Safe Handling of Infectious Agents

Primary and Continuous Cell Cultures

Cell cultures, in general, present few biohazards in the laboratory, as evidenced by their extremely wide usage and the rare cases of transmitted infections to laboratory personnel. Primary cell cultures initiated with tissues from infected humans or animals are recognized hazards.

Continuous cell cultures present no real documented risk in the laboratory unless they are carelessly contaminated with an infectious agent. All continuous cell lines should be regularly monitored for contamination with infectious agents, and it should be emphasized that all nutrient media or other reagents that may contain ingredients of biologic origin must be treated as though they contain potentially infectious agents.

The Eight Basic Rules of Biosafety

The most common means of exposure can be essentially eliminated as occupational hazards by following the seven basic rules of biosafety:

1. Do not mouth pipette.
2. Manipulate infectious fluids carefully to avoid spills and the production of aerosols and droplets.
3. Restrict the use of needles and syringes to those procedures for which there are no alternatives; use needles, syringes, and other "sharps" carefully to avoid self-inoculation; and dispose of "sharps" in leak- and puncture-resistant containers.
4. Use protective laboratory coats, eye protection, closed toed shoes, and gloves.
5. Wash hands following all laboratory activities, following the removal of gloves, and immediately following contact with infectious materials at the end of an operational at the end of the day.
6. Do not wear gloves outside the lab area.
7. Decontaminate work surfaces before and after use, and immediately after spills.
8. Do not eat, drink, store food, or apply cosmetics in the laboratory.

Labeling of Specimens With the Laboratory

Some form of labeling is necessary to maintain the identity of specimens in the laboratory and to ensure that the analytical results obtained are properly recorded and reported. In addition, it is the practice in many cases that special hazard warning labels be affixed to specimens that are known to be hazardous.
(e.g., specimens obtained from patients known to be infected with hepatitis B virus (HBV) or human immunodeficiency virus (HIV).

The need for such special labeling is concerned more with ethical or regulatory issues (e.g., workers' right-to-know) than with laboratory safety. All clinical material must be considered to be infectious, and must be handled with exactly the same precautions as are used for processing specimens with hazard warning labels.

Containment Equipment

Introduction

The risk of exposure of laboratory personnel can be minimized by the use of carefully selected safety equipment. A primary objective of containment is to control aerosols, but in a broader sense safety equipment should serve effectively to isolate the worker from the toxic or infectious material being processed. In many situations, however, the need is just the reverse: i.e., to protect the product or the work from contamination originating with the worker or the environment. Finally, there is often the need to protect both the worker and the product, as in handling cell cultures.

Biological Safety Cabinets

Most laboratory procedures generate aerosols that may spread infectious material in the work area and pose a risk of infection to the worker. Biological safety cabinets are used extensively to prevent the escape of aerosols or droplets and to protect materials from airborne contamination. There are three major types of this very useful safety device, referred to as Class I, Class II, and Class III. These instruments are distinct from horizontal or vertical laminar flow "clean benches," which should never be used for handling infectious, toxic, or sensitizing material.

The type used at California State University, Fullerton is the Class II biological safety cabinet, which provides protection to the worker, the environment, and the products. The airflow velocity at the face of the work opening is at least 75 linear feet per minute (lfpm), and both the supply and the exhaust air are HEPA-filtered. These cabinets are partial containment devices, which, if used in conjunction with good laboratory practices, can dramatically reduce the risk of exposure of operators to infectious aerosols and droplets.

It is emphasized that biological safety cabinets are not chemical fume hoods. Some of the air (30 to 70 percent) drawn in through the work opening of these cabinets is re-circulated within the cabinet. Accordingly, users should be aware of the possible buildup of hazardous concentrations within the cabinet if toxic, flammable, or explosive materials are used. In addition, users of Class IIA type cabinets should know that non-particulate toxic, flammable, or explosive materials are not removed by HEPA filters, and are thus discharged back into the laboratory room.

The operational efficiency of each biological safety cabinet should be specifically tested and the system certified before the instrument is placed in operation after installation, and subsequently on an annual basis. Recertification is also required if the unit is relocated or if maintenance that may affect
performance is done. Maintenance work on biological safety cabinets should be performed by trained service personnel only. In addition, cabinet users should understand the operation of the equipment, its limitations, and the proper procedures to be followed. Laboratory directors are responsible for providing such training.

**Clothing, Masks, and Face Shields**

Laboratory coats, gowns, safety glasses, face shields, masks, closed toed shoes, and gloves offer some personal protection and are often used in combination with other safety devices such as biological safety cabinets. Special laboratory clothing protects street wear from contamination. It should not be worn outside of the laboratory. Each of these items has a particular use in protecting the worker and should be used when circumstances require. Gloves are especially important when handling any potentially infectious material such as blood or other biological specimens. Safety glasses, face shields, and masks may protect mucous membranes of the eye, nose, and mouth from splash or droplet hazards during operations performed outside of a biological safety cabinet.

**Waste Handling**

The primary responsibility for the safe handling and disposal of infectious waste resides with the generator of the waste. This responsibility extends to the ultimate point of disposal even when there are other parties involved in handling the waste. No waste management program is functional unless all appropriate personnel are cognizant of the aims of the program and trained in the procedures for handling the waste. Training should be a continuing process.

Persons who generate laboratory waste are responsible for preparing the waste so that potential occupational exposures and environmental contamination are minimized. Cell culture and infectious wastes need to be segregated by the generator from all other waste streams.

**Containment and treatment**

After use, disposable Petri plates, flasks and other containment growth containers must be placed in autoclavable bags which are clearly marked with the biohazard symbol. Autoclave bags should be placed inside leak proof containers while they are being filled. When full, bags should be lightly closed with tape or a rubber band, then autoclaved. Open bags slightly or puncture the bag with several holes to allow venting of steam during autoclaving. Place waste bags in metal pans to catch any leakage that occurs during autoclaving. Autoclave on the "liquids" cycle for 45 minutes at 121 degrees C (250 degrees F) and at least 15 pounds of pressure. The autoclaved bags must then be taken directly outside to the trash dumpsters for immediate disposal.

Liquids should be treated with 0.01% to 0.05% (final concentration) bleach. Household bleach solutions are 5-7% sodium hypochlorite, so diluting them 100 fold in the liquid to be treated is sufficient to destroy most cultures. For example, add 1 ml of bleach for every 100 ml of culture. Leave the solution for at least ten minutes for maximum effectiveness. This solution can then be safely dumped in the
laboratory drain, but it must be flushed with copious amounts of water to flush the solution out of the drain trap.

Large volumes of cultures, or cultures which are pathogenic or toxic, must be inactivated by autoclaving. Place culture containers in secondary containers whenever a chance of spillage may occur. Autoclave on the "liquids" cycle at the parameters described above.

**DO NOT LET FULL BAGS (AUTOCLAVED OR NOT) OR OLD CULTURES SIT AROUND. TAKE CARE OF THEM IMMEDIATELY AND DISPOSE OF THEM IN THE OUTSIDE DUMPSTERS!**

If you have any questions about cell cultures, or any other infectious agents, please contact the Environmental Health & Safety Office in T-1475 OR CALL X4346(SAFE).

*Most of this information is taken directly from Biosafety in the Laboratory, National Academy Press, Washington, DC. 1989.*