

CHAPTER III: GENERAL BIOSAFETY PRINCIPLES

A. RISK ASSESSMENT

To apply biological safety principles rationally while handling a potential pathogen, one must perform a risk assessment, which considers:

1. The biological and physical hazard characteristics of the agent,
2. The sources likely to harbor the agent,
3. Host susceptibility,
4. The procedures that may disseminate the agent, and
5. The best method to effectively inactivate the agent.

Globally, numerous government agencies have classified microorganisms pathogenic for humans into risk groups (RG) based on the transmissibility, invasiveness, virulence or disease-causing capability, lethality of the specific pathogen, and the availability of vaccines or therapeutic interventions. Risk groupings of infectious agents usually correspond to biosafety levels (BL or BSL), which describe recommended containment practices, safety equipment, and facility design features necessary to safely handle these pathogenic microorganisms. The list of pathogenic microorganisms includes bacteria, viruses, fungi, parasites, and other infectious entities. The scheme ascends in order of increasing hazard from Risk Group 1 (RG1) agents, which are nonpathogenic for healthy human adults, to RG4 agents, which display a high morbidity and mortality and for which treatments are not generally available.

The risk group listing of the NIH *Guidelines* is an accepted standard and can be accessed electronically at:

<http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>.

The American Biological Safety Association also provides a comprehensive risk group listing and references international agencies. This list is accessible at:

<http://www.absa.org/riskgroups/index.html>.

Another reliable source of information about human pathogens is available from pathogen safety data sheets posted by Health Canada:

<http://www.phac-aspc.gc.ca/msds-ftss/>.

Microorganisms that are RG1 require standard laboratory facilities and microbiological practices, whereas those in RG4 require maximum containment facilities. Many of the agents likely to be handled experimentally at the University of Chicago are RG2 or RG3 pathogens, designated as moderate and high hazard, respectively. These agents typically

require more sophisticated engineering controls (e.g., facilities and equipment) than standard laboratories, as well as special handling and decontamination procedures.

Risk Group 1 agents are not associated with disease in healthy adult humans. Examples: *E. coli* K-12, *Saccharomyces cerevisiae*.

Risk Group 2 agents are associated with human disease that is rarely serious, and for which preventive or therapeutic interventions are *often* available. Examples: *E. coli* O157:H7, *Salmonella*, *Cryptosporidium*

Risk Group 3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may* be available (high individual risk but low community risk). Examples: *Yersinia pestis*, *Brucella abortus*, *Mycobacterium tuberculosis*.

Risk Group 4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk). Examples: Ebola virus, Macacine herpesvirus (formerly Cercopithecine herpesvirus 1, also called Herpes B or Monkey B virus).

Microorganisms classified as RG2 or higher have been reported to cause infection and disease in otherwise healthy adults. Many RG2 agents have been associated with laboratory-acquired infections. The progression from invasion to infection to disease following contact with an infectious agent depends upon the route of transmission, inoculum, invasive characteristics of the agent, and resistance of the person exposed (whether innate or acquired). Not all contacts result in infection and even fewer develop into clinical disease. Even when disease occurs, severity can vary considerably. It is important to assume virulence and handle such agents at the prescribed biosafety level.

B. ROUTES OF INFECTION

Depending on the organism in question, pathogens are transmitted via several possible routes of infection. The most common routes of infection are inhalation of infectious aerosols, exposure of mucous membranes to infectious droplets, ingestion from contaminated hands or utensils, or percutaneous inoculation (injection, incision, or animal bite). Appropriate precautions should be implemented to reduce the risk of such exposures.

C. EXPOSURE SOURCES

1. CLINICAL AND PATHOLOGICAL SPECIMENS

Any specimen from human patients or animals may contain infectious agents. Specimens most likely to harbor such microorganisms include blood, sputum, urine, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, feces, and tissues. Personnel in

laboratories and clinical areas handling human blood, body fluids, non-human primate material, or even human cell lines that have been screened for pathogens should practice *universal precautions*, an approach to infection control wherein all human blood and certain human body fluids are treated as if known to be infectious for Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), Hepatitis C (HCV) and other bloodborne pathogens. Such personnel are required by Federal law (OSHA 29 CFR 1910.1030) to undergo bloodborne pathogen (BBP) training. At the University of Chicago, this training requirement can be satisfied either online or by attending an in-person training session. For information on obtaining this training, go to https://training.uchicago.edu/course_detail.cfm?course_id=32.

Animals may harbor endogenous pathogens that are virulent for humans. For personnel handling these animals or their tissues/body fluids, we recommend an analogous approach to infection control, *universal precaution*, which assumes these animals and their blood and body fluids to be infectious.

