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Original article

Brief Review on Segregation and Treatment of Biomedical Waste in Medical Laboratories in light of WHO and CDC Guidelines

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ABSTRACT

The proper management of biological waste in laboratory settings is a critical aspect of biosafety in microbiological and biomedical laboratories. Beyond protecting lab workers, biological waste management safeguards public health, protects the environment, and ensures compliance with legal and regulatory frameworks. Segregation, decontamination of liquid waste, decontamination of solid waste, and techniques for inactivating toxins are all main aspects of biological waste management that will be outlined, aligned, and summarised with the main institutional biosafety protocols for biological waste management, published by major authorities, including CDC and WHO. This research found that the information from the two institutions complements each other. Therefore, this brief review aimed to compile and unify information from both institutions to construct a single, coherent text that provides a practical summary for medical laboratory professionals seeking to enhance the implementation of international, harmonized protocols that strengthen biosafety, environmental stewardship, and regulatory compliance.

Introduction

Laboratory biosafety and biosecurity activities are essential to protect the laboratory workforce and the wider community from accidental exposure or release of biological pathogens. These activities should be carried out using a risk assessment framework aligned with necessary safety instructions to ensure a safe working environment, with adequate measures in place to minimize the likelihood and severity of potential exposure to biological agents. Biosafety awareness and expertise have been greatly enhanced by the publication of the WHO Laboratory Biosafety Guidelines [1-5], and the US Centers for Disease Control and Prevention (CDC) biosafety guidelines [6-7]. Moreover, the development of new technologies, such as molecular diagnostic methods, has reduced the number of diagnostic activities that require the spread of live biological agents at high concentrations [4].

The management of a typical laboratory should include handling of waste potentially contaminated with biological or chemical agents at varying rates. Decontamination methods should be adopted after risk assessment [4]. According to the US EPA, each facility should assign a responsible person or committee to prepare an infectious waste management plan that outlines policies and procedures for managing infectious waste. This plan should include the following elements: Classification and segregation, packaging (containment), storage, transport, treatment, and disposal [8]. Treatment can precede storage and transport. As with any waste management system, strategies such as contingency planning, staff training, waste minimization, recycling, and reuse must be considered.

This review will highlight three of the most important procedures required to manage waste within microbiological and biomedical laboratories. The procedures include segregation, packaging, and treatment. These procedures will be reviewed in the light of the most important recommendations published so far by the World Health Organisation (WHO) and the U.S Centers for Disease Control (CDC) regarding these stages of waste management.

Types of Medical Waste

During medical laboratory activities, both solid and liquid waste are generated, which might be contaminated_or uncontaminated with pathogens. It is almost impossible to accurately separate them according to the state of the material (solid or liquid),_as well as the amount and type of agents causing the contamination. The most practical approach to medical waste management is to identify wastes that pose a sufficient potential risk of causing infection during handling and disposal, and for which certain precautions need to be taken.

The Environmental Protection Agency (EPA) and WHO Laboratory Biosafety Manual consider any instruments or materials in the waste that have come into contact with people or animals infected with highly infectious agents to be considered infectious waste [4,8]. However, certain laboratory medical wastes are not infectious. For example, gloves that have been worn and have not come into contact with an

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infectious agent; should they be treated in the same way as infectious waste? According to the CDC guidelines, such waste is treated as infectious waste if the country or state legislation stipulates that it is treated as such, and is defined as regulated waste [7]. Thus, all infectious waste is regulated waste, but not all regulated waste is infectious. The main types of regulated waste that may be infectious include the following:

Sharps Waste

Sharps are tools that can cause cuts or punctures, including needles, scalpels, blades, knives, glass slides, coverslips, and pipette tips. Whether contaminated or not, these items are usually considered high-risk healthcare waste and should be treated as if they were contaminated [9].

Infectious and Pathological Waste

Infectious waste refers to materials suspected of containing pathogenic microorganisms—such as bacteria, viruses, parasites, or fungi—in quantities sufficient to cause disease in susceptible individuals. This category encompasses waste contaminated with human body fluids, including blood, saliva, and other secretions, as well as items such as gloves, tubing, bandages, and swabs that have been exposed to these fluids. It also includes cultures and stocks of infectious agents generated during laboratory procedures, in addition to autopsy waste, animal carcasses, and any materials that have been inoculated, infected, or otherwise exposed to highly infectious agents.

Pathological waste constitutes a specific subcategory of infectious waste and primarily consists of anatomical materials. Although it falls under the broader classification of infectious waste, it is often managed separately due to the specialized handling, treatment, and disposal procedures required for its safe management.

