Personnel and Product Protection During Manipulation of Blood Products

Pamela Zabel, Normand Robichaud, and Anne Hiltz

University Hospital, London, Ontario, Canada; Health Protection Branch, Bureau of Radiation and Medical Devices, Ottawa, Ontario, Canada; and Victoria General Hospital, Halifax, Nova Scotia, Canada

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 which 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.

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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 first paper (*1*) discussed facility design, equipment, and sterilization with special emphasis on biological safety cabinets. This paper will address personnel and product protection.

Most radioactive agents for cell labeling are nonselective and will label all cell types to some extent in a blood sample. It is therefore, generally necessary to isolate the particular cell fraction from whole blood before labeling (*2*). The numerous isolation steps must incorporate the concepts and techniques of universal precautions and aseptic technique to protect personnel and products.

There are two reasons for exercising great care when manipulating blood for the purpose of cell radiolabeling. The first is to protect the cells from microbial or viral contamination and cross-contamination from other biological materials during the labeling procedures. This requires understanding and implementing aseptic technique. The second reason is to protect the health care worker from the blood, which potentially could be infected (e.g., hepatitis or HIV viruses). This requires an understanding of and implementation of universal precautions.

IMPORTANCE OF ASEPTIC TECHNIQUE

Aseptic technique is defined as "the use of procedures in the preparation and administration of parenteral products, which minimize or prevent the introduction of microorganisms" (*3*). Strict adherence to aseptic technique principles to prevent microbial contamination is critical when working with blood products.

Blood is an ideal medium for the propagation of microorganisms. Microorganisms reinjected into the circulation of a patient are disseminated throughout the body within a few minutes. When some microorganisms are arrested in small vessels with slow blood flow rates, like capillaries, they have an opportunity to initiate infection in the surrounding tissues (*4*).

A relatively healthy person can generally combat the transient bacteremia since the reticuloendothelial cells and immune system usually remove and inactivate the circulating bacteria. The situation becomes more serious when the resistance of a patient is significantly impaired or when the circulating bacteria reach vulnerable exposed tissues.

If an infection develops and the microorganism is an environmental contaminant (*Bacillus sp.* for example), the identification of the microorganism isolated from the clinical specimen may be delayed or ignored. The microbiology laboratory may discard the isolate believing it to be a contaminant that originated during sampling or that was introduced during plating (*5*).

In order to protect the patient, the cell labeling procedures must be performed using strict aseptic technique and be conducted in the appropriate biological safety cabinet and facilities (*1*).

UNIVERSAL PRECAUTIONS

Universal precautions or "universal blood and body fluid precautions" address the issue of protection of health care

For reprints contact: Normand Robichaud, MSc, Radiopharmaceuticals Section, Bureau of Radiation and Medical Devices, 775 Brookfield Rd., Ottawa, Ontario, Canada K1A 1C1.

workers against infectious diseases, which may be present in patients but have not yet been recognized. The major concerns are human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other bloodborne pathogens. An important aspect of universal precautions is the "window" period in diseases caused by pathogens such as HIV, during which infection is present but cannot yet be diagnosed.

The basis of universal precautions is that all blood and body fluids from any patient, regardless of diagnosis, should be considered potentially infectious. Risk management involves the use of different barriers, e.g., gloves, masks, gowns, and eye protection when there is a known or potential contact with body fluids or tissues. For complete details on universal precautions, refer to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) "Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids and Tissue" (*6*) and publications of the Centers for Disease Control and Prevention (*7,8*).

Protective Barriers

Proper use of barriers is essential to protect staff from being infected. As well, proper disposal or disinfection of the barrier is necessary to prevent transmission of infection to other patients or staff. Barriers are selected that are appropriate for the particular procedure being followed. These barriers are only to be used when there is actual or potential exposure to body fluids or tissue, and they must be removed upon completion of the procedure (*9*).

Gloves and Gowning. Gloves are to be worn during all procedures with blood products in clinical or laboratory situations. Gloves should be chosen that have been shown to be a suitable mechanical barrier against HIV and other pathogens. It is important that the skin on the hands remain intact since skin is an excellent physical barrier. Any nonintact skin should be covered with an approved occlusive dressing. Handwashing is the simple most important means of preventing laboratory acquired infection: This cannot be overemphasized. Hands should be washed even if gloves have been worn.