2. CULTURES

BSL-2 practices should be used for cell lines of human origin, even well established lines such as HeLa and HEK293, and for all human clinical material (e.g., tissues and fluids obtained from surgery or autopsy). Non-human primate cell cultures derived from lymphoid or tumor tissue, cell lines exposed to or transformed by a non-human primate oncogenic virus, and all non-human primate tissue should also be handled at BSL-2. When a cell culture is inoculated with (or known to contain) an etiologic agent with higher biosafety level, it should be classified and handled at the same biosafety level as the agent.

When manipulations of these types of cell cultures present a potential to create aerosols, use a biological safety cabinet. Do not use a clean bench as it will not protect you from potential pathogens. Conversely, a fume hood will protect you but will not protect your sample from contaminants in the ambient air. A disambiguation of biological safety cabinets, clean benches, and fume hoods is provided below.

Accidental spilling of infectious liquid cultures is an obvious hazard due to the generation of aerosols and/or small droplets. However, even routine manipulations of cultures may release microorganisms via aerosol formation:

EXAMPLE OF PROCEDURES THAT GENERATE AEROSOLS:

- Popping stoppers from culture vessels.
- Opening closed vessels after vigorous shaking.
- Spattering from flame-sterilized utensils.
- Expelling the final drop from a pipette.

- Spinning microfuge tubes in a standard microfuge.
- Vortexing liquid samples.

WHAT TO DO TO LIMIT AEROSOLS GENERATION/DISSEMINATION:

- Manipulate cultures of infectious material carefully to avoid the uncontrolled release of aerosols or the generation of large droplets or spills.
- Centrifuge cultures using gasket-sealable tubes, carriers, and rotors, when available.
- Seal microplate lids with tape or replace them with adhesive-backed Mylar film.
- When vortexing infectious samples, ensure there is a tight seal.
- Load, remove, and open tubes, plates, and rotors within a biological safety cabinet or fume hood. Keep in mind that the fume hood will protect you from your sample but will not protect your sample from potential contamination from room air.

When preparing aliquots of infectious material for long-term storage, consider that lyophilization of viable cultures may release high concentrations of dispersed particles if ampules are not properly sealed. Breakage of ampules in liquid nitrogen freezers may also present hazards because of survival of pathogens in the liquid phase.

Considerations for shared/core facilities:

Equipment used for manipulations of infectious materials, such as cell sorters and automated harvesting equipment, must be evaluated to determine the need for secondary containment and to consider decontamination issues. Costly equipment of this type is often operated at multi-user or core facilities; the inherent variability in risk from one project to another makes it imperative that operators and users of these facilities understand risks and methods for risk mitigation.

3. ANIMALS

Exercise care and thoughtfulness when using animals to isolate and propagate microorganisms, study pathology, or produce antibodies. Laboratory animals may harbor microorganisms that can produce human diseases following bites, scratches, or exposure to excreted material. In the process of inoculating animals, an investigator can be exposed to infectious material by accidental self-inoculation or inhalation of infectious aerosols. During surgical procedures, necropsies, and processing of tissues, aerosols can be produced unintentionally, or the operator can inflict self-injury with contaminated instruments. Since animal excreta can also be a source of infectious microorganisms, investigators should take precautions to minimize aerosols when changing bedding and cleaning cages. The Animal Resources Center (ARC) offers required training for any personnel working with

animals. For information on obtaining this training, contact the ARC at <https://animalresources.uchicago.edu/>.

D. LABORATORY EXPOSURE POTENTIAL

1. TEACHING LABORATORIES

Whenever possible, we recommend the use of avirulent strains of infectious microorganisms in teaching laboratories. However, even attenuated microbes should be handled with care. Students should be cautioned against and trained to prevent unnecessary exposure, as exposure to “avirulent” strains may be problematic in immunocompromised individuals. Establishment of safety consciousness is integral to the conduct of good science.

2. RESEARCH LABORATORIES

The risk of exposure increases with experiments in research laboratories using high concentrations or large quantities of pathogens. The use of animals in research on infectious diseases also presents greater opportunities for exposure.

3. CLINICAL LABORATORIES

Personnel in laboratories performing diagnostic work-up of clinical specimens from humans or animals are often at risk of exposure to infectious agents. The absence of an infectious disease diagnosis does not preclude the presence of pathogens. This is especially true of materials from patients who have received immunosuppressive therapy since such treatment may activate latent infections or make hosts more likely to harbor infectious agents.

E. HEALTH STATUS

Some unusual circumstances warrant special considerations or measures to prevent infection of laboratory personnel by certain microorganisms.

Regardless of the risk group of the organism you work with, it is good practice to inform your personal physician about your occupational risks, especially work with biohazardous or potentially biohazardous agents, so he or she may have a record of this information. Certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can include, but are not limited to: diabetes or other metabolism disorders, pregnancy, certain autoimmune diseases, immunodeficiency or immunosuppression, animal-related allergies, chronic skin conditions or respiratory disorders, and steroid therapy, even if only temporary.