). For most sterile products, the required efficiency is 99.997%. Gases and vapors are not trapped, but particles and liquid droplets are removed by the filter through a combination of forces, such as interception, inertial impactation, diffusion, electrostatic attraction, and sedimentation. The HEPA filter cannot be cleaned and must be replaced once it is loaded to its capacity (*6*).

Initially, the air is drawn in through a prefilter which entraps dust and large particles from the environment; then the prefiltered air is propelled by the motor blower through the air duct and pressure chamber (Fig. 3) (The Baker Company) (*9*). The air is purified as it passes through the HEPA filter and moves with uniform velocity along parallel lines, giving it a laminar flow characteristic. The HEPA-filtered clean air descends over the work area and splits to exit through both the rear and front exhaust grills.

Escape of pathogens into the workers' environment is prevented by an air barrier of inward flowing room air at the front opening and the cleaning action of the exhaust filter

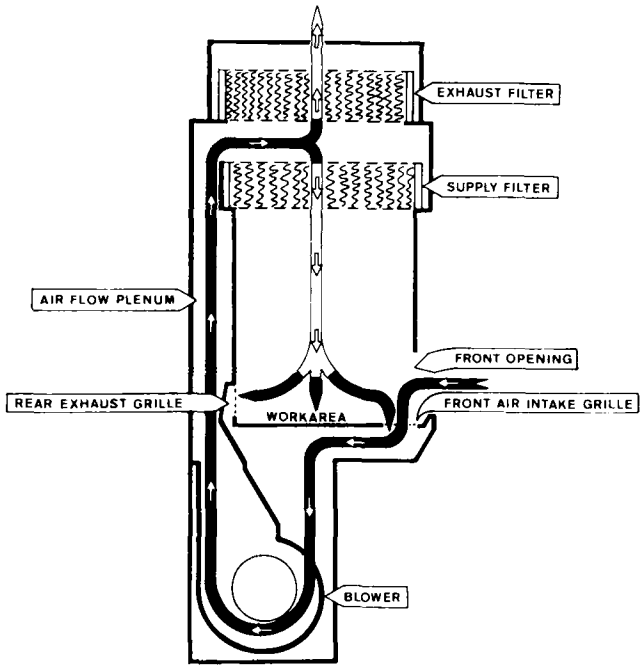

FIG. 3. Schematic cross-section of a generalized Class II biological safety cabinet. (Reprinted by permission from Ref. 9.)

(*9,10*). The clean air streams, within the designated chamber, will remain intact unless overcome by turbulence caused by the introduction of objects and personnel movement (*10*). The purified laminar flow air cannot sterilize contaminated articles brought into the biological safety cabinet.

Types of Biological Safety Cabinets. Laminar flow hoods that have been developed solely for product protection are not appropriate for cell labeling procedures because they provide no operator protection. A horizontal laminar flow clean work station is not recommended, since the air flowing over the product may disperse aerosols from the product into the working environment and onto the operator (*6*). A biological safety cabinet must be selected that protects the personnel as well as the product (*6*).

The National Sanitation Foundation (NSF) developed a classification system for biological safety cabinetry, which has been adopted as the standard by government agencies, end users, and manufacturers. NSF Standard 49 was issued in 1976 and revised in 1983 and 1987 (*11*). Biological safety cabinets are classified into three categories, Classes I, II and III, depending on the degree of operator and product protection. Refer to the Standard for details of the various classes (*9*). Table 1 outlines the uses of Class I and Class II biological safety cabinets (*11*).

Cabinets used in cell labeling should belong to Class II. Protection is provided for personnel, product, and environment. An open front with inward air flow provides personnel protection, HEPA-filtered laminar air flow aids in product protection, and HEPA-filtered exhaust air protects the environment (*6*). Class II cabinets are further defined into subcategories Type A and B.

Class II, Type A is a recirculating cabinet with a minimum of 30% exhaust and a minimum calculated average inflow velocity of 0.38 m/sec (or 75 fpm) through the work area access opening (*12*). A schematic representation is shown in Figure 4A. The exhaust air is HEPA-filtered and returned directly into the laboratory. The cabinet is suitable for work with low- to moderate-risk biological agents in the absence of volatile toxic chemicals and volatile radionuclides. Where possible it is desirable to discharge the filtered exhaust air to the outside of the building (*6*).