Gowns are to be worn throughout the labeling procedures. Gowns should have tight wrists that fit underneath the glove cuff so as not to expose bare wrists. The procedures to use with gloves, scrubbing, and gowning are discussed, together with aseptic technique, later in this document.

Facial and Eye Protection. Masks and eye protection are worn during procedures that are likely to generate droplets of blood or body fluids, which may enter the mouth, nostrils, or eyes. If the appropriate vertical biological safety cabinet is being utilized (*1*) in the proper manner during cell labeling procedures, the air flow patterns and viewing panel barrier typically provide protection so that utilization of a mask and eye protection are not necessary. While working with blood products, the operators must ensure that they never unconsciously touch themselves, particularly near mucous membranes such as the mouth and eyes.

Outside of the vertical biological safety cabinet, it is advisable to wear all forms of protection (gown, glasses, gloves, and mask). This is an important precaution in the event of an accidental breakage or spill, which could occur during transfers from the centrifuge to the biological safety cabinet.

Disposal of Waste

Needles and Sharps. An important element of the concept of universal precautions is the proper disposal of sharps and needles. Approved puncture-proof sharps containers must be readily available in all appropriate areas for direct placement of all disposable sharps. Sharps containers are to be sealed once they are three-quarters full. Recapping of needles is not recommended unless an approved device is used that allows a single-handed procedure.

Blood and Blood Byproducts. Blood samples in tubes should be autoclaved before disposal to landfill sites. All clinical material and blood products are to be placed in autoclavable bags held in autoclavable metal cans. Before autoclaving, 200–500 ml of water must be added to autoclave bags. Bags are then loosely sealed with autoclave tape so air can be removed and steam can penetrate. The autoclaving cycle for all material to be decontaminated is 121°C and is typically for 1 hr depending on the load size. Verify the cycle with biological sterility indicators (*9*).

Accidental Needle Punctures

If an accidental needle puncture occurs from a needle that has been in contact with body fluids, the following steps should be taken: (1) wash the wound in warm soapy water; (2) make the puncture wound bleed; (3) apply povidone iodine; and (4) cover the wound and contact supervisor and employee health services. Ascertain infection status of patient from whom sample was taken (e.g., antibody determination for HIV or antigens of HBV). This will give some estimate of real risk associated with the incident.

Accidental Biological Spills

If an accidental biological spill occurs (e.g., blood), the following steps should be taken. Appropriate barrier precautions (gown, gloves, mask, and eye protection) should be worn. Allow aerosols to settle and then cover spill with disposable absorbent material such as paper towels. The spill should be contained and mopped up with absorbent material and placed in an autoclave bag. Apply an approved disinfectant which must be tuberculocidal. (Example: A strong hypochlorite solution consisting of one part commercial bleach mixed with four parts water, which is prepared fresh each week and stored in a sealed plastic bottle. Caution: Do not use this solution on metal surfaces. The disinfectant should be carefully poured, starting at the spill perimeter and working toward the center. Allow a disinfectant contact time of at least 15 min before clean-up (*9*).

ASEPTIC TECHNIQUE IN BIOLOGICAL SAFETY CABINETS

Aseptic technique and strict adherence to procedure should be used by all personnel labeling white blood cells, platelets, or during any other aseptic procedure involving an open system.

The quality of the labeled cells is directly related to the manipulative skills and conscientious attitude of the staff preparing them. Written procedures should be available for cleaning, disinfecting, cell labeling, and general working techniques. The work should be performed in a certified biological safety cabinet, while wearing a sterile gown and sterile latex gloves. Personnel performance should be reviewed periodically.

Operators must not acquire a sense of false security when working in the biological safety cabinet. The laminar air flow does not remove microbial contaminants from surfaces and it is not a sterilization process. Therefore, the significance of the microbial load present on materials placed in the biological safety cabinet should be recognized (*10*). Persons working in the cabinets should be aware of the direction of laminar flow air currents within the units so that potentially contaminating substances (e.g., blood from an HBV patient) do not transfer infectious agents to other exposed sterile items which are downwind.

Scrubbing and Gowning

The human body is an important source of microbial and particle contamination. An individual is capable of generating millions of particles per minute. The clothing worn by personnel is, therefore, extremely important when performing aseptic operations such as cell labeling (*11–13*).

