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Biological Safety Manual

2.1 Introduction

The purpose of this manual is to serve as a resource for researchers and staff and to support and encourage their activities in a manner that:

- Protects all University personnel and visitors from laboratory-acquired infections;
- Maintains the security and integrity of specimens and other research materials;
- Provides environmental protection to minimize risks to those outside the laboratory and beyond the confines of the campus; and,
- Ensures compliance with existing Federal, State, and City health, safety, and environmental regulations and guidelines.

No single document can address every contingency. When additional activity- or agent-specific information is required, EH&S will assist investigators in developing and implementing appropriate practices to minimize the risk of laboratory infection or environmental contamination. When indicated, the expertise of the Institutional Biosafety Committee or other resources may be called upon for additional input. **Principal Investigators are responsible** for seeking out these and other resources. They must also ensure that **all personnel** under their supervision are appropriately trained, informed of applicable regulations and guidelines and that they are capable, based on academic background and hands-on experience, of working within these regulations and guidelines.

Biological Safety is a dynamic field; researchers use organisms that were not even identified a few years ago and the technology (and the nature of the hazards) associated with them is continually evolving. Between periodic revisions of this manual, EH&S will continue to communicate pertinent biosafety information to the research community via e-mail, newsletters and other media, and during periodic laboratory surveys.

2.2 Biosafety Levels and Risk Assessment

Risk is the probability that harm, injury, or, in the context of this document, disease will occur. The foundation of any safety program is the use of control measures appropriate for the risk posed by the activities and the agents in use. To characterize their risk, microorganisms and clinical materials are assigned to one of four Biosafety Levels (BSLs). For each BSL there is a unique set of safety equipment, facility design features, and practices that will reduce the risk of laboratory-acquired infections.

A complete description of work practices, safety equipment, and facility design features for BSL-1 through BSL-4 is available in the CDC/NIH publication **Biosafety in Microbiological and Biomedical Laboratories (BMBL 5) 5th Edition**, specifically, **Section IV.** The following excerpts should be considered as general summaries and users are encouraged to review the more comprehensive information on the aforementioned web sites. The NIH's Guidelines for Research Involving Recombinant DNA Molecules provide additional information along these lines as well

as guidance for risk assessment of microorganisms and materials containing recombinant DNA, which may increase or decrease the risk of the activities.

2.2.1 Biosafety Level 1 (BSL-1)

- Agents: defined and characterized strains of microorganisms not known to consistently cause disease in healthy adults e.g., *B. subtilis*, *S. cerevesiae*, non-pathogenic *E. coli*. Includes recombinant DNA activities using such non-pathogenic organisms as hosts for the expression of genes incorporated into bacterial plasmids or low risk viral vectors such as baculovirus or Adeno Associated Virus.
- Work practices: standard microbiological practices/aseptic technique.
- Safety equipment: none required-gloves, lab coats and eye protection recommended.
- Facilities: bench top sink available for hand washing.

2.2.2 Biosafety Level 2 (BSL-2)

- Agents: associated with human diseases of varying severity, e.g., Hepatitis B and C, HIV, *S. typhi*, human retroviruses, *S. aureus*. Includes recombinant DNA activities using viral vector systems such as Adenoviruses and some Retroviral vectors, particularly Lentiviral vectors, and expression of recombinant DNA in BSL-2 organisms.
- Transmission: inoculation and other percutaneous injuries, ingestion, mucous membrane exposure
- Work practices: BSL-1 practices, with the addition of: limited access, 'Biohazard' signs, 'sharps' precautions, defined procedures for Regulated Medical Waste (RMW) disposal and medical surveillance (as needed).
- Safety equipment: Class I or II Biological Safety Cabinet (BSC) or equivalent containment for manipulations with potential for aerosolization or splashing; lab coats, gloves, eye/face protection.
- Facilities: BSL-1 facilities, with the addition of: available autoclave, directional airflow, no air recirculation, disinfection/decontamination procedures in place.

2.2.3 Biosafety Level 3 (BSL-3)

- Agents: serious or lethal diseases transmissible via aerosols, e.g., *M. tuberculosis*, SARS. Recombinant DNA activities using genetic material from BSL-3 organisms or such organisms as host cells.
- Transmission: aerosol inhalation, inoculation and other percutaneous injuries, ingestion, mucous membrane exposure
- Work practices: BSL-2 practices, with the addition of: controlled access, on-site decontamination of all waste and lab clothing, medical surveillance.
- Safety equipment: Class I or II Biological Safety Cabinet (BSC) or equivalent containment for all open manipulations of agents; lab coats, gloves, eye/face, and respiratory protection (as needed).
- Facilities: BSL-2 facilities, with the addition of: physical separation from access corridors, double-door entry, directional air flow into lab, no recirculation of exhaust air, back-up ventilation and filtration systems, in-lab autoclave.

2.2.4 Biosafety Level 4 (BSL-4)

Organisms in this category are of such extremely high risk that only a handful of laboratories nationwide work at this level. No such facilities exist at the University.

2.2.5 Agent Summary Statements

Agent Summary Statements, including recommended BSL for many laboratory microorganisms, are included in BMBL 5.

Other resources for biosafety hazard classification are noted on the American Biological Safety Association (ABSA) page, Risk Group Classification for Infectious Agents.

2.2.6 Risk Assessment

Biosafety Level classifications are appropriate for typical laboratory operations. The Principal Investigator or laboratory director is responsible for implementing more (or less) stringent practices based on laboratory specific conditions. Such a decision is ultimately the result a risk assessment process that accounts for the following:

- Pathogenicity - the ability of an organism to cause disease.
- Virulence - the severity of disease.
- Transmission route - parenteral, ingestion, mucous membrane exposure, or inhalation. The latter route is of the greatest concern which is why organisms such as M. tuberculosis require more stringent control than organisms that are transmitted via direct contact, e.g., HBV.
- Agent stability - survival in environment or otherwise prolonged viability (spore formation).
- Infectious dose - the dose required to cause infection in humans or animals (ID 50 refers to the dose needed to infect 50% of the exposed population).
- Antibiotic resistance.

The use of recombinant DNA may alter any of the above risk factors and investigators should take these modifications into consideration when working with recombinant microorganisms.

All of the above factors are inherent to a particular microbe; external factors to be considered in a risk assessment include:

- Titer/volume of material used - titer may increase several orders of magnitude compared to levels in clinical samples, upon culturing.
- Availability of effective treatment or vaccine.
- Nature of activities - e.g., potential for splashes, volume used, complexity of manipulations, skills and training level of investigators.
- Health status of investigator - e.g., immune state, pregnancy, vaccination status.

2.3 Engineering Controls

Engineering controls are devices and equipment that isolate and contain a hazard. The best engineering controls function with a minimum of user input and may, to a degree, compensate for human error.

2.3.1 Biological Safety Cabinets (BSCs)

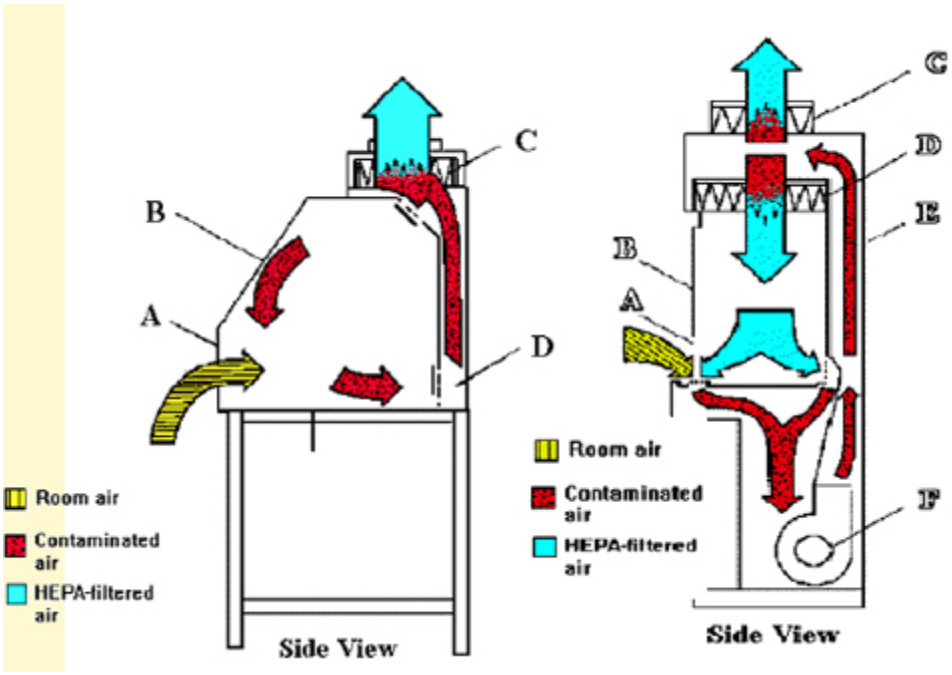
Biosafety cabinets are the primary engineering control for the minimization of exposure to potentially infectious materials. BSCs combine directional air flow and high efficiency particulate air (HEPA) filters to protect researchers and the environment from aerosolized microorganisms. Air enters the cabinet through the face (where the investigator sits), preventing contaminants generated at the work surface from entering the laboratory. Air discharged from the cabinet first passes through a HEPA filter, removing 99.97% of particles with an aerodynamic diameter of 0.3 microns; smaller or larger particles are removed with greater efficiency. Most BSCs also protect materials used within them from contamination. **All open manipulation of organisms requiring BSL-3 containment and activities with a BSL-2 organism having potential for splashes or aerosol generation, must be performed in a BSC or similar type of containment device**.