Pharmaceutical waste

including genotoxic and cytotoxic drugs. Genotoxic wastes may have carcinogenic, mutagenic, or teratogenic properties. This kind of waste includes residues of certain cytostatic drugs or urine, vomit, and feces from patients treated with cytostatic drugs, radioactive material, and chemicals.

Chemical waste

Chemical waste from healthcare is considered hazardous if it is toxic or corrosive, such as acids with a pH less than 2 and bases with a pH greater than 12, flammable, reactive (explosive, water-reactive, shock-sensitive), or oxidizing. Chemical wastes that do not have any of the above characteristics, for example, sugars, amino acids, and some organic and inorganic salts, are classified as non-hazardous chemical wastes [5].

Radioactive waste

contains radionuclides with short half-lives (i.e., half of the radionuclide content decays within hours or a few days); therefore, the waste loses its radioactivity relatively quickly. Encapsulated radioactive materials with low radioactivity may be present [5].

Segregation

The simplest waste separation system involves separating all hazardous waste from general, non-hazardous waste. To provide a minimum level of safety for laboratory workers and patients, the hazardous waste category is usually divided into two categories: used sharp instruments and potentially infectious items such as culture plates, tubes, dressings, gloves, and other single-use items. Therefore, the separation of general non-hazardous waste, potentially infectious waste, and used sharp instruments into separate containers is often referred to as the "three-bin system"[5].

Other types of containers may be used for different waste categories, such as chemical and pharmaceutical waste, or for separating pathological waste, "anatomical waste", which is treated and disposed of differently from other types of waste [5]. More detailed waste separation may be required as it is stipulated by the state laws and regulations. For instance, solid radioactive waste is separated from liquid radioactive waste, infectious waste is sorted according to its level of hazard (RG1 or RG2), and broken glass may be separated into coloured and clear glass. The general waste in the laboratory may also include a separate category for paper waste to facilitate recycling, and so on [10].

According to WHO guidelines, liquid chemical waste should not be disposed of down drains. Instead, it must be stored in leak-proof containers made of strong materials, with no mixing of different solutions in the same container; each container should hold only one type of solution. A label must be placed on the container

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clearly indicating the chemical name or symbol and the type of hazard using appropriate symbols. Low-energy light bulbs (compact fluorescent lamps) contain small amounts of mercury and must be separated along with batteries and sent to a recycling company. Radioactive waste should be collected in lead-lined containers [5].

Waste containers and their colour coding system

Generally, in the laboratory, containers with a tight lid are preferred, and the lid should be opened manually or ideally with a foot pedal. Many modern waste containers are designed for automated systems that empty, wash, and mechanically sterilize their contents. Waste containers are made from a variety of materials, including cardboard, reusable plastic, and metal. Waste containers come in various shapes and sizes. Therefore, the type and amount of waste generated by the laboratory should be assessed to select the appropriate container. For instance, sharp tools should never be disposed of in cardboard boxes, which might be used for broken glassware [7]. Because sharp waste can cause injuries that increase the risk of infection, both contaminated and uncontaminated sharps must be collected in puncture-resistant, waterproof containers that are difficult to open once sealed. Sharps containers can be either single-use or designed for sterilization and reuse. Single-use containers are typically made of cardboard or molded plastic, while reusable designs are made from plastic or metal.

FDA-approved sharps disposal containers are available. However, if reusable containers are not available, strong, puncture-resistant plastic bottles, such as those used for some laundry detergents, can be used. In this case, original labels should be removed or obscured, and the bottle must be clearly marked as a sharps waste container [11], which is known as Bio-Can. In medical laboratories and microbiology labs, it is important to ensure that the plastics used for containers or bags are chlorine-free. In addition, plastic bags, which are used for disposing of biologically hazardous waste, should be leak-proof and relatively durable [5]. According to the International Organization for Standardization (ISO 7765-1, 1988), the thickness of these bags should be 70 µm [5,12]. It should also be noted that not all plastic bags can withstand the steam sterilization temperatures of up to 121ºC. Therefore, bags that are autoclavable must be chosen if sterilizing the waste inside plastic bags is planned later. Both the container and the bag should have the same colour to avoid confusion, and the colour should be appropriate for the type of waste they are designated for [5]. Colour coding makes waste sorting easier for laboratory staff to place waste in their appropriate container. This system also facilitates waste segregation during transport, storage, treatment, and disposal. Moreover, it provides a visual indicator of the potential hazard posed by the waste in that container. It is worth noting that there is no objection to having the waste segregation categories and color-coding system for waste containers determined by national legislation, as long as the same segregation system is applied nationwide [5]. In the absence of national legislation, the colour-coding system issued by the World Health Organization (as summarised in figure 1) can be used as a reference. Each container should be clearly labelled with an international hazard symbol to indicate the hazard category.