In a *Class II, Type B* cabinet, operator exposure is reduced to the lowest practical limits. A flow rate of 0.5 m/sec (100 fpm) of room air into the cabinet through the work access opening helps to provide a barrier against exchange of air inside and outside the cabinet. The recycling of air is decreased to <30% (Class II, Type B1) or eliminated entirely (Class II, Type B2), and the cabinet air is exhausted to the outside of the building through HEPA filters (*6*).

Class II, Type B cabinets are suitable for work with low- to moderate-risk biological agents. They may also be used with biological substances treated with toxic chemicals and radionuclides. An external blower system is typically required within the exhaust system of the building, and an adequate air supply is needed within the room to supply the large volume of air eliminated during use of the cabinet. The ducting and air flow requirements must be discussed with the building maintenance engineer.

A total exhaust system (Class II, Type B2), as depicted in Figure 4C, does not recirculate any of the exhaust air and is required if volatile radionuclides (e.g., radioiodines) are used. Ideally, only total exhaust cabinets should be installed in nuclear medicine departments, provided the air supply is sufficient in the area where the cabinet is to be installed.

TABLE 1. Classes of Biological Safety Cabinets

Class	Protection	Use
I	personnel environment	Low- and moderate-risk biological agents where no product protection is required
II	personnel environment product	Low to moderate risk biological agents . . .
II-A		in the absence of volatile toxic chemicals or volatile radionuclides
II-B1		treated with minute quantities of toxic chemicals and trace amounts of radionuclides
II-B2 (Total exhaust)		treated with toxic chemicals and radionuclides
II-B3 (Convertible)		treated with minute quantities of toxic chemicals and trace quantities of radionuclides that will not interfere with the work if recirculated in the downflow air