2.3.1.1 Class I BSC

Room air enters at face (A), circulates within the work space, and exits through the HEPA filter (C) after passing through rear plenum (D). Class I cabinets closely resemble the earliest manufactured BSCs; they are infrequently used for modern research activities, because while protecting the investigator and the immediate environment, they do not protect research materials from environmental contamination.

2.3.1.2 Class II, type A1 BSC

Room air (A) is drawn through the supply grille at the front of the work surface and passed through fan (F), entering the rear plenum, (D). Portions of airstream pass through exhaust filter (C) or supply filter (E). Only HEPA-filtered air contacts the work area, providing protection from environmental contamination of research materials. Class II, type A1 BSCs are the most common type of BSC used in Columbia research facilities.



Please note, Class I and Class II, cabinets exhaust filtered air **back into the laboratory**. Because HEPA filters do not capture gases or vapors, **volatile, toxic chemicals must not be used in these BSCs**. Limited quantities of these materials may be used in Class II, type B cabinets, which discharge into building exhaust systems.

2.3.1.3 Class III BSC

Often referred to as 'glove boxes,' these are totally enclosed, gas-tight cabinets designed for work with the highest risk pathogens. Exhaust from Class III cabinets is filtered before being discharged through dedicated ventilation systems.

2.3.1.4 Clean benches

Some laboratories have Clean Air Benches, devices that may be confused with BSCs because of their physical similarities. Clean Air Benches draw air through a filter and direct a filtered airstream, (and any contaminants, if present) **from** the inside of the work space **into** the laboratory- a pattern just the opposite of a BSC. They are designed for handling sterile materials or when a dust-free environment is needed. They **are not** safety devices and should never be used for handling infectious materials.

2.3.1.5 Procedures for Effective Use of BSCs

Appropriate user protection and contamination prevention provided by a BSC is directly related to the activities of the operator. Below are the steps to ensure that the BSC functions effectively.

- Cabinets **must be** certified under the following conditions:
    - Annually

    - Following relocation (including within-room). BSC on castors may be moved carefully without subsequent recertification.
    - Following HEPA filter change
    - Following service that may have affected containment ability.
    - Semi-annual certification is recommended when cabinets are used for work with airborne-transmitted organisms or other high risk agents, e.g. *M. tuberculosis.*
    - If the airflow, indicated by magnehelic gauges fall out of an established range.
- A list of qualified vendors can be found on EH&S's web site.
- To maintain proper directional airflow, do not block the front air intake or the rear exhaust grille and minimize the amount of material kept inside the cabinet.
- Heat from a Bunsen burner may damage HEPA filters and disrupt the protective airflow pattern. The use of disposable inoculating supplies combined with the sterile atmosphere of the BSC, should eliminate the need for heat decontamination throughout the procedure. When heat sterilization is necessary, use a microincinerator - a small oven that eliminates the need for an open flame. Microincinerators are available from lab supply vendors, e.g. VWR.
- Work 4-6 inches from the front of the cabinet, over the tray and not over the grille; avoid rapid arm movements that can disrupt airflow.
- In order to minimize arm movement in and out of the cabinet, place all needed materials in BSC at the start of procedures, arranging them to so that 'dirty' items do not pass over 'clean' ones. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials
- Allow cabinet fan to run 5 minutes prior to and at the completion of work; wipe interior with 70% ethanol before and after work.
- Locate BSCs in low-traffic areas away from air supply grilles and doorways; drafts may disrupt protective air flow.
- Many BSCs are equipped with UV lights, but routine disinfection of work surfaces is more critical in ensuring a contaminant-free work area, and relying heavily upon the disinfection activity of the UV light is not recommended. Turn off UV lights when the cabinet is in use. UV lights should be wiped with an alcohol-moistened cloth weekly; a dust covered bulb is ineffective. Bulbs must be disposed using EH&S's hazardous waste program.
- Close the room door when working in a BSC, particularly if it is close to a laboratory door.
- Most BSCs have a removable work surface tray and front grille, and the space beneath it requires regular cleaning to avoid contamination problems. A schedule for regular removal of the work surface tray and disinfection of the space beneath with 10% bleach followed by 70% ethanol is recommended. The drain valve under the work surface can facilitate cleaning.

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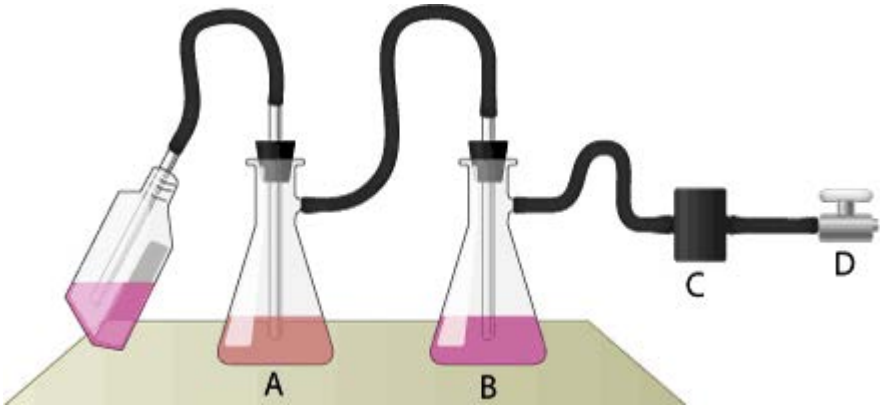
2.3.2 Vacuum Line HEPA Filters

Vacuum lines require periodic maintenance by University personnel and it is vital to ensure that exposures to research materials are prevented. All vacuum lines, both inside BSCs and on bench tops must be protected with a HEPA filter and a disinfectant-filled collection flask. See the following diagram for setup instructions.

The left suction flask (A) is used to collect contaminated fluids into a suitable decontamination solution; the right flask (B), serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms. Use flask(s) large enough to collect a day's worth of aspirate, nothing larger. Compression of the Tygon tubing may indicate that the filter requires replacement. Keep flasks in the BSC, not on the floor, to avoid accidental breakage or spilling. If flasks must be kept on the floor, use secondary containment such as a plastic bucket. Filters may be obtained through VWR, Fisher, and other lab supply companies. Empty flasks daily, providing fresh disinfectant each day, to reduce the likelihood of contamination problems.



The left suction flask (A) is used to collect contaminated fluids into a suitable decontamination solution; the right flask (B), serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms. **Use flask(s) large enough to collect a day's worth of aspirate, nothing larger. Keep flasks in the BSC, not on the floor, to avoid accidental breakage or spilling. Filters may be obtained through VWR, Fisher, and other lab supply companies. Empty flasks daily**.

