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Report

Cytogenetic Biodosimetry in Radiation Emergency Medicine: 2. Biosafety and Chemical Safety in Biodosimetry Laboratory

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In a biodosimetry laboratory, blood collected from exposed patients is cultured and the exposure dose is estimated based on the frequency of chromosome aberrations. Blood is defined as an infectious specimen because it may contain hepatitis virus and human immunodeficiency virus (HIV) and must be handled in a biosafety level (BSL) 2 facility. Due to the recent coronavirus pandemic with SARS-CoV-2, further strengthening of infection control measures is required. This article outlines the requirements for setting up a BSL2 laboratory, personal protective equipment for infection control, treatment of infectious biological waste and emergency response measures. Furthermore, it is essential to safely manage hazardous chemicals used in biodosimetry. Biodosimetry laboratories should conduct risk assessments of blood handling and chemical use and consider risk mitigation measures. In addition, laboratory personnel must educate workers on infection control and chemical safety.

Key words: cytogenetic biodosimetry, laboratory management, biosafety, chemical safety, emergency response

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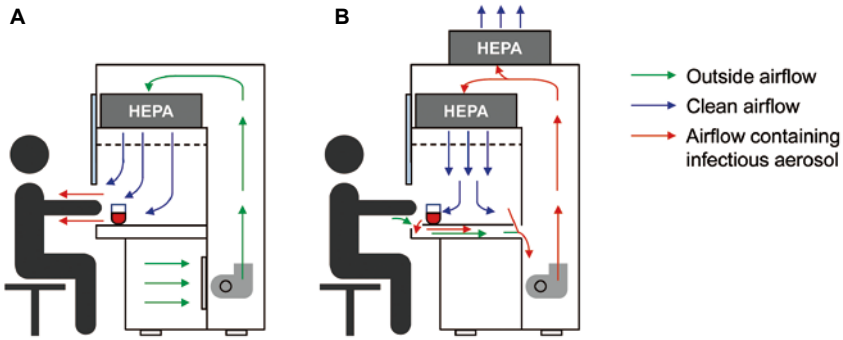

Fig. 1. Comparison of a clean bench and a safety cabinet. A. clean bench, B. safety cabinet.

1. Introduction

Laboratory facilities are designated to biosafety levels (BSL) 1 to 4 depending on the type of pathogens handled. BSL1 has the most basic level of containment while BSL4 has the maximum level of containment. The risk levels of pathogens are categorized based on the pathogenicity of infectious microorganisms, transmission route and host range of microorganisms, availability of effective preventive measures and availability of effective therapeutic methods[1].

In biological dose assessment (biodosimetry), blood collected from exposed patients is cultured and chromosome aberrations are analyzed. Human blood is an infectious specimen that is classified as a United Nations (UN) standard category B biological substance[2]. The World Health Organization (WHO) laboratory biosafety guidelines recommend a BSL2 environment for blood handling. While handling blood specimens, it is necessary to take thorough infection control measures and properly dispose of infectious waste[1]. In addition, methanol, acetic acid and other hazardous and flammable substances are used when preparing chromosome specimens. Chemicals used should also be properly handled with care.

Biodosimetry laboratories are required to manage their laboratories in accordance with national laws and guidelines, as well as international guidelines. Biosafety guidelines and chemical handling guidelines must be established for each facility. Efforts must be made to ensure rigorous safety management and laboratory users must be educated in workplace health and safety. This paper introduces the establishment of a biodosimetry laboratory that complies with the laws and guidelines in infection control, chemical safety, infectious biological waste treatment and liquid chemical waste disposal.

2. Infection control and BSL2 laboratory maintenance

Peripheral blood used for biodosimetry may contain pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and the novel coronavirus (SARS-CoV-2). Therefore, human blood must be treated as an infectious specimen. In particular, as the SARS-CoV-2 virus is transmitted by aerosols, infection protection against aerosols and other biohazard control measures should be taken when handling blood collected from patients with suspected radiation exposure and positively diagnosed with COVID-19[3].

Blood is usually handled in a BSL2 laboratory as microbial infectious agents may cause moderate levels of disease in the event of exposure. Laboratories responsible for biodosimetry in radiation emergency medicine should set up BSL2 laboratories in accordance with WHO's laboratory biosafety guidelines[1] to prevent infections and contamination. In addition, BSL2 laboratory certifications and approval are required and regulated by the laboratory's respective institutes.

The requirements of the BSL2 laboratory and their details are shown below. These requirements are not exhaustive. For more information, please refer to your institutes' BSL2 certification criteria.

2.1. BSL2 laboratory requirements

a) The laboratory has the structure and equipment of a normal biological laboratory.

b) The laboratory is equipped with a biological safety cabinet (only when performing operations that are prone to aerosols).

Note 1. Aerosols may be generated when the blood collection tube or cell culture tube is opened.

Note 2. The safety cabinet needs to be inspected at least once a year.