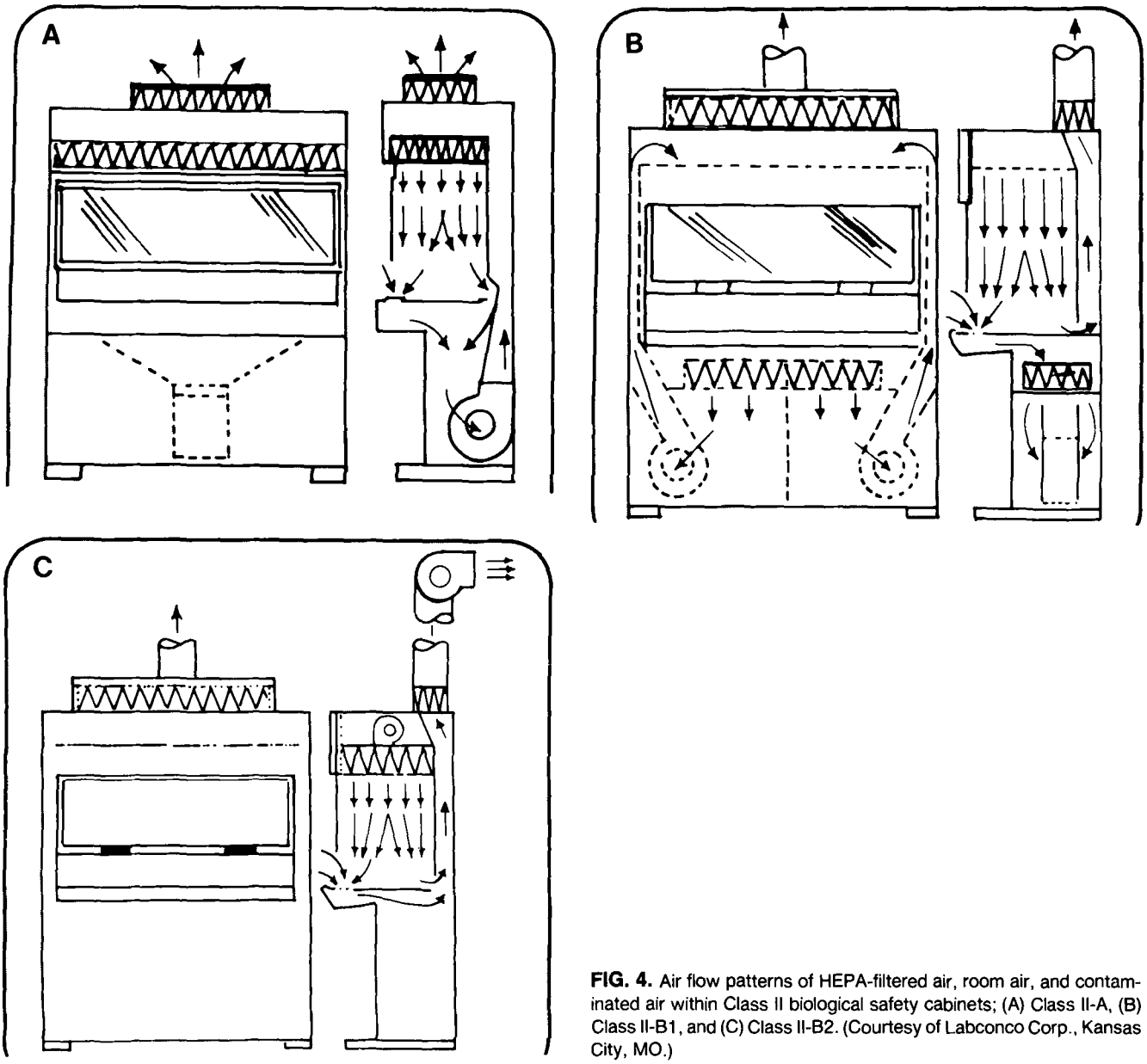

FIG. 4. Air flow patterns of HEPA-filtered air, room air, and contaminated air within Class II biological safety cabinets; (A) Class II-A, (B) Class II-B1, and (C) Class II-B2. (Courtesy of Labconco Corp., Kansas City, MO.)

Class II, Type B1 biological safety cabinets, which recirculate <30% of the air through a HEPA filter (Fig. 4B), may be permissible if no volatile contaminants are produced and any droplets and aerosols generated are trapped indefinitely by the HEPA filters.

Performance Requirements for Biological Safety Cabinets. Certification of the biological safety cabinet to NSF standards is necessary after installation or repairs, relocation of the unit, or replacement of the HEPA filter (*11*). Cabinets should be performance-tested at least annually to determine HEPA filter and gasket integrity (leak test and pressure test), filter loading, air velocity profiles over the work surface and opening, and air flow patterns (*11,13–16*). When on-site expertise and testing equipment are not available, commercial testing services are available, which can serve as a reliable alternative.

For working with sterile products, the purified air supply within the cabinet should meet Grade A conditions of the Canadian Good Manufacturing Practices (GMP) guidelines (*14*) or Class 100 of the U.S. Federal Standard 209D (*15*). The nearest British equivalent is Class I of the British Standards Institute BS 5295 (*16*). Grade A standards of the Canadian GMP include the following.

- 99.997% HEPA filter efficiency
- minimum air flow rate of 0.3 m/sec (vertical cabinet) [Note that the requirements for air flow velocity are higher in the Class II biological safety cabinets: a minimum of 0.45 m/sec (90 ± 20 fpm), and for using radioactivity (*5*), it is advisable that the linear face velocity be between 0.5 and 1 m/sec (100–200 linear fpm).]
- less than 3500 particles/m^3 larger than 0.5 μm
- less than 1 particle/m^3 larger than 5 μm
- less than 1 viable particle per m^3

Accessories. It is preferable to irradiate the biological safety cabinet with a short-wave ultraviolet (UV) light when the cabinet is turned off. This does not circumvent the need to disinfect the cabinet prior to use, since the UV radiation only affects a very thin exposed surface and is not sufficient to sanitize or sterilize large areas.

Some units have a sloped recessed viewing panel, which allows the operator to be positioned over the work for better viewing and comfort.

Since the biological safety cabinet only performs well within a given range of air velocities, this range must be monitored and maintained. Variable speed controls are necessary to adjust the blower speed as the HEPA filter becomes loaded. Air flow alarms are desirable to alert users if the air flow velocity falls below a minimum rate (*6*) or to monitor velocity pressure differential within the cabinet.

A few other accessories to consider are IV bars, electrical outlets, connections for gas, air, and vacuum. The location of accessories should be selected to avoid disruption of the laminar flow in the work area.