2.3.3 Sharps Containers and Safe Needle Devices

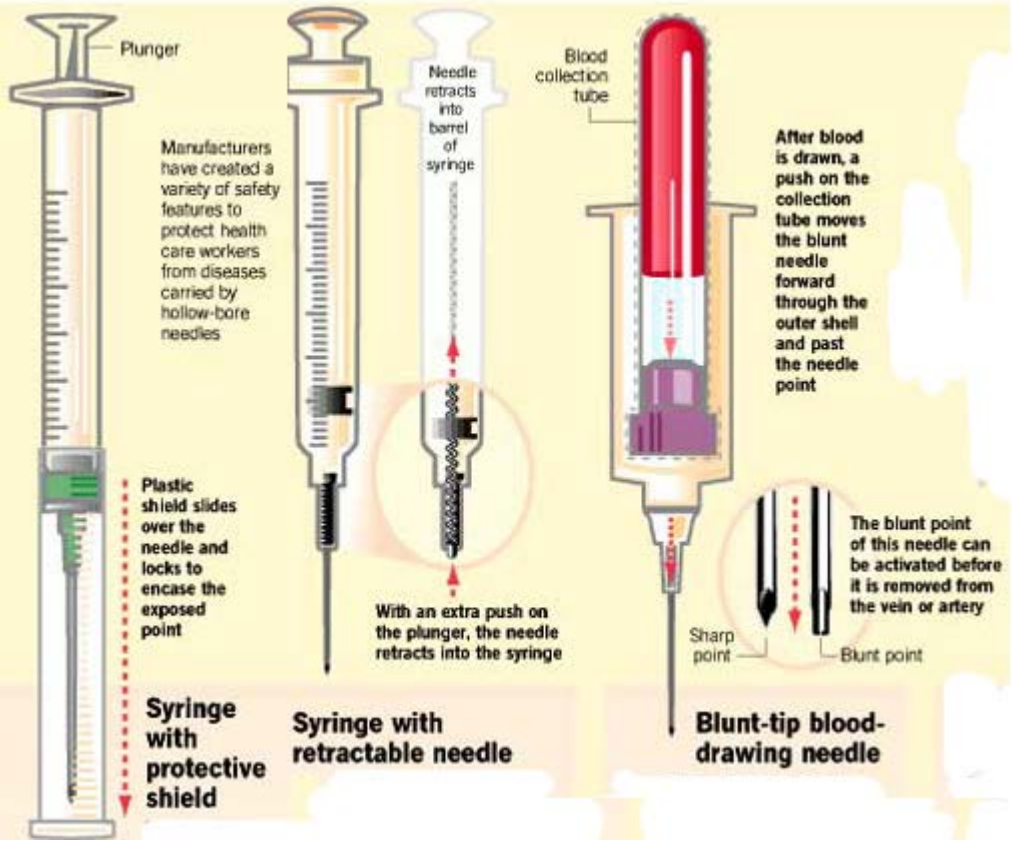
2.3.3.1 Sharps Containers

Needles, razor and scalpel blades, Pasteur pipettes, serological pipettes, micropipette tips and similar items must be discarded as Regulated Medical Waste, including organisms and materials containing recombinant DNA in puncture-resistant sharps containers. Many 'sticks' and cuts are caused by improperly disposed sharp items or sharps that were left 'lying around'; keep a sharps container as close as possible to where these items are used, if possible within arm's reach.

Glass items (pipettes, test tubes) should be substituted whenever possible by plastic ones. The use of needles, blades and other sharp objects should be limited to those situations where no other alternative exists.

2.3.3.2 Safe Needle Devices

These include 'needleless systems' and sharps incorporating automatic protection features. They allow for the elimination of exposure to or automatic shielding of needles during use, minimizing the risk of 'sticks' and cuts. While mostly applicable to clinical settings, they must be incorporated whenever there is the risk of exposure to materials containing recombinant DNA, human blood, body fluids, cells, unfixed tissue or any other material covered by OSHA's Bloodborne Pathogens Standard.



2.3.4 Centrifuge Safety

Centrifuge accidents may release large volumes of infectious, aerosolized material.

- Keep accurate rotor use logs; decommission rotors as per manufacturers' recommendations.
- Inspect rotors, particularly the chambers, for corrosion and pitting.
- Use "safety cups" or covers (gasketted containers into which tubes are placed during centrifugation). If a tube breaks, the material will be contained. **An example of a centrifuge safety cup is illustrated on the next page.** These safety devices can be obtained from the manufacturer. Check with personnel in your laboratory to confirm that they have already been obtained for your lab's centrifuge(s).
- If a safety cup is unavailable, be sure the rotor cover or chamber lid is tightly closed - never use an uncovered rotor.
- For infectious materials or materials containing recombinant DNA, fill tubes and load/unload rotors or safety cups inside a BSC.

If a tube breaks during centrifugation:

- Allow aerosols to settle for 15 minutes before opening the chamber.
- Don personal protective equipment as described in Section 2.8, Spill Procedures (below).
- Use a squeeze bottle to carefully apply disinfectant solution to contaminated surface, taking care to minimize splashing.

- Allow 20 minutes contact time, remove buckets and rotors to nearest BSC, aspirate residual disinfectant, and wipe down surfaces with clean water.
- Place debris in sharps containers or red bags.
- Follow manufacturers' instruction for selection of disinfectants for use on rotors and buckets. These items are usually corrosion-sensitive.

2.4 Work Practices

Principal Investigators are responsible for ascertaining that their staff are appropriately trained to carry out their assigned laboratory functions, but ultimately individuals bear primary responsibility for their own safety and health. Never hesitate to inquire of senior lab staff if there is an activity that you are not sure you can perform safely. You may also contact EH&S in such a situation.

2.4.1 Basic Precautions

- All laboratories must have a door sign that states the name, and phone number of the PI, emergency contact number(s), any entry restrictions, and for labs working at BSL-2 or -3, the universal Biohazards symbol. These signs are provided by EH&S.
- Keep laboratory doors closed when working with BSL-2 or BSL-3 organisms.
- Drinking, chewing gum, applying cosmetics, or handling contact lenses in work areas is strictly prohibited, as is the storage of food or beverages in refrigerators/freezers used for research materials.

- Cover work surfaces with 'bench-kote' or other absorbent; use disinfectant-soaked towels for work with highly infectious material or when splashing/spattering is anticipated.
- Decontaminate work surfaces at the end of procedures and immediately after a spill. Limit bench-top items to those in immediate use; cluttered areas are more likely than well-maintained spaces to be the sites of accidents and are harder to clean and disinfect.
- After use, place reusable sharps such as surgical instruments, in puncture-resistant containers with disinfectant solution and labeled with the biohazard symbol. Detailed protocols must be developed for handling, cleaning, disinfecting and/or sterilizing reusable sharps. See Segregation and Disposal of Regulated Medical Waste.
- Minimize splashing and aerosol generation. When pipetting, expel liquids against the side-wall of a tube rather than against the tube bottom. If aerosols of infectious materials will be generated, work in a BSC.
- Use secondary containers (trays, specimen transport bags) for the prolonged storage or transport of infectious materials. Whenever possible, replace glass lab ware with plastic; glass Pasteur pipettes are particularly prone to breakage.
- Never pipette by mouth.
- Use only mechanical pipetting devices and cotton-plugged pipettes; do not expel air through a pipette to mix suspensions containing infectious or toxic materials.

2.4.2 Fire Prevention and Biological Safety

Efforts to eliminate contamination, as practiced in some settings, run counter to basic fire prevention principals. This may occur in two types of activities:

- Dipping cover slips, cell spreaders and other items for which contamination-free status is required, into alcohol followed by flaming with a Bunsen burner. This may result in a fire when ignited alcohol drips onto flammable material.
    - Use disposable cell spreaders, which may be collected and re-sterilized by autoclaving for subsequent use.
    - Instead of dipping and flaming cover slips, autoclave batches in a glass petri dish. If kept covered in a biological safety cabinet, the slips will remain sterile.
- The use of burners in a biological safety cabinet to flame pipets, bottle and tube necks and inoculating loops needlessly places an ignition source in the work area. The environment inside the cabinet is microbiologically sterile. Using wrapped sterile pipets in this environment eliminates the need to flame. Single use inoculating loops are available and like cell spreaders may be collected and re-sterilized by autoclaving for subsequent use. Opening and closing of tubes and bottles inside the cabinet eliminates the need for flaming the necks of these containers.

If you must use a flame or heat either purchase a model that comes with a low-flame pilot light or a micro-incinerator that provides a heat source without an open flame.

2.4.3 Pipetting and Repetitive Stress Injuries

Repetitive pipetting, particularly with multi-channel devices may result in harmful stress on the arms, wrists, or shoulders. Some of this stress can be eliminated by using devices designed with

ergonomic considerations. Ask your supplier about such models or contact EH&S to obtain product information. To reduce the risk of repetitive stress injuries:

- Rotate pipetting tasks among several people.
- Take short pauses of a few seconds when you can't take a longer break.
- Choose pipetters requiring the least pressure; use only the force necessary to operate it.
- Work with arms close to the body to reduce strain on shoulders.
- Keep head and shoulders in a neutral position (bent forward no more than 30 degrees).
- Use adjustable chairs or stools; high stools will force you to work with a bent neck.
- Don't elevate your arm for lengthy periods without support.

Frequent pipetting requiring thumb action to expel liquids may cause inflammation of the tendon or sheath used for this motion; use pipettes that expel liquids with a "clenching-the-fist" motion.

2.5 Personal Protective Equipment

The appropriate use of personnel protective equipment (PPE) is critical in reducing exposure to potentially infectious materials. PPE use must be put into its proper context, however. PPE is to be considered the 'last line of defense' when risk assessment does not indicate that engineering controls and work practices can be relied upon for adequate protection. These situations frequently exist, necessitating the use of PPE.

2.5.1 Gloves

Gloves must be worn whenever handling infectious materials. Users of latex gloves are at risk for developing allergies to latex or the chemicals used in manufacturing these gloves. Nitrile or vinyl gloves should be used instead of latex. Those who prefer latex should use only powder-free gloves that are designated "low protein" by the manufacturer. Glove manufacturers should be able to document their products' resistance to permeation.