Figure 1. The diagram shows the waste containers' colour coding. This diagram has been designed based on the World Health Organization (WHO) published text [5]

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Waste containers should be placed within easy reach of laboratory staff when needed. For example, sharps containers should be placed on the workbench, while the general waste container should be placed near the handwashing sink or under the tissue dispenser. This encourages laboratory staff to place tissues in the non-infectious waste container. Infectious waste containers should not be placed in public areas, as patients and visitors may use them and come into contact with potentially infectious waste [5].
The waste-management committee is responsible for seeing that segregation rules are enforced and waste audits carried out to quantify the amount of waste being produced. In addition, segregation posters for medical and waste workers help to raise knowledge about segregation practices and improve the quality of separated waste components [5].

Waste Management
The required level of disinfection
Regulated medical waste treatment protocols may vary between countries. Fortunately, general guidelines and procedures are available. From a microbiological perspective and according to the definition of the Centers for Disease Control and Prevention (CDC), regulated medical waste is treated or disinfected to reduce the microbial load, not to make it "sterile" [7]. The WHO states more details by adopting the third level of microbial inactivation standard, which is issued by the State and Territorial Association on Alternate Treatment Technologies (STAATT). This level requires inactivation of bacteria (vegetative cells), fungi, hydrophobic/hydrophilic viruses, parasites, and mycobacteria at a rate of 6 logs or more; Whereas *Geobacillus stearothermophilus* spores and *Bacillus atrophaeus* spores should be reduced by 4 logs [5].

Methods for Treating Biological Waste
Steam sterilization using autoclaves is a common method to treat regulated medical waste. Sterilizers must be designed to operate at a pressure ranging from 1 to 2 bar or higher. The efficiency of waste treatment by autoclaves depends on an appropriate rate of temperature, pressure, and sterilization time, and this depends on several factors including the load, the types of bags or containers used and their integrity, the physical properties of the materials in the waste (such as bulk density, heat capacity, and thermal conductivity), the amount of residual air, and the moisture content in the waste. In the past, a minimum recommended temperature and exposure time was proposed, which is 121ºC for 30 minutes. However, Exposure of waste for up to 90 minutes at 121°C in a steam sterilizer (depending on the load size and type of container) might be necessary to ensure an adequate sterilization cycle.7 The Robert Koch Institute recommends treating prions, which cause Creutzfeldt-Jakob disease, using steam sterilization at 134 ºC for 60 minutes due to their high resistance [5]. After steam sterilization, the rest of the waste is safe for handling and can be discarded with all other nonhazardous solid waste [5].
Quality control tests should be conducted using waste samples that represent the actual waste produced in a healthcare facility to determine or verify the minimum conditions that are required to achieve the microbial inactivation standard. At regular intervals (typically once a month, or every 40 hours of use, or once a week, depending on consumption), biological indicators should be used for verification tests. Colour-changing chemical indicators, like strips with thermal indicators (chemical compounds that change colour when a specific temperature is reached) or integrators (indicators that respond to both time and temperature), can be used as an extra check. Each waste load can be used to record reaching the necessary temperature [5].
The second generation of steam sterilization-based systems has been developed with the aim of improving heat transfer to waste, and/or making the treatment system a continuous process (instead of a batch process). These systems combine steam processing with various types of mechanical processing, such as shredding, drying, and mixing before, during, or after steam processing [5].
Chemical disinfection is the most suitable method for treating solid and liquid waste, such as blood, microbial cultures, urine, feces and feces suspensions, and sharps. The speed and efficiency of chemical treatment depend on several factors, which include: the quantity and type of the used chemical, the amount of waste, its organic load, and other physical factors such as temperature, humidity, pH, duration, and extent of contact between the chemical disinfectant and the waste. Some differences between chemical disinfection methods used to treat the same type of waste have been noted between different institutions. For example, the treatment of tissue culture according to the biosafety manual at the University of California, San Diego (USA) is done by adding bleach to achieve a final concentration of 10%,13 while the University of New South Wales (Australia) uses a 1% bleach concentration as a treatment for tissue culture waste [14,15]. This might refer to the use of commercial bleach with different concentrations of sodium hypochlorite. Commercially available bleaching solutions typically contain 3-8% (w/v) sodium hypochlorite [16].

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The microbial sensitivity to chemical disinfectants is least in prions, followed by bacterial spores, then mycobacteria, non-enveloped and small viruses, fungi, vegetative bacterial cells (except biofilm cells), and finally enveloped and medium viruses, which are considered the most sensitive to chemical treatment [5,6]. Prions have a high resistance to a variety of physical, chemical, and gaseous methods. Fortunately, there is no epidemiological evidence linking the transmission of spongiform encephalopathy to the disposal methods of medical waste related to this disease. The most important chemicals used in biomedical waste treatment, their effective concentrations, and the type of impact were mentioned in the Biological Safety Manual for Microbiological and Biomedical Laboratories issued by the CDC [6].