Disinfection Procedures for Biological Safety Cabinets

Disinfection of the biological safety cabinet with a suitable disinfectant must be performed before and after each use of the cabinet. Disinfection is defined as the destruction of organisms capable of causing infectious disease. This is different from sterilization, which can be defined as the process of destroying all living organisms (including spores). There are hundreds of disinfectants available on the market, and it would be impractical to try to discuss them all. Disinfectants are categorized in classes, based upon the active ingredient present in the formulation. The most common classes are alcohols, chlorine, formaldehyde formulations, phenolic formulations, and glutaraldehyde formulations. Many disinfectants found in the latter group are also called "cold sterilants" because they sterilize at room temperature, provided the contact time is sufficient.

Alcohol is the most widely used disinfectant in nuclear medicine departments. The use of ethyl alcohol (ethanol) for disinfection or sanitization is widespread in many departments, mainly because ethanol is generally readily available in the hospital. However, isopropyl alcohol is more effective (*17,18*).

The fact that alcohols are surface tension reducers, lipid solvents, and protein coagulating agents contributes to their disinfecting properties. However, they are also potent dehydrating agents; a property that interferes with their coagulating powers (*17*).

The disinfecting activity of aliphatic alcohols is directly proportional to the length of their carbon chain and molecular weight. The bactericidal activity increases for carbon chains of up to 8 to 10 carbon atoms and then decreases as the water solubility decreases. This explains why isopropyl alcohol is a slightly more potent disinfectant than ethanol. As well as being less volatile than ethanol, its effectiveness is greater, given the same surface contact period. In addition, unlike ethanol, isopropyl alcohol is not subject to legal regulations and restrictions (*18*).

The disinfecting action of alcohols, like their denaturing effect on proteins, requires the presence of water to penetrate the bacterial membrane (*18*). Many people have a false sense of security when they use 95%–100% alcohol to disinfect their biological safety cabinet. Contrary to widespread belief, alcohol is most effective in 50%–70% aqueous solutions. A 95%–100% alcohol content results in a low contact time because it evaporates too quickly and does not provide the necessary water content.

The main disinfecting property of alcohol is due to its action on the bacterial cell membrane. The structural integrity of the bacterial membrane, composed of proteins and lipids, depends on the orderly arrangement of those proteins and lipids. By penetrating into the hydrocarbon region of the bacterial membrane, alcohol disorganizes the membrane, thus interfering with its normal functions. The net effect is the release of metabolites from the bacterial cell and interference with active transport and energy metabolism of the cell. In addition to this effect, alcohol also denatures cellular proteins, which adds to the disinfecting action (*19*).

As bacterial spores have been reported to remain dormant in alcohol preparations, 70% isopropyl alcohol should be filter-sterilized through a sterile 0.22-μm membrane filter into sterilized containers (*20*).

The disinfection procedure protocol for biological safety cabinets is listed below (*21,22*).

1. Turn off the UV light and turn on the HEPA filter blower for at least 15 min prior to working in the cabinet.
2. Disinfection of the biological safety cabinet should take place before and after use and also between patients.
3. Use only filter-sterilized disinfectants (e.g., 70% isopropyl alcohol) and sterile wipes inside cabinet. Do not use paper, as it is an important source of contamination and also sheds particles.
4. Pour solutions from container onto sterile wipes. Do not immerse cloth in the alcohol.
5. Wash the inside surfaces of the biological safety cabinet and allow a sufficient disinfectant contact time (minimum of one min). Do not lift the cloth from the surface while performing the procedure. Use a new cloth for each surface. Wipe the back panel from the top and work downstream from side to side. Wipe the side panels and front panels from back to front working from the top and moving downstream.
6. If possible rotate different disinfectants to decrease the risk of selecting resistant microorganisms. Do not use chlorine (e.g., bleach) routinely on metal surfaces (it will rust the metal). If bleach is utilized to disinfect a spill in the cabinet, rinse immediately with sterile water.
7. With some disinfectants, it may be necessary to rinse with sterile water for irrigation after sufficient time has elapsed. This will prevent buildup of residue that may eventually become a source of contamination.

CONCLUSION

During the isolation of blood cell fractions, both specialized facilities and equipment and sophisticated techniques are necessary to protect the injectable product and the people performing the procedure. This first part of the teaching editorial concentrates on the facilities and equipment, while the second part (*5*) will discuss universal precautions, aseptic technique, and working with open sources of radioactivity.

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