Corrosives and organic solvents may penetrate gloves or diminish their protective ability; it may be necessary to stock more than one type of glove for the full range of a laboratory's activities. Glove compatibility information is available from glove manufacturers, or consult the EH&S website.

When using any glove:

- Check for visible tears and other defects.
- Remove rings and other jewelry if they may rip gloves.
- Protective ability diminishes as gloves are worn due to stretching and abrasion; change gloves regularly or as soon as possible if they are overtly contaminated.
- Wash hands immediately after removing gloves.
- Remove gloves when leaving the laboratory; even if they are "clean", their presence in an elevator or other common area justifiably causes misgivings among other building occupants - they do not want to turn the same door knob. (Proper decontamination of the

exterior surfaces of containers used to transport infectious materials is required and will eliminate the perceived need to wear gloves during transport on campus.)

2.5.2 Eye Protection

Eye injuries are among the most preventable types of laboratory accidents. Glasses routinely worn for vision correction do not provide the appropriate level of protection for work with hazardous materials.

- Safety glasses with side shields provide the minimum level of protection for handling any hazardous material.
- Goggles, which unlike safety glasses fit tightly all around the eyes are required for activities with a small splash hazard or work with organisms transmissible through mucous membrane exposure.
- Goggles are used with a face shield when an elevated risk of large quantity splashes exists or when working with highly toxic, corrosive, or infectious materials. Face shields must also be used for protection against UV radiation (be sure that the face shield carries the manufacturer's validation of UV protection) and when handling liquid nitrogen.

2.5.3 Lab Coats

Lab coats must not be worn outside of the laboratory if they were used during work with infectious materials. Wear coats that are resistant to liquid penetration for activities with splash potential or use a plasticized apron. For high risk activities, use a rear-fastening lab coat. Provision, laundering, and replacement of lab coats is the responsibility of the Principal Investigator, or Department; employees must not launder contaminated lab coats at their home.

2.5.4 Surgical Masks

Masks will help prevent ingestion and protect the mucous membranes of the nose and mouth. They **do not** provide sufficient protection against infection from organisms transmitted by inhalation, e.g., *M. tuberculosis.*

2.5.5 Respirators

Respirators are used when there is the risk of airborne exposure to organisms transmitted by inhalation and containment devices are unavailable or unable to provide sufficient protection. Respirators use must be preceded by medical clearance, training, and fit testing. These services must be arranged through EH&S.

2.6 Laboratory Equipment

In additional to electrical and mechanical considerations, laboratory equipment poses hazards related to the materials used in them. Some equipment may be one-of-a-kind with each requiring

its own learning curve before it can be safely used. Some equipment becomes obsolete relatively quickly and with each new piece, comes the need to relearn some operational aspect.

Be sure that 'owners' manuals' are readily accessible and when in doubt, contact a customer service representative. Do-it-yourself fixes are not only dangerous but may invalidate warranties. Senior lab personnel should be responsible for ensuring that new staff are familiar with the safe operation of equipment.

There also may be specific requirements for moving sophisticating machinery in which case a customer service official should be contacted or the users' manual carefully reviewed.

2.6.1 Water Baths

Water baths may become contaminated by organisms incubated in them or through amplification of water or airborne organisms. Iodine-based or phenolic disinfectants are recommended for intermediate temperature baths. A 1/1,000 dilution of household bleach is also effective but may corrode water bath components. It has been reported that placing a few pennies (copper) in the bath will inhibit microbial growth. **Never** use sodium azide; it is fairly toxic and drain disposal is illegal and may result in the formation of explosive metal azides. Consult the manufacturer to determine the recommended disinfectant. **Do not leave water baths on overnight or when they will be unattended for an extended period of time**.

2.6.2 Cryostats

Cryostats should be regularly decontaminated with a tuberculocidal hospital disinfectant (see Decontamination, section 2.7). Trimmings and tissue sections should be treated as potentially infectious. Never attempt to clear debris from a blade with your finger; always use a brush or other mechanical device to prevent contact with the blade. When changing blades use protective gloves and handle the blades with forceps or tongs. Pre-soaking blades in a disinfectant solution prior to cleaning (removal of debris) will reduce the number of viable microorganisms.

2.6.3 Mixers, Sonicators, and Blenders

Mixers, sonicators, and blenders produce large quantities of aerosols. Models designed to contain aerosols are available. These devices should be operated within a BSC with a disinfectant-moistened towel placed over the top. Open only after allowing time for aerosols to settle. If possible, avoid using glass bowls. Sonication may be safely performed by placing a tightly capped specimen tube in a beaker of water and putting the probe in the **water**, not in the tube.

2.6.4 Needles and Syringes

Sharps should only be used when no other alternative is available.

- Use blunt needles, pipettes, or canulas to aspirate fluids instead of hypodermic needles; substitute plastic for glass when possible.

- Use only needle-locking units or units in which the needle is an integral part of the syringe.
- Dispose all needles properly in a "sharps" container immediately after use.
- Dispose of unused needles in sharps containers.
- Never recap, shear, break, or bend needles under any circumstances. Expel air and bubbles into a disinfectant-moistened pad.
- Refer to section 2.3.3.2 for more information on safe needle devices.

2.6.5 Lyophilizers

Lyophilizers produce a dry solid that is very easily dispersed. They should be fitted with a HEPA filter or vented to a BSC when used for drying suspensions of infectious material. Ampoules of lyophilized solids should be opened only in a BSC; place a disinfectant-moistened pad over the scored line when opening the ampoule. Disinfect chamber surfaces and any material collected in the vapor trap.

2.7 Decontamination

Decontamination refers to any activity that reduces the microbial load to a level deemed suitable to prevent contamination or infection. The appropriateness of a decontamination procedure is situation-dependent. For example, surgical instruments must be sterile but this level of microbial killing is unnecessary for environmental surfaces such as floors and walls.

Antisepsis refers to the application of a chemical to living tissue to prevent infection. Examples include iodine compounds and antimicrobial soaps for hand washing.

2.7.1 Sterilization

Sterilization refers to the destruction of all microbial life, including bacterial endospores.

2.7.1.1 Autoclaves

Autoclaves provide the most efficient and reliable method of sterilization for most laboratory applications. The critical process factors are temperature, exposure time, **and** ensuring that materials are packaged to allow the steam to penetrate throughout the load. Sterilization time will vary in relation to the size of the load and the packing density of the chamber. Typical laboratory autoclaves operate at 121°C and 15 psi. All users must review the operating manual periodically. Instructions should be prominently posted.

Use heat resistant gloves and face protection, particularly when removing processed material; crack the door slowly and wait a few minutes before fully opening it.

For dry loads, add 250-500 ml. of water to the load pan to aid in steam generation. Autoclave bags should be closed loosely to allow steam to penetrate; do not tightly cap bottles and test tubes.

Autoclave tape is not a fail-safe indicator of sterilization; it blackens after only brief exposure to a temperature of 121°C. When used for sterilizing infectious waste, autoclave performance must be periodically validated by using *B. stearothermophilus* spore vials. Place a vial in a hard-to-reach area of a mock challenge load and attach a string to facilitate removal after autoclaving. Incubate as directed; a lack of turbidity indicates that the autoclave is achieving sterilizing conditions.

Some autoclave tapes contain lead which makes it necessary to dispose of these tapes as Hazardous Waste. Laboratories must use lead-free autoclave tape to eliminate this hazardous waste stream Information concerning lead-free autoclave tape can be found in the Get the Lead Out pdf.

2.7.1.2 Dry Heat

Dry heat is used for materials (some glassware, instruments, and anhydrous materials) that are sensitive to moisture or the corrosion it may cause. Consult the manufacturers of such items for recommendations for appropriate sterilization procedures. Dry heat requires higher temperatures and a longer exposure times than autoclaving. Dry heat for 2-4 hours at 160°C is needed to sterilize a load requiring 30 minutes at 121°C in an autoclave. This method may also be validated by using spore vials; see autoclave section (above).

2.7.1.3 Chemical Sterilization

Chemical sterilization is chiefly used for heat-sensitive patient-care instruments that enter body cavities or normally sterile areas. This process requires prolonged contact times with relatively highly concentrated solutions. As a result, these products, especially prior to dilution, may be toxic and should be treated as hazardous chemicals. Carefully follow manufacturers' directions regarding dilution, contact time, personal protective equipment. Some sterilants require that specific ventilation systems be in place to remove hazardous gases and vapors.

Manufacturers of "Cidex" (active ingredient: glutaraldehyde) have developed "Cidex-OPA". Its active ingredient, ortho-phthalaldehyde, is less toxic and irritating than glutaraldehyde and is the preferred product at the University for aldehyde-based sterilization.

2.7.2 Disinfection

Disinfection refers to the elimination of virtually all pathogenic microorganisms on inanimate objects with the exception of large numbers of bacterial endospores.