A sterile gown that completely covers the body should be worn at all times. The gown fabric should be of a tight weave and not prone to emitting fibers. The gown will then act as an effective barrier to the passage of microorganisms from the body to the environment and will also serve as one of the protective barriers necessary for universal precautions (*11–13*). Disposable, powder-free, latex, sterile gloves should also be worn at all times. A disposable face mask should be worn if the operator has a cold or infection, to protect the cells from contamination.

Facilities should be designed so that a sink is readily accessible for handwashing before and after completing the procedure (*1*). The taps should preferably utilize foot or elbow operating controls. Use of protective garments should be restricted to the cell radiolabeling room. If a prolonged incubation allows departure from the room, then hooks to hang gowns should be provided near the exit or entrance of the cell radiolabeling room.

Scrubbing and Gowning Procedure. The following scrubbing and gowning procedures should be followed to ensure an aseptic environment (*11,12*).

1. Remove all hand, wrist, or other exposed jewelry.
2. Scrub hands and forearms with surgical cleanser. Work up a lather and scrub well.
3. Dry hands with a lint free cloth towel, wiping from the hands to the elbows in long strokes.
4. Put on sterile gown and gloves, pulling gloves over the cuffs of the gown. Ensure that the gloves are tight fitting. Reglove at least hourly or if gloves become punctured or torn while working. Gloves should be put on just prior to and taken off immediately after completing the procedure. Gloves that have been inside the biological safety cabinet should be considered potentially infected and disposed of as such. A clean gown should be worn every day or in the event of a contamination.
5. Before starting work in the biological safety cabinet, wipe gloves with sterile filtered 70% isopropyl alcohol to remove powder or utilize hypoallergenic, sterile, nonpowdered gloves.
6. Hands must be washed after removal of gloves, regardless of the amount of time the hands have been covered.
7. Degowning: Gowns should be placed immediately into a laundry bag after the procedure is completed. Gloves should be discarded into a biohazard bag if they are potentially contaminated with blood products; otherwise they may be discarded in the regular garbage.

General Working Procedures for the Biological Safety Cabinet

The following procedures should be followed when working in the biological safety cabinet (*11,12*).

1. Disinfect the biological safety cabinet with 70% isopropyl alcohol as outlined previously (*1*).
2. Wipe *all* items placed in the biological safety cabinet with sterile, filtered, 70% isopropyl alcohol.
3. Remove all items from cardboard cartons and outer wrappers before bringing them into the work area.
4. Open articles in sterile packaging, such as syringes, within the first six inches of the biological safety cabinet; place contents deep into the cabinet; and discard the packaging.
5. Keep movement in the work vicinity to a minimum.
6. Always keep in mind that the purified laminar air flow comes from above in a vertical biological safety cabinet. It is critical not to block this air flow and to minimize manipulations above the sterile item.
7. Keep the number of transfers into and out of the cabinet (i.e., equipment and hands) to a minimum. Wipe gloves with sterile, filtered, 70% alcohol if gloved hands go outside the sterile area. Reglove when gloved hands contact nonsterile surfaces (e.g., cardboard boxes or phone).
8. Minimize talking while working in the cabinet. Do not sneeze or cough into the work area.
9. Do not write in the work area. Keep all unsterile items (e.g., pencils, papers, and wrappers) out of the biological safety cabinet.

10. In order to maintain proper air flow and avoid air turbulence, equipment should not obstruct the front and rear return grills.
11. Keep all unnecessary items out of the work area. When finished with materials, disinfect them and remove them from the biological safety cabinet. Throw garbage (wrappers for needles and swabs) out of the cabinet and into the garbage. Place all essential items at the sides of the major site of activity to minimize movements and keep turbulence under control.

REAGENTS, SUPPLIES, AND CELL TREATMENT

All reagents and supplies used in a cell labeling procedure should be of the highest possible quality, sterile, pyrogen-free, and particulate-free. Sterile, plastic, disposable material is preferable to breakable glass when handling infectious material. All reagents should be checked for visible turbidity, leaks, cracks, particulate matter, and for the manufacturer's expiration date before use. If a problem is found, the product should not be used.

Single-use vials should be used whenever possible. When multiple-use containers and reagents are used, they should be marked with the date and time the container was entered. The product label or package insert should be consulted to determine if refrigeration of the container is necessary.

Sterilization of supplies or equipment in the facility must be done using a validated sterilization process. If a waterbath is used during the procedure, this may be a source of contamination (*14*). Disinfect and empty the waterbath frequently. Adding one part vinegar to twelve parts water will decrease the pH to 3.5. This pH has been found to be effective in inhibiting growth of bacteria in the waterbath (*15*). The acidic pH is not suitable if the waterbath contains exposed copper. Surveillance cultures can be done periodically to verify a low contamination level (*14*).

