BIOLOGICAL SAFETY

FOR RESEARCH LABORATORIES

BIOSAFETY MANUAL

MOUNT SINAI SCHOOL OF MEDICINE

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INSTITUTIONAL BIOSAFETY PROGRAM

Environmental Health and Safety Office

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Notify the Biosafety Officer in all situations

Chapter 1: Emergency Procedures

A. Biological Agents

1. Small spills that can be handled with disposable pads, paper toweling and disinfectants on hand can be contained by laboratory personnel under the direction of the Principal Investigator.

2. Large spills (greater than five liters) of biohazardous agents should not be handled by laboratory personnel. Personnel should hold their breath and exit the lab immediately. ***Notify the Biosafety Officer immediately!*** Personal decontamination and follow-up medical treatment should be performed as soon as practical.

3. Do not re-enter the lab under any circumstances, until given clearance to do so by the Biosafety Officer. Decontamination personnel should not enter the area until a minimum of 30 minutes time has elapsed after the spill.

4. Contaminated clothing is to be removed and autoclaved ***with no exceptions.*** If showers are available, wash all exposed areas of the body with a germicidal surgeon's scrub or soap and water. Contaminated items may be decontaminated or discarded at the discretion of the Biosafety Officer.

5. Place a dry towel or pad over the spilled material, and absorb as much spilled material as possible. These towels or pads can be autoclaved and disposed of in red bags. Place a towel or pad soaked in disinfectant directly over the spill area, and allow sufficient contact time for the disinfectant to neutralize any organisms present. Place dry pads or toweling over the wet ones and carefully remove and contain for disposal.

6. Spills in a biological cabinet pose little aerosol hazard to lab personnel. A wipe down with 3% *Wescodyne*, 1/100 dilution of Household Chlorine Bleach, or 70% ethanol or 70% isopropanol can be used to disinfect the cabinet.

7. Do not open internal surfaces of the biological safety cabinet without prior decontamination of the cabinet. Exposure to viable infectious organisms within these areas could result. This procedure should be performed only by certified individuals knowledgeable in NSF and NIH decontamination procedures.

8. **SPILLS, PATHOGENIC AGENT EXPOSURES AND NEEDLESTICKS**
   All spills resulting in personal exposure, exposures to BBFE (BloodBorne Fluid Exposure) and needle sticks occurring in ISM-MS Research Labs must be reported to the Biosafety Officer immediately. *

   In the event of a BBFE, the exposed individual is directed to follow the procedures given below:

   - **Employees and volunteers;** contact EHS x 57691 or 646-951-7223 or GO TO 19 East 98th Street, 2nd floor.
   - **Medical Students;** contact Jack Martin Fund Clinic (212-824-7395, ext 57395)
   - *Contractors and all others staff*; go to Emergency Department for follow-up care
   - **When EHS is closed**, go to the Emergency Department and call the Nursing Administrator - **[Dial "O"].**

9. An emergency action procedure should be drafted by the Principal Investigator and made available in written form, to all laboratory personnel and support staff associated with a hazardous project. Treatments, post-exposure vaccination, hospitalization or other interventions should be addressed in this procedure.

10. In laboratories handling large-scale RG-2 and higher agents, the risks associated with an exposure, the procedures and equipment used to protect personnel from the associated hazards and risks of working in that laboratory must be written out in a ***Standard Operating Procedure***. All lab personnel and support staff must have access to the written procedures at all times, and should receive annual training in those procedures. Bloodborne Agents have specific requirements as stipulated in ***OSHA's 29 CFR 1910.1030 Bloodborne Pathogens Standard:*** https://www.osha.gov/SLTC/bloodbornepathogens/index.html

B. Carcinogenic Agents

1. Small amounts of aqueous solutions of dilute carcinogens can be contained by personnel under the direction of the Principal Investigator.