Note 3. A clean bench is a device that protects the cell culture from contamination by providing an aseptic working environment. Aerosols generated from an infectious

Fig. 2. Representative image of a Biohazard sign for a BSL2 laboratory. Contact personnel listed in the sign must be contactable at all times.

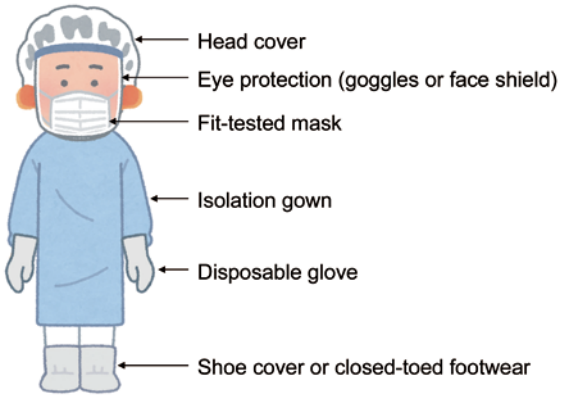

Fig. 3. Personal protective equipment for biodosimetry.

Fig. 4. Centrifuge bucket with a lid for biohazardous aerosol protection.

sample can be released outside of the cabinet. It is not designed for personal protection (Fig. 1A). Clean benches are thus not recommended for biodosimetry. On the other hand, infectious aerosols are not released outside the Class II safety cabinet and can be used for personal protection (Fig. 1B).

c) Install the autoclave in the building where the laboratory is located if an autoclave is used for sterilization.

2.2. BSL2 laboratory display and access restrictions

a) Internationally recognized biohazard symbols must be displayed on the doors outside of rooms which handle infectious specimens such as blood (Fig. 2).
b) Only certified personnel are allowed access to the laboratory work area.
c) Laboratory doors must be closed.
d) Food and drink cannot be stored in the laboratory work area.
e) Eating, drinking, smoking, makeup application and contact lens handling in the laboratory work area are prohibited.

2.3. Personal protective equipment (PPE)

a) Wear the following personal protective equipment (Fig. 3) when handling blood, bodily fluids or other potentially infectious samples.
   - Isolation gown
   - Surgical mask
   - Goggles or face shield
   - Disposable gloves
   - Head cover
   - Closed-toed footwear
b) The gloves used must be removed aseptically. Wash your hands after removing your gloves.
c) It is prohibited to wear lab coats outside the

laboratory, such as in the office, library and staff room.

d) Do not wear exposed footwear in the laboratory. Feet and toes should be fully covered.

e) Lab coats should not be stored in the same lockers or cupboards as everyday clothing.

2.4. Other biohazard safety measures required for biodosimetry

a) Centrifuge

Centrifuges are used for lymphocyte isolation, cell wash and cell harvest and fixation. To prevent biohazardous aerosol transmission and contamination during centrifugation, it is highly recommended to attach the lid to the bucket of the centrifuge (Fig. 4) or to install the centrifuge in the biological safety cabinet. Pathogen contamination in the working environment can be prevented by removing the centrifuge tube from the closed bucket in the safety cabinet.

b) CO_2 incubator

In biodosimetry, blood or isolated peripheral blood mononuclear cells are directly cultured in a CO_2 incubator without pathogen inactivation. Disinfection and sterilization inside the CO_2 incubator should be regularly performed with its in-built heat-sterilization function. Moreover, CO_2 is often supplied from a compressed gas cylinder connected to the incubator. In accordance with the safety regulations outlined by your country on the use of compressed gases, efforts must be made to prevent accidents caused by pressurized gas, such as fall prevention measures. Compressed gas cylinders should also be used before the inspection expiration date, or be replaced or re-inspected if expired. Valves should also be maintained and inspected regularly. This reduces the risk of chemical and physical hazards associated with compressed gas cylinders.

3. Infectious liquid and solid biohazardous waste handling

After using infectious specimens that may contain pathogens, pathogen spread must be inhibited. Leftover blood, culture supernatant after blood culture, reagents used for blood cell washing and other equipment where blood and its components were in contact with must be disinfected or sterilized by an appropriate method and discarded.

3.1. Biological liquid waste disinfection

Any liquid reagents in contact with blood should be equally treated as an infectious specimen like blood. After collecting these liquids in an appropriate storage container, sodium hypochlorite solution (effective chlorine concentration 0.1% as a regular disinfectant, effective chlorine concentration 0.5% for large amounts of organic matter) can be added as a first step to decontamination[1]. Proper waste disposal can be finally performed after high pressure thermal sterilization (121 ℃, 20 minutes). If there is a waste disposal facility capable of handling biological liquid waste disposal according to national guidelines, biological liquid waste in the lab should be first treated with suitable disinfectants before contacting the facility for disposal.