Disinfection encompasses a continuum of outcomes in terms of the types of organisms destroyed. Microorganisms can be grouped in terms of decreasing resistance to disinfectants as follows: bacterial endospores (*B. subtilis, clostridium spp*); Mycobacteria; nonlipid or small viruses (poliovirus, rhinovirus); fungi; vegetative bacteria; and lipid or medium sized virus (herpes simplex, HIV, HBV).

The table at the end of this section provides a framework for the selection of the appropriate disinfectant. The label on a commercial product will note its type of 'cidal' action ('tuberculocidal', 'sterilant'). These claims may not appear unless the manufacturer has submitted data to the EPA supporting such claims. The lists of EPA registered disinfectants can be obtained from your campus EH&S office.

The EPA does not independently audit such results and research indicates that in real life situations some products do not perform as claimed. This result from manufacturers testing their products in best-case situations, e.g., on a smooth surface, at an optimal pH, in a buffer solution instead of a solution containing organic material which partially inactivates some disinfectants. For high risk pathogens, investigators may devise their own test to confirm a product's claim or consult EH&S.

When using any disinfectant:

- Follow label instructions for dilution and contact time needed for desired level of disinfection.
- Disinfectants that require pre-use dilution should be treated as hazardous chemicals during mixing. Wear a lab coat, the correct type of chemical-resistant glove, and goggles, not glasses.
- Clean contaminated surfaces as soon as possible and any surface that may have become contaminated at the end of the task.
- Select the disinfectant with the lowest toxicity possible

Considerations for selecting and using disinfectants:

- Nature of surface-rough surfaces require a longer contact time than smooth ones.
- Surface compatibility-bleach will corrode many metals, rinse with water after use; instruments vary in their ability to withstand disinfectants based on their composition.
- Organic matter will inactivate some disinfectants; a second application may be necessary once visible contamination (and hence, most organic debris) has been removed. **The removal of visible 'soil' may be the single most critical factor in assuring effective decontamination.**
- Resistance of microorganisms, e.g. bacterial endospore vs. vegetative bacteria.
- Number of microorganisms present, overnight culture vs. a recently inoculated one.

The Bloodborne Pathogens Standard requires that products labeled "tuberculocidal hospital disinfectant" be used on surfaces and equipment when the Standard is in force. Household bleach, usually at a 1/10 dilution, also satisfies this requirement and may be used in these cases. Bleach solutions lose potency over time and should be prepared fresh daily.

Summary of Disinfectant Activities

Disinfectant	Disinfection Level	Bacteria	Lipophil. Viruses	Hydro-Philic Viruses	M. tuber-culosis	Fungi	Comments
Alcohols (ethyl and isopropyl) 60-85%	intermediate	+	+	-	+/-	+	Not sporicidal; evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable.
Phenolics (0.4%-5%)	intermediate	+	+	+/-	+	+	Not sporicidal; phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue.
Glutaraldehyde (2-5%)	high	+	+	+	+	+	Used to sterilize surgical instruments that cannot be autoclaved; strong odor; sensitizer; use with adequate ventilation. Not for use on Environmental surfaces.
Quaternary Ammonium (0.5-1.5%)	low	+	+	-	-	+/-	May be ineffective against Pseudomonas and other gram – bacteria; recommendation limited to Environmental sanitation (floors, walls). Low odor, irritation.
Iodophors (30-1,000 ppm iodine)	intermediate	+	+	+	+/-	+/-	Inactivated by organic matter.
Chlorine (100-1,000 ppm)	intermediate	+	+	+	+/-	+	Not sporicidal; inactivated by organic matter; fresh solutions of hypochlorite (chlorox) should be prepared daily; corrosive; irritating to eyes and skin.

2.7.3 Using bleach as a disinfectant

The sodium hypochlorite in household bleach is a strong oxidizing agent that is an effective disinfectant for the known, and potential, infectious materials at Columbia University. However, over time the sodium hypochlorite breaks down to salt and water. When bleach and water are mixed together, 1:10, to create a cleaning or disinfecting solution, the solution rapidly begins to lose needed disinfecting properties. Therefore, it is recommended that the solution is made fresh daily.

Stock bleach should be stored in an opaque plastic bottle at room temperature. The rate of degradation depends on the initial hypochlorite concentration, the ambient temperature and the volume remaining. Manufacturers are not required to put an expiration date on the bottle. A good practice is to mark the bottle with the receive date, and replace bleach that was received more than 6 months prior. Colorimetric test strips for hypochlorite concentration provide an easy and useful monitoring tool.

Not all bleach is created equal; the potency of commercial bleach is between 3.25 and 6.15% hypochlorite, depending on manufacturer. Ultra regular CLOROX liquid bleach contains a higher concentration. As an additional measure of surety, it is possible to determine the production date of this product from the **last four digits** of the serial number on the bottle. The first of these digits identifies the year of production (3=2013, 4=2014) and the other three indicate the day of the year

of production (002= Jan 2nd, 364=Dec 30th). For example, E614293 = production date 10/20/2014 (October 20th is the 293rd day of 2014). Note that this scheme is only applicable to Clorox bleach.

Gloves should be worn while handling bleach. Bleach can be corrosive on some surfaces, including steel. Bleach residue on non-porous surfaces should be wiped off with water or 70% ethanol. Bleach should not be used in conjunction with other household cleaning products that contain ammonia; the two can react to produce a highly toxic product. Pre-filled spray bottles that mix at the nozzle are a convenient way of generating a 1:10 mixture for use in the lab.

Aspiration of tissue culture media into a collection flask, under vacuum, is one of the most commonly performed laboratory procedures. University Policy requires that such media be decontaminated prior to disposal in the municipal sewer system.

Effective decontamination is simple, following these instructions. Before aspiration, add undiluted bleach to fill 10% of the final volume of the collection flask. Bleach is an effective decontaminant with the added advantage that its strong oxidizing properties will turn the phenol red indicator in tissue culture media from pink to yellow/clear. Aspiration flasks containing pink liquid indicate insufficient bleach concentration, and should be topped off with fresh bleach until a yellow/clear color is achieved prior to additional aspiration or disposal. Empty the collection flasks when they are 3/4 full, or at least weekly.

An adhesive magnet (shown below) is available to post on biosafety cabinets that summarizes effective aspiration flask and bleach management. Please email biosafety@columbia.edu for a complimentary copy.

COLUMBIA UNIVERSITY
Environmental Health and Safety

Tissue culture aspiration flasks and bleach management

- Aspirated tissue culture media must be decontaminated. Before aspiration, add undiluted bleach to fill 10% of the final volume of the collection flask .
- Bleach will turn the phenol red indicator in tissue culture media from pink to yellow/clear. Aspiration flasks containing pink liquid indicate insufficient bleach.
- Empty flasks into the drain when they are 3/4 full, or at least weekly.
- Bleach breaks down over time; bleach stock bottles must be labeled with a receipt date and used, or discarded, within 6 months.
- Bleach used for routine decontamination must be diluted to 10% and prepared fresh daily.

A — Collection flask, B—Overflow collection, C—HEPA filter, D—Vacuum line

- Filters must be replaced when they become clogged. Never operate a vacuum line for aspiration of liquid culture media without a HEPA filter in place.
- For reordering filters, ensure that the replacement filter has a pore size ≤ 0.45 µm.
- Fisher Scientific catalog numbers: Pall: NC0151261, Whatman: 09-744-79, Millipore: SLFH05010.

For more information, contact EH&S at 212-305-6780
biosafety@columbia.edu www.ehs.columbia.edu

2.8 Biological Spills – Response and Clean-Up Policy

A. Purpose

This policy addresses the need for University laboratories and patient care areas to develop spill response plans addressing foreseeable occurrences. Materials for clean-up should be assembled in one place, and personnel should be trained in the appropriate response to clean up spills or how to request assistance. Designations should be established between those spills and events that laboratories are capable of handling, versus those circumstances that require outside assistance.

B. Applicability/scope

1. This policy applies to areas where infectious materials (including recombinant microorganisms), clinical specimens/tissues, or regulated medical waste are handled.

2. This policy covers biological spills in laboratories, patient care environments and common areas of Columbia University.

3. Biological spills at BSL1 or BSL2 differ from chemical and radiological spills in that only direct contact with the spilled materials represents a hazard. Access to only the immediate area around the spill needs be restricted.

4. Biological spills at BSL3 or ABSL3 require a higher level response. Investigators that work at BSL3 or ABSL3 have written response protocols that are not detailed in this policy.

5. EH&S has written internal "Procedures for Responding to a Hazardous Materials Incident", should a large scale biological spill require National Incident Management System or NIMS-equivalent command, resource and support staff.

C. Responsibilities

Spills in the University laboratory:

Investigators are generally responsible for cleaning up biological spills they create in the lab. Laboratories are required to maintain basic materials for response to routine spills (please see below; biological spill kits). EH&S is available to consult on clean-up procedures and will assume responsibility for cleaning the spill if it is beyond the scope of the lab staff's ability, due to hazard level or resource limitation.