The cells and blood products should be treated gently at all times to ensure cell viability. Low centrifugal speeds for short periods may help to minimize mechanical trauma to cell fractions (*2*). The cells should be reinjected as soon as possible. All tubes should be labeled appropriately so that no confusion occurs during the procedure.

It may be advisable for new personnel to perform the entire procedure on blood from a normal volunteer and to send samples for sterility testing. A sample that has not been manipulated should also be submitted as a control.

USE OF OPEN SOURCES OF RADIOACTIVITY

Working with Radioactivity in the Biological Safety Cabinet

The safe handling of radioactive materials in the laboratory or hospital must be done under the constraints of the appropriate regulatory agency (i.e., the Nuclear Regulatory Commission [NRC] or the agreement state agency in the United States and the Atomic Energy Control Board in Canada). The guidelines set by the regulatory agency through individual licenses or general regulations must be followed. All regulatory users should implement the ALARA (as low as reasonably achievable) principle. Every protocol should be evaluated and necessary steps should be taken to limit radiation exposure. The allowable limits of radioactivity in various laboratories will periodically change with new regulations. Specific requirements for the fume hood and facilities are often detailed in guidelines to regulations (*16*).

Large lead bricks or drawing-up stations are generally not desirable in the biological safety cabinet as they may disturb the laminar air flow and the airflow barrier at the front of the unit. It is preferable to shield the tubes containing radioactivity in individual lead pots that have been wiped with isopropyl alcohol.

The following are a set of guidelines developed for laboratory users of open sources of radioactivity greater than 3.7 MBq (100 μCi) of indium-111 or 370 MBq (10 mCi) of technetium-99m within a fume hood (*16*). These limits correspond to the limit of 100 scheduled quantities which must be handled in a fume hood for a basic radioisotope laboratory (*17*). The actual limits are periodically reevaluated and revised by the appropriate regulatory agency.

Requirements for Laboratory Users of Open Sources of Radioactivity

Required Apparel. In addition to the gown used in the blood cell isolation steps, the following additional disposable protection is advisable when dealing with open sources of radioactivity: disposable sleeves, a disposable apron, and disposable gloves (double gloving while handling open source of radioactivity) (*17*).

Required Equipment and Materials. The following equipment and materials are required when working with open sources of radioactivity (*17*).

1. Biological safety cabinet with adequate shielding for radionuclides to be used.
2. Radiation monitor.
3. Spill kit.
4. Shielded high-level waste container.
5. Waste can labeled "Radioactive Material" for combustible wastes (gloves and apron).
6. If necessary, waste can labeled "Radioactive Material" for noncombustible wastes.
7. Absorbent paper.
8. Trolley or adequate container to safely transport materials, stock radionuclides, products, and if necessary, wastes between laboratory and work site.
9. "Radioactive Material" labels for products.

Required Procedures. The following procedures are required when working with open sources of radioactivity (*17*).

1. Prepare a written document describing the procedure in detail.
2. Include a description of the most efficient placement of materials within the cabinet and the order of their use.

This is required in order to reduce personnel exposure by reducing the time of the procedure and to increase the distance or shielding of the radionuclide.

3. All transportation between the hot lab and cell labeling room must be done in a safe manner, which reduces the possibility of a spill and exposure.
4. Update the inventory sheet for the radionuclide.
5. Operators must wear a laboratory coat or gown, on top of lab coat wear disposable sleeves and apron which must not leave the work site, and double disposable gloves. The outer pair must remain in the cabinet until the work is finished at which time they may be disposed of in the appropriate waste can, depending on the amount of contamination.
6. Upon completion of the work, operators must monitor the work site and clean it if necessary, monitor wastes and dispose of or store them appropriately, monitor the products and stock for spills and clean if necessary, monitor self and if contaminated clean affected areas and monitor again, and if there has been a major spill or contamination, contact the radiation safety officer.

CONCLUSION

During the course of this paper, the theoretical and practical aspects of aseptic handling of blood and blood fractions for radiolabeling purposes have been reviewed. When adequate precautions are taken, cell labeling can be achieved in a safe manner for the health care worker performing the procedure, while minimizing the risk of contaminating the end-product, which will be reinjected into the patient.

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