2. Exposure to concentrated stocks of carcinogens is to be avoided at all costs. In the event of a spill of any volatile, concentrated material, do not attempt to contain the

spill, but ***leave the area immediately*** and contact the Mount Sinai Security Office at ***"60"*** Security will notify the Chemical Hygiene Officer or Env Health and Safety individual covering after-hours and weekends***. Give as much information as you can about the incident.***

a. **DIAL '60'** *and give YOUR:*
b. NAME
c. LOCATION OF SPILL AND YOUR CURRENT LOCATION
d. CONTACT NUMBER (from where you called if different from the lab)
e. APPROXIMATE AMOUNT OF MATERIAL INVOLVED
f. (1ml, 500mls, 1liter, several (dry)ounces, ¼ pound container etc.)
g. NAME(S) OF ANY / ALL EXPOSED INDIVIDUALS
h. NAME(S) OF ANY / INJURED INDIVIDUALS
i. Follow-up with EnvHS as soon as possible
j.

3. Do not re-enter the area under any circumstances until given authorization to do so by the Chemical Hygiene Officer.

4. All contaminated clothing is to be removed at once, and preferably discarded.

5. Report to Employee Health Service or to the Emergency Department on weekends or off-hours for medical attention if indicated. It is advisable to have a copy of the carcinogen's Safety Data Sheet in order to assist medical staff.

6. Written Emergency reaction procedures should be drafted and available to all personnel working in the laboratory, especially for the chemicals required in ***OSHA's 29 CFR 1910.1450 Laboratory Standard :*** ***https://www.osha.gov/SLTC/laboratories/standards.html***

   The specific 29 CFR 1910 Standards for select chemical hazards can be found at: https://www.osha.gov/pls/oshaweb/owasrch.search_form?p_doc_type=STANDARDS&p_toc_level=1&p_keyvalue=1910 under each sub-section reference, i.e.".1450"**.**

7. Safety Data Sheets must be available to all laboratory personnel at all times that are working with or have exposure to carcinogens and other toxic compounds.

C. Personal Exposures

Depending on the nature of the injury and the material being utilized at the time, different approaches to providing first-aid will be exercised in response to the gravity of the incident. Obviously, contaminated wounds with blood or materials known to contain pathogenic agents carry a higher risk of infection than “clean” wounds. One must consider whether the site was clean at the time of injury, i.e. a cut with a clean scalpel through a bloody glove is as dangerous as a cut with a contaminated object. If there is any doubt as to whether the site or the instrument causing the injury was contaminated, treat the incident as if it was an exposure to a pathogenic agent.

1. When working with known human pathogenic agents or with HUMAN source material, i.e. blood, body fluids and tissues, wash the wound with copious amounts of soap and water immediately, generating a good lather through gentle friction of the hands. This also applies to carcinogens and promoters.

2. Do not use strong disinfectants, scrub brushes or any other object that can abrade the skin. These activities could cause additional damage and increase the chances of penetration of either a pathogen or toxic or carcinogenic chemical.

3. If the wound is a puncture or laceration produced by a contaminated sharp, allow the wound to bleed under a steady gently flowing stream of water. Do not squeeze the wound, since this may cause additional trauma to the site and force pathogens and carcinogens deeper into the tissues. Wash the wound site with soap and water.

4. If any of the materials described in paragraph 2 (above) enter the eye, or splash into the mouth, rinse with water only, immediately. For an eye exposure, care must be taken to rinse the affected eye in a manner that will not introduce the contaminated rinsate into the unaffected eye. Rinse for a full ten minutes at a minimum with a gentle stream of water, or use an eye cup if one is available.

5. Once the injury or site of exposure has been cleansed, report to the Employee Health Service or Emergency Department immediately. If you are a student, go to the appropriate service for follow-up evaluation and treatment.