Spills in University patient care areas:

Clinicians are generally responsible for cleaning up biological spills that contact their equipment (e.g. dental chairs) and work surfaces. Facilities are generally responsible for cleaning up biological spills that are on the floor. Clinicians should call Facilities for service (212-305-4357). Facilities will then reach out to EH&S if a consultation on clean up procedures is

warranted. Departments are encouraged to contact Facilities to establish specific agreements regarding the scope of spill clean-up services.

Spills in common areas:

Facilities are generally responsible for cleaning up biological spills that are, for example, on the hallway floor or in a bathroom. EH&S is available to consult on clean up procedures and will assume responsibility for the spill if it is large.

D. Definitions

BSL – Biosafety Level

BSC - Biological Safety Cabinet

NIMS - National Incident Management System

PPE – Personal Protective Equipment

RMW – Regulated Medical Waste

E. Procedures

1. Personal exposure takes priority over clean up.

If exposure occurs, immediately remove contaminated clothing and other protective equipment and wash affected areas with soap and water. If medical follow-up is warranted it should be sought immediately (see Medical Surveillance, below). For additional detail regarding response to personal exposures to biological materials, please see relevant sections of the University's Bloodborne Pathogens Exposure Control Plan.

2. Materials in a biological spill kit

The following materials should be set aside apart from those that are in regular use in the lab to ensure their availability in an emergency.

· Goggles or face shield, gloves, wrap-around lab coat, shoe covers (optional)

· Disinfectant solution*

· Paper towels or other absorbent

· Forceps, tongs, broom, dust pan

· Red bags for regulated medical waste, sharps container

*A 1/10 dilution of household bleach, prepared fresh daily is effective in most situations; contact EH&S for more information about selection of disinfectants, particularly for any organisms atypical in their sensitivity to disinfectants.

3. Spill response procedures involving microorganisms, including recombinant microorganisms, requiring BSL1 or BSL2 containment.

· Alert personnel in vicinity to leave the immediate area.

· Don protective equipment (gown/lab coat, gloves, eye protection).

· Cover an area twice the size of the spill with paper towels, or other absorbent material.

· Pour disinfectant solution onto the spill, starting at the perimeter and working inward from the edges of the towels. Avoid splashing.

· Allow 20 minute contact period.

· Wipe down any contaminated stationary equipment or furniture twice with disinfectant. Contaminated fabric-covered furniture or porous material should generally be treated with disinfectant and then discarded. EH&S can provide a consultation on other contingencies.

· Use forceps, tongs, or broom to remove broken glass and other items; place in sharps container or red bag, as appropriate.

· Remove towels and re-clean area with disinfectant solution.

· Collect and dispose in Regulated Medical Waste (RMW) container.

· Decontaminate (autoclave, or use a chemical disinfectant) reusable clean-up items and other permanent equipment.

· Inform laboratory personnel when the clean-up is complete.

Procedures for BSL-1 and BSL-2 laboratories should incorporate a degree of flexibility. One could safely abridge the procedures above if 1 ml were spilled over a small bench top area. However, dropping 50 ml of culture on the floor necessitates the more detailed procedure.

4. Spills inside a Biological Safety Cabinet (BSC)

· Keep the cabinet running.

· PPE should be use at the time of the spill, but if it is not, don gown/lab coat, gloves and eye protection.

· Clean-up as per directions above, making sure to wipe down back and side walls of cabinet.

· If material has spilled into the catch tray beneath the work surface, add a volume of disinfectant roughly equivalent equal to the quantity of the spill in the tray, wait 20 minutes, and absorb with paper towels. For more details on disassembly of BSC, see the EH&S biosafety manual section 2.3.1.5; Procedures for Effective Use of BSCs.

· After completion, allow cabinet blower to run for ten minutes before resuming work.

5. Spills inside a centrifuge

· Shut centrifuge off and do not open the lid for 20 minutes to allow aerosols to settle.

· Put on PPE, see above.

· Use a squeeze bottle to apply disinfectant to all contaminated surfaces within the chamber, taking care to minimize splashing.

· Allow 20 minute contact period and then complete clean-up of the chamber.

· Remove buckets and rotors to nearest BSC; disinfect and clean as per manufacturer's instructions.

The use of centrifuge safety cups can limit the extent of contamination to the cup itself, which can be reopened inside a BSC for cleaning.

6. Spills outside the laboratory in common areas

· Viable organisms should only leave the laboratory in a well-sealed primary (inner) and secondary (outer) container with a closable top. A test-tube rack inside a tray is not acceptable for transport.

· The exterior of the secondary container should be wiped down with disinfectant prior to leaving the laboratory so that it can be transported without wearing gloves.

· In the unlikely event of a spill, post someone to notify people in the immediate area, collect PPE and clean-up material and then proceed with clean-up. Public Safety can help restrict access to contaminated areas.

7. Spill procedures in clinical areas

· Healthcare providers can clean up spills on the floor in clinical areas if they feel comfortable doing so. Alternatively, Facilities should be notified, please see Section C, above.

· Alert personnel in vicinity to avoid the immediate area.

· Procedures are the same as those detailed in section E.2.

8. Abandoned spills in common areas.

· Biological spills encountered in a hallway (e.g. leaking red bag placed inappropriately on the floor). Notify personnel to avoid the immediate area. Notify EH&S. Public Safety can help restrict access to contaminated areas.

· Blood spills encountered in a bathroom (e.g. menstrual blood or nosebleed). Such spills should be reported to Facilities.

F. Emergency contacts

Public Safety Morningside: 212-854-2797, Emergency: 212-854-5555

Public Safety Medical Center: 212-305-8100, Emergency: 212-305-7979

Facilities: 212-305-4357

EH&S Morningside: 212-854-8749

EH&S Medical Center: 212-305-6780

Click locations and contact info for health care providers.

G. Medical Surveillance

Personal exposure takes priority over clean up. If medical follow-up is warranted it should be sought immediately. For details on health care providers see above link for locations and contact information.

For more information on post exposure response, review the Bloodborne Pathogens Exposure Control Plan.

H. Recordkeeping

N/A

I. Appendices

None

J. Forms

None

K. References

Health & Safety Manual - Biological Spills

Biological Spills: Clean-up Procedures

Bloodborne Pathogens Exposure Control Plan

Locations of health care providers.

2.9 Tissue Cultures and Cell Lines

Cell lines obtained from commercial sources may become contaminated with adventitious agents while used in the laboratory. The extent of screening varies among providers and while most test for bacteria, mycoplasma, and fungi, they do not routinely include testing for viruses other than those categorized as 'Bloodborne Pathogens'.

Cell cultures known to contain an infectious agent or oncogenic virus should be manipulated at the Biosafety Level appropriate for the agent, usually BSL-2.

For activities with materials not known to contain infectious agents, the following hazard classification applies:

BSL-1 is appropriate for well-established lines of cells of sub-primate origin if they do not harbor a primate virus and are free of bacteria, fungi, and mycoplasma. However, working with these materials at BSL-2 is recommended because of the additional degree of protection from contamination provided by BSL-2 practices, particularly the use of a Biological Safety Cabinet.

BSL-2 is appropriate for activities with: all primate cell lines, even well established ones, all cells derived from primate lymphoid or tumor tissues; all primate tissue; all human clinical material*; cultured cells new to the laboratory until proven contaminant-free; and, cells exposed to or transformed by a primate oncogenic virus.

*These activities and the use of any cells purposely infected with or suspected of harboring agents defined as bloodborne pathogens are covered by the Bloodborne Pathogens Standard). Laboratories using human cell strains (non-transformed cells) propagated from primary explants must also comply with the Standard because they are considered "unfixed human tissue" which is covered by the regulation.

2.10 Select Agents and Toxins; Biosecurity

2.10.1 Select Agents and Toxins

Select Agents and Toxins are high risk microorganisms (BSL-3 and 4 agents) and toxins that may pose a public health threat to individuals, animals, or plants due to accidental or intentional release. Their use and possession must be consistent with biological safety and biosecurity requirements promulgated by the USDA and/or CDC. Laboratories wishing to acquire and use

Select Agent microorganisms or toxins must **first** obtain CDC or USDA approval by demonstrating that the materials will be used in appropriately engineered facilities, with adequate security. Approval is also contingent upon demonstration of appropriate SOPs relating to security, incident and emergency response.

A list of Select Agent microorganisms can be found at National Select Agents Registry site.

For Select Agent toxins, quantity limits exist. Laboratories possessing amounts below these limits are exempt from the CDC/USDA approval process. It is presumed that no laboratory at Columbia will need to use any of these toxins above the quantity limits, however manufacturers and suppliers often require documented attestation of quantity limit compliance when ordering *any* quantity of a Select Agent toxin. Please contact EH&S if you are requested to provide such documentation.

Refer to EH&S' website for a summary of the requirements for the use of Select Agents and Toxins.