Discharging Blood and Body Fluids

There is no evidence of the transmission of bloodborne pathogens through contact with sewage water (raw or treated) containing blood waste or other body fluids. The reason is attributed to the pathogens of these diseases not being resistant to environmental factors [7]. According to the CDC guidelines, the contents of containers that hold more than a few millilitres of blood remaining after laboratory procedures, suction fluids, or loose blood can be poured into sinks or toilets in healthcare facilities without treatment. The national regulations should specify a volume for these fluids that does not exceed a few millilitres, or after treatment according to the processing techniques approved by the legislator [7]. There are no specific guidelines published by WHO.

Inactivation of toxins

Risks arise during the laboratory use of biological toxins through accidental injection, absorption through the skin or mucous membranes, inhalation, or ingestion. Although most toxins can be inactivated by steam sterilization (121°C, for one hour), others cannot be inactivated by steam, such as microcystin, saxitoxin, and tetrodotoxin. These toxins can be inactivated by dry heat ranging from 260-815 ºC, or by adding sodium hydroxide in concentrations ranging from 0.1 - 0.25 N or by adding sodium hypochlorite solutions of 0.1 - 2.5% (weight/volume) [6].

Microwave processing techniques

Microwave waste treatment technology is essentially a steam-based process where treatment occurs through the action of moist heat and steam generated by microwave energy. Water contained in the waste is rapidly heated by microwave energy at a frequency of about 2450 MHz and a wavelength of 12.24 cm. In general, microwave-treatment systems consist of a treatment area or chamber into which microwave energy is directed from a microwave generator (magnetron). The types of waste commonly treated in microwave systems are identical to those treated in autoclaves [5].

Incineration

Among the options for processing pathological and anatomical waste (such as large animal carcasses) are incineration or alkaline digestion. Incineration is not suitable for waste with a high proportion of wet waste and has much lower calorific values. It is also not suitable for waste that contains large amounts of reactive chemicals or pressurized gas containers. Waste incinerators typically operate at temperatures ranging from 850 ºC to 1200 ºC. The furnaces are designed to completely incinerate this waste while keeping gas emissions within the limits set by the Environmental Protection Agency [5].

Commercial systems might combine incineration, shredding, and/or alkaline digestion. Nevertheless, any treatment technology, such as shredding, that causes the formation of aerosols from medical waste that might carry airborne pathogens should be avoided. Unless aerosols can be effectively contained and workers can be equipped with proper PPE. Overall, the Federal state and local guidelines and regulations should define the categories of medical waste subject to regulation and specify the requirements associated with their treatment and disposal.

Treatment of Sharps

The methods generally entail the following steps: using onsite mechanical needle cutters or electric needle destroyers, then shredding the treated plastic parts, followed by burying the metal pieces in sharps pits, and remelting the plastics for recycling. Alternatively, the sharps waste can be autoclaved, shredded, and then encapsulated in cement blocks that later become useful items such as hospital benches [5].

Where does waste decontamination take place (on-site or off-site)

The basic principle is to treat all contaminated materials or liquids coming out of the laboratory on-site to ensure safe handling or to package and transport them safely to another treatment site [6,7].

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Laboratory guidelines for working with infectious microorganisms at BSL1 or 2 laboratories allow for these materials to be decontaminated off-site before disposal. However, on-site decontamination by a known effective method is preferred. On the other hand, Laboratory guidelines of BSL3 & BSL4 recommend that all laboratory waste be decontaminated on-site before disposal by an approved method, preferably within the laboratory. On-site treatment is required bioterrorism strategy [6,7].

To help out making the decision about which biosafety level should be followed, the Pathogen Safety Data Sheets (PSDSs), previously titled Material Safety Data Sheets for infectious substances, provide useful data on the risk group of the microorganism and which biosafety level should be applied when it is handled in the lab [17].

In conclusion, both organizations WHO and the CDC, agree on basic aspects of waste management in biomedical laboratories. Some details that are missed to be covered by the WHO guidelines can be found in the CDC guidelines and vice versa. Importantly, the two organizations give space to local legislation to determine their own way of disposing of biological waste in a manner that is consistent with the country's capabilities and the nature of the work.

Conflict of Interest statement

There is no conflict of Interest.

Authorship confirmation/contribution statement

Hala Almshawit: Conceptualisation, formulation of research aims, data curation and analysis and writing original draft. Weam Tahar: Writing - review and editing.

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