6. Report the incident to the Biosafety Officer immediately after treatment using an Incident Report form.

D. Emergency Information Cards

It is a useful practice to develop and distribute among all laboratory personnel and support staff Emergency Information Cards that can be carried in a lab coat pocket or in a person's wallet or handbag. This card should contain the name and synonyms of a particular chemical, the Chemical Abstract Service (CAS) number, the Registry of Toxic Effects of Chemical Substances (RTECS) number, and a short synopsis of signs and symptoms of exposure and toxification with the subject chemical. International Safety Cards developed by NIOSH can be obtained here for some chemicals, https://www.cdc.gov/niosh/ipcs/ and also at this site: https://www.cdc.gov/niosh/npg/

In similar fashion, cards can be made for biological agents and toxins, using information found in The CDC publication, ***BMBL*** ***(ref.6)***, available at http://www.cdc.gov/biosafety/publications/bmbl5/index.html or the Health Canada biological MSDS list, available at http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/index.html (This site is Canada's equivalent of the CDC's OHS, http://www.hc-sc.gc.ca/pphb-dgspsp/ols-bsl/index.html).

These cards are invaluable in an emergency situation, providing much needed information to health care professional or emergency department personnel providing treatment. The Biosafety Officer can assist you in preparing these cards.

Adenovirus types 1, 2, 3, 4, 5 and 7

NAME, SYNONYM OR CROSS REFERENCE: ARD, acute respiratory disease, pharyngoconjunctival fever

MODE OF TRANSMISSION: Directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person; outbreaks have been related to swimming pools; possible spread through the fecal-oral route

INCUBATION PERIOD: From 1-10 days

COMMUNICABILITY: Shortly prior to and for the duration of the active disease

FIRST AID/TREATMENT: Mainly supportive therapy

IMMUNIZATION: Vaccine available for adenovirus types 4 and 7 (used for military recruits)

SPILLS: Allow aerosols to settle; wearing protective clothing gently cover the spill with absorbent paper towel and apply 1% sodium hypochlorite starting at the perimeter and working towards the center; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization, incineration, chemical disinfection

Examples of Emergency Information Cards

Acetonitrile CH_3CN **CAS# 75-05-8** **RTECS# AL7700000**

Synonyms & Trade Names

Cyanomethane, Ethyl nitrile, Methyl cyanide [Note: Forms cyanide in the body.]

Exposure Routes inhalation, skin absorption, ingestion, skin and/or eye contact

Symptoms Irritation nose, throat; asphyxia; nausea, vomiting; chest pain; lassitude (weakness, exhaustion); stupor, convulsions; in animals: liver, kidney damage

Target Organs respiratory system, cardiovascular system, central nervous system, liver, kidneys

First Aid . Get medical attention promptly after first aid.

Chapter 2: Biological Safety

A. General Biological Procedures

In *Stedman's Medical Dictionary,* 27th Edition, one can find the following definition:

"bi-o-safe-ty: ***Safety measures applied to the handling of biological materials or organisms with a known potential to cause disease in humans …"***

While the definition is relatively simple and straightforward, the principles and practices employed in biosafety range in complexity and detail in direct relation to the relative risks associated with a given microorganism or its products.

Our working definition for a ***biological hazard*** or ***biohazard*** is any bacterium, prion, rickettsia, virus, fungus, parasite or its products (i.e. toxins) that can self-replicate, invade a host and cause an infection, or can cause morbidity or mortality in humans or animals. While the definition appears to be broad, it is limited primarily to those agents that can infect humans or are suspected of infecting humans primarily.

Hazard is a specific property or set of properties that are a hallmark for a given pathogen, such as spore formation in *Histoplasma capsulatum,* or toxin production in *Clostridium botulinum.* In a simple sense, it is what makes the organism dangerous and noteworthy, and allows it to establish an infection or produce a series of symptoms and *sequelae* in a host.

Risk is simply determining what your odds are of coming down with that series of symptoms and *sequelae* once you and your organism of choice have come in contact with one another. There is an intricate interplay of infectious dose, host susceptibility, virulence of the pathogen, route of exposure, and the individual's immune status that begins once an exposure has occurred. It is to be noted that not all exposures result in an infection.