2.10.2 Biosecurity

The need to safeguard and limit access to biological materials is not limited to laboratories using Select Agent materials. Consult the Principles of Laboratory Biosecurity section of the BMBL 5 for guidance in establishing laboratory-specific biosecurity procedures.

2.11 Hazardous Materials: Registration and Approval

2.11.1 Recombinant DNA

Recombinant DNA refers to either: (i) molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecule that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

The NIH's Guidelines for Research Involving Recombinant DNA Molecules apply to **all rDNA activities** at Columbia, regardless of the funding source for a particular project. The NIH's risk assessment criteria for most viral vectors give very little weight to 'replication deficiency' alone. This requires applying the same hazard assumptions as if wild type virus were being used, as well as commensurate biological safety procedures.

All uses of rDNA must be described in a submittal to the University's Institutional Biosafety Committee. The NIH defines protocols which are 'exempt' from submission requirements, but this category is narrower than most people assume and investigators must, at a minimum, submit an initial application for the IBC to make this determination. To submit your lab's rDNA work:

- Go https://www.rascal.columbia.edu.
- Select 'Hazardous Materials' from the menu on the left side of the welcome screen
- Log in with your CU UNI and password

- Under **Create a New Request, select "**Recombinant DNA (Appendix A)"
    - If recombinant DNA will be used as part of an animal care protocol, check the "Used/Administered as part of a protocol" box at the top of the form.
    - If recombinant DNA will be used only *in vitro* check the "Invitro/Invertebrates only" box. Complete **and save the** application

- The completed application can then be **either** attached to an IACUC protocol **or** submitted directly to EH&S for in vitro-only activities

2.11.2 Other Hazardous Materials Requiring Registration and Approval

The intended use of Infectious Agents (Appendix B), use of human materials (blood, body fluids, tissues, cells, human cell lines (Appendix C), or use of hazardous chemicals or biological toxins (Appendix E) must also be documented by using the same RASCAL-based submittal process as described for recombinant DNA.

2.12 Regulated Medical Waste

Regulated Medical Waste (RMW) Regulated Medical Waste (RMW) is material that may be contaminated with blood, bodily fluids, or other infectious materials, as well as sharps. RMW must be properly handled, collected, segregated, packaged, stored, labeled, transported and disposed of in order to minimize the risk of transmitting infection or endangering human health.

2.12.1 Containers for Regulated Medical Waste

2.12.1.1 Sharps Containers are for disposal of items contaminated with infectious materials or recombinant DNA that may rip or poke a hole in a red plastic bag, including:

- **All** hypodermic needles, suture needles, syringes, and scalpel blades, **even if unused**.
- Pasteur pipettes (glass or plastic) blood vials, razor blades, serological pipettes (glass or plastic), slides, cover slips, and glass culture dishes and test tubes containing or that were in contact with cultures/stocks of microorganisms, or **if they are unwrapped/unpackaged or appear as anything other than unused**.
- Devices and materials listed in the bullet above, may be placed in **cardboard boxes** (see below) if they are unused and in their original packaging.
- Broken or unbroken glassware that were in contact with infectious agents, such as used slides and cover slips.

2.13 Bloodborne Pathogens

The OSHA Bloodborne Pathogens Standard covers all employees with "reasonably anticipated" exposure to human blood, blood products, or other material capable of transmitting HIV, HBV,

HCV and other bloodborne diseases. The law requires that employers develop and implement an Exposure Control Plan that:

- Identifies job titles and tasks where exposure may occur.
- Describes the procedures that will be used to minimize exposure risk:
- Working at BSL-2 with emphasis on engineering controls as the preferred type of control measure
- Adopting Universal Precautions-treating all human blood, certain body fluids, and other materials as if they were known to be infectious for bloodborne diseases.
- Details procedures to ensure rapid follow-up treatment consistent with current medical recommendations for employees, paid for by the employer, in the event of an exposure incident.
- Offers affected employees the HBV vaccine free of charge and the option of accepting it at any later date if the initial offer is declined.
- Provides a schedule for the regular cleaning and decontamination of work surfaces.
- Provides employees with initial and annual update training focused on work practices that will minimize their risk of exposure.

EH&S conducts mandatory safety training sessions in a classroom setting monthly. Personnel receiving training for the first time must attend a live training session. The requirement for annual re-training may be satisfied by either attendance at a classroom session or by logging on to RASCAL where review material and a short quiz are provided online.

A hard copy of the required Exposure Control Plan is available from EH&S.

PIs are responsible for modifying the plan with site (laboratory)-specific information as required by the regulation.

Biosafety brochure

Bloodborne Pathogens/Biological Safety

All University personnel working with: human blood/body fluids/unfixed tissue, human or non-human primate cell lines or any materials deemed capable of transmitting HIV, HBV, HCV or other bloodborne diseases; infectious microorganisms classified at Biosafety Level-2 (agents capable of causing disease n healthy adults); or viral vectors classified by the NIH as requiring Biosafety Level-2 procedures. *Refresher training is required annually.* First time attendees must attend a classroom training (see schedule below); annual refreshers may be taken on-line via RASCAL as course TC0509*

Shipping Biological (infectious and potentially infectious) Materials, Genetically Modified Microorganisms, and Exempt Specimens

This course is designed for all University personnel responsible for the preparation, packaging and documentation for shipping infectious or potentially infectious materials, including genetically

modified microorganisms or any unfixed human or animal specimens. This training is offered on RASCAL as course TC0507*.

Shipping with Dry Ice, Exempt Specimens, and Excepted Quantities of Dangerous Goods Dry ice is considered a 'Dangerous Good' regardless of other package contents and also requires training. This module is intended for shippers of dry ice and also covers information pertaining to the shipment of unfixed, exempt human or animal specimens and specimens or samples preserved in small quantities of preservatives such as ethanol or formalin (excepted quantities). This training is offered on RASCAL as course TC0076*.

Recombinant DNA Training All researchers and personnel included on any Appendix A submitted for the use of recombinant DNA are required to complete this training to be in compliance with the NIH Guidelines for Research with Recombinant DNA. This training is offered on RASCAL as course TC0508*.

Biosafety Training for Research with Viral Vectors All researchers and personnel working with viral vectors for which the submission of an Appendix B is required must complete this online course. This training is offered on RASCAL as course TC1150*.

*RASCAL training courses:

Go to: https://www.rascal.columbia.edu/. Log in and select: 'training center' and then 'safety courses'. Select the appropriate training module

Shipments of certain biological materials are regulated domestically by the Department of Transportation (DOT) as well as the Federal Aviation Administration (FAA), and the International Air Transportation Association (IATA), if sent internationally. Any students or University employees involved in packaging materials, preparing samples for shipping, handling such packages, preparing related paperwork, or signing to authorize shipments must undergo specific training and maintain records of this training (i.e; by printing a copy of your training certificate from RASCAL).

There are 4 basic classifications for shipments of biological materials:

- Non-regulated biological material
- Exempt Human or Animal Specimens
- Biological Substance, Category B
- Biological Substance, Category A

Following the completion of appropriate training, University personnel can be qualified to prepare and ship packages containing exempt specimens or category B substances. Prior to initiating any shipment of a Category A Biological Substance , an "Intent to Ship" form must be completed and submitted to EH&S. After review, EH&S will advise of additional procedures and requirements that apply to your shipment.