If chemical disinfectants other than a hypochlorous acid-based solution are used, ensure that the concentration and type of the disinfectant used is suitable for effective sterilization of the target pathogen and liquid waste volume. In addition, if other chemicals are found in the biological liquid waste such as acids or ammonium-containing materials, hypochlorous acid treatment will generate hazardous gases. In that case, another suitable disinfectant should be used instead.

3.2. Disposal of solid biohazardous waste in contact with infectious specimens

Pipette tips, cell culture tubes, blood collection tubes and other biological disposable equipment which were in contact with infectious specimens must first be sterilized by high pressure thermal sterilization (121 ℃, 20 minutes). They can then be discarded or separately disposed of as medical waste. If the laboratory is unable to perform autoclaving within its premises, solid biohazardous waste must be sent to a waste disposal facility capable of handling solid biohazardous waste disposal according to national guidelines.

4. Chemical safety in biodosimetry

Several hazardous chemicals and organic solvents are used in biodosimetry and its related studies. The person responsible for the biodosimetry laboratories must be educated and trained in the hazardous chemical handling in accordance with relevant laws and guidelines. Safety data sheets (SDS) for each hazardous chemical used in the laboratory must be prepared and managed so that they can be viewed promptly. All hazardous chemicals used should also be labelled with their appropriate Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard pictograms[4].

4.1. Hazardous chemicals

Proper management of hazardous chemicals used in the lab is necessary for workplace and health safety. Any hazardous chemicals used must be made known to the institute, locked and stored in their respective chemical cabinets, and the amount of the chemical stored and used must be recorded. Laboratories that use hazardous chemicals must be well-equipped with precautions regarding health hazards and have an emergency

response plan in place. In addition, laboratory personnel must educate users on chemical safety and potential health hazards associated with hazardous chemicals used in the lab.

4.2. Flammable chemicals

Flammable chemicals such as methanol are used in biodosimetry research. Flammable chemicals should be stored in a flammable safety cabinet for fire protection. When using these chemicals, it is also necessary to evaluate the risk of fire and explosion to workers and consider measures to reduce the associated risks.

4.3. Local exhaust ventilation

When using hazardous chemical substances or organic solvents, exhaust equipment specific for each chemical classification is required. Local exhaust devices such as fume hoods must be used to reduce the risk of harmful health effects in researchers by preventing prolonged exposure to noxious chemical fumes.

4.4. Liquid chemical waste

The chemicals used must be classified according to facility regulations and disposed of in an appropriate manner.

5. Measures related to emergency response

In laboratories that handle infectious samples and toxic substances, emergency response must be established in case of potential exposure accidents, spills, fires and natural disasters. In the event of an exposure accident (e.g. needle stick injuries, ingestion, aerosol exposure) while working in the laboratory, the worker must promptly contact the laboratory manager and treat the affected area immediately. In the event of a biological or chemical spill, laboratory staff should be trained to handle and clean up spills with an appropriately prepared spill kit. All accidents and spills should be promptly reported to the respective organization's Occupational Safety and Health department.

In addition, emergency contact networks and evacuation routes must be established in case of natural disasters such as earthquakes and fires. In the event of a natural disaster or fire, it is important to take appropriate actions in response to the disaster, giving top priority to ensuring personal safety. The laboratory manager must educate these emergency responses during the laboratory safety briefing.

6. Conclusion

As blood used in biodosimetry in radiation emergency medicine is an infectious biological sample, efforts must be made to protect researchers against infection in accordance with various laws and guidelines set by each country and international guideline. In addition, there are various regulations regarding the use of chemicals and the handling of liquid waste. The person in charge of the biodosimetry lab must understand these laws and guidelines to equip the laboratory and educate its users.

BSL2 laboratory

- Install a Class II biological safety cabinet.
- Install an autoclave in the building where the laboratory is located.
- Display the biohazardous signs and emergency contact information on the BSL2 laboratory entrance door.
- Only authorized personnel can enter the laboratory.
- Wear personal protective equipment.

Infectious biohazardous waste

- Pathogen spread should be inhibited.
- Any solution in contact with blood must be sterilized and treated appropriately with a disinfectant.
- Any solid waste in contact with blood must be disposed of properly.

Chemical safety and management

- Safety data sheet (SDS) of each chemical must be prepared.
- Strictly manage the use of hazardous chemicals in accordance with national regulations.
- Install a local exhaust system such as a fume hood. Chemicals emitting hazardous fumes or aerosols should be used inside the fume hood.
- It is necessary to carry out a risk assessment of all hazardous chemicals and consider measures to reduce the risks.
- Liquid chemical waste must be sorted according to regulations and disposed of properly.

Measures related to emergency response

- Ensuring personal safety is a top priority.
- In the event of a spill or an exposure accident while working in the laboratory, the worker must promptly contact the laboratory manager.
- Promptly report to the Occupational Safety and Health department set up in each organization.
- Emergency contact networks and evacuation routes must be established.

Conflict of Interest

The authors declare that they have no conflict of interests.

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