Risk Groups are the four categories that the CDC and the NIH use in order to classify microorganisms with respect to **the most probable outcomes** of infection after exposure to a given organism has occurred.

Biological Safety Levels are the recommended set of microbiological practices, facilities, equipment and procedures that are recognized as being effective in controlling exposures and the hazards of working with a given group of microorganisms. It is good practice to regard each level as a ***minimum*** level of practice, to be built upon until the next level is reached.

These levels have long replaced the earlier "P-1, P-2" physical containment levels recommended by the NIH Guidelines many years ago, making these terms archaic in current biosafety usage.

While there is some correlation between the biosafety levels and the risk groups, the practice of saying *"Risk Group 2 uses Biosafety Level 2"* is no longer acceptable, and has been replaced with a "sliding-scale" concept of looking at the specific hazards and risks of a particular strain of an organism, and then custom building the biosafety level needed to provide *maximum* protection from

those hazards. This can be accomplished by taking a given safety level and adding components from the next higher level to it to increase the level of protection using facilities and equipment at hand. This allows an investigator to customize the Biosafety Levels to address the specific hazards of a given strain of a microorganism.

In order to simplify the process of evaluating the risks and the hazards associated with one's organism(s) of choice, The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH), both separately and jointly, have developed several guidelines and procedural guides to cover biosafety in the laboratory. Much of the content of this manual has been "lifted" directly from these publications, with some minor changes in order to provide a more concise reference for you.

In summation, there is ample evidence that working with microorganisms can be hazardous to your health, if you do not protect yourself and use good safety procedures. In the introduction to ***BMBL*** (ref. 6), the statistic of 3, 921 cases of laboratory-acquired infections reported over 27 years is cited from Sulkin and Pike's studies (1976). Their observation was…"*fewer than 20% of all cases were associated with a known accident…*", and probably more than 80% of the cases were attributable to exposures to infectious aerosols. Pike is quoted as stating in 1979:

> **…" the knowledge, the techniques, and the equipment to prevent most laboratory infections are available…".**

If anything in this manual is not clear to you, please contact the Biosafety Officer, at 241-5169. If you are not sure of the hazards or risks involved in working with a given agent, contact the Biosafety Officer ***before*** initiating a project.

The decision to work safely with biological agents rests squarely with you. No safety program, set of procedures, safety equipment or training will protect you if you disregard or decide not to practice biosafety in your laboratory.

See ***Infection Control*** available under the **"Policies and Manuals"** icon on the MSMS Intranet Site at: http://intranet1.mountsinai.org/msmc/manuals_toc.asp

B. Bloodborne Pathogens Used In Research

There is a specific section outlining in detail what the established practices based on risk assessments pertain to work with these agents. The pathogens covered are not limited to Human Immunodeficiency Virus and Hepatitis B Virus, but all recognized Bloodborne agents and "Other Potentially Infectious Materials" derived from human sources (including commercial human sera etc.).

The Standard Biosafety Level used is Biosafety Level 2 as found in the BMBL

http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf , and contained in http://www.osha.gov/SLTC/bloodbornepathogens/index.html .

The Bloodborne Pathogen Standard found at: :http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051 addresses those laboratories working with the actual agents or large amounts of a bloodborne agent specifically at section 1910.1030(e) *HIV and HBV Research Laboratories and Production Facilities*.

C. Genetically-Modified Microorganisms (GMOs), Non-Recombinant DNA and Expression Products

Recent advances in recombinant DNA technology are pushing the former definitions and risk-assessments into to grayer areas continually. Chimera organisms can be synthesized across Kingdoms and Phyla allowing the creation of animals with vegetable genes and visa-versa. It is also possible to create a new bacteria or virus with several properties that none of the parent organisms had. In like manner, it is possible to synthesize *de novo*, an artificial chromosome (BAC) that behaves like a regular bacterial chromosome.