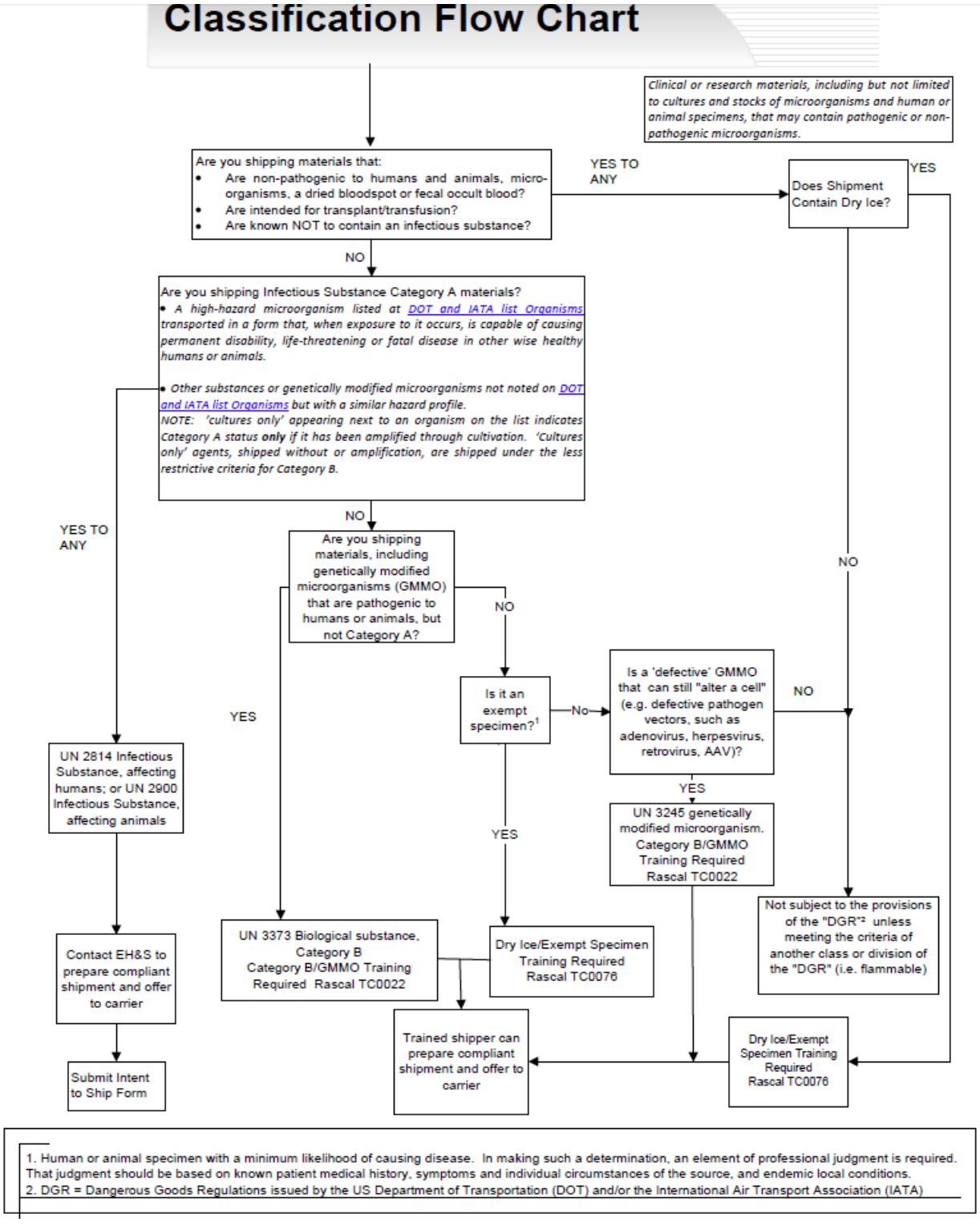
International shipments also require a commercial invoice. A commercial invoice template is available. Please contact EH&S well in advance of shipping internationally for help completing this invoice and to determine whether export/import permits are required.

EH&S is available for technical consultation throughout this process regardless of shipment type or class.

Inter-campus transport by investigators is permitted for specific classifications of biological materials. Refer to section 12 of the Biological Materials Shipping Manual.

- If your shipment contains a material indicative of a Category A Biological Substance, please contact EH&S for further instruction or submit the intent to ship form.
- If your shipment is an exempt specimen for which, based on professional judgment, there is minimal likelihood that a pathogen is present, you must first complete the RASCAL training module TC0076 “Dry Ice and Exempt Specimens Training” prior to shipment
- If your shipment is a Genetically Modified Microorganism or Category B Biological Substance , you must complete the RASCAL training module TC0022/0052/0058 “Shipping Category B and Genetically Modified Microorganism Training” prior to shipment.
- Please note that “Diagnostic Specimen" and "Clinical Specimen” are no longer valid shipping names and should not be written on any shipping materials.

For assistance in classifying biological material and instructions for shipment please see the biological materials shipping flow chart.



Requirements for Submission and Approval for the use of Recombinant DNA

[Columbia University Institutional Biosafety Committee Policy/Operating Procedures (Charge)]

Recombinant DNA use is inseparable from research progress in the life sciences and other fields. Many of the same hazards associated with standard microbiological activities carry over into this field based on the use of potentially infectious vectors and genetic materials from recognized pathogens. The University seeks to address and mitigate these hazards through the appropriate application of biological safety processes and adherence to relevant regulatory mandates.

Introduction

Recombinant DNA refers to either: (i) molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecule that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

The University's Institutional Biosafety Committee (IBC) is charged with facilitating compliance with the NIH's Guidelines for Research Involving Recombinant DNA Molecules. The "Guidelines" are actually requirements and the failure to adhere to them may result in suspension of NIH funding to an individual PI or the entire institution. The Guidelines apply to all recombinant DNA (rDNA) activities at an institution where any work with recombinant DNA receives NIH funding. Stated another way, the Guidelines apply to all rDNA work at Columbia regardless of the individual project's funding source. See also Investigator responsibilities under the NIH guidelines" brochure.

Responsibilities

- Recombinant DNA Activities Committee (RAC) group within the NIH is responsible for carrying out the functions specified in the NIH Guidelines, as well as others specified in its charter or assigned by the Secretary of Health and Human Services or the NIH Director.
- Office of Biotechnology Activities (OBA) serves as a focal point for information on recombinant DNA activities and provides advice within and outside NIH including institutions, Biological Safety Officers, Principal Investigators, Federal agencies, state and local governments, and the private sector.
- Columbia University's Institutional Biosafety Committee (IBC) is responsible for facilitating compliance with the Guidelines through activities that include education and training, review of rDNA proposals, and periodic reporting to the NIH as required by the Guidelines.
- Principal Investigators and laboratory staff are responsible for submitting their rDNA proposals to the IBC in a timely manner, adherence to the biological safety practices appropriate to the risk of their research materials, and seeking IBC assistance on any safety or compliance issues related to their work with rDNA or other biological materials.
- Columbia University Environmental Health & Safety (EHS) provides technical support to the IBC and has primary role in development and implementation of research safety policies.

Approval of rDNA Activities

The Guidelines specify different levels of approval and registration requirements (Sections III-A through Section III-F) that must be met prior to or upon initiation of work. Certain uses of rDNA (Section III-F) are exempt from any approval or registration requirements.

Section III-A: Experiments that Require Institutional Biosafety Committee Approval (IBC), RAC Review, and NIH Director Approval Before Initiation.

The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.

Section III-B : Experiments That Require NIH/OBA and IBC Approval Before Initiation.

Experiments Involving the Cloning of Toxin Molecules with LD50 < 100 nanograms per kilogram body weight.

Section III-C : Experiments that Require IBC and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment.

Deliberate transfer of recombinant DNA, into one or more human research participants(eg: a clinical trial); Institutional Review Board (IRB) approval is also required.

Section III-D: Experiments that Require IBC Approval before Initiation

- Use of Risk Group 2, 3, 4 or Restricted Agents as host-vector systems *(ex. lentiviral or adenoviral vectors)*
- Cloning of DNA from Risk Group 2 or higher agents into non-pathogenic prokaryotic or lower eukaryotic host-vector systems
- Use of infectious viruses or defective viruses in the presence of helper virus in tissue culture
- Experiments involving transgenic or non-transgenic animals administered rDNA *(does not include generation or breeding transgenic rodents, see Section III-E)*
- Experiments involving more than 10 liters of culture
- Experiments involving high risk Influenza Viruses

Section III-E: Experiments that Require IBC Notice Simultaneous with Initiation

- Formation of recombinant DNA molecules containing no more than 2/3's of the genome of any eukaryotic virus if it is demonstrated that the cells lack helper virus for the specific Families of defective viruses being used
- Generation or interstrain breeding where one or both of the strains is transgenic if the experiment requires BL1 containment; experiments that require higher levels of containment are covered under Section III-D, IBC approval required before initiation. Note: propagation of a single transgenic strain is exempt.

Section III-F: Exempt Experiments

Those that are not in organisms or viruses

- Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
- Recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. (see Appendix A of the *NIH Guidelines).*
- Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome that are propagated and mainta ined in cells in tissue culture.
- Escherichia coli K-12, Saccharomyces, Bacillus subtilis or Bacillus licheniformis Host-Vector Systems.
- Propagation of a single transgenic strain is exempt.

The NIH provides additional guidance for the use of rDNA in animals, including transgenics.

Submitting Your Recombinant DNA Application

- Go https://www.rascal.columbia.edu.
- Select 'Hazardous Materials' from the menu on the left side of the welcome screen.
- Log in with your CU UNI and password.
- Select "Recombinant DNA (Appendix A)":
    - For activities that will NOT involve the administration of recombinant DNA to vertebrate animals, select "Invitro/Invertebrates only". Once the Appendix is saved, it can be directly submitted to the IBC.
    - For activities that will involve the administration of recombinant DNA to vertebrate animals, or the generation or breeding of transgenic animals select "Invitro/Invertebrates only" which then must be attached to the pertinent IACUC protocol.
    - For activities requiring BSL-2/ABSL-2 containment or greater, the submission of an Appendix B to describe the use of infectious materials is also required.
    - For human gene transfer protocols complete and sign form "Application for the use of Recombinant DNA (rDNA) Molecules in Human Gene Transfer" and follow the submission instructions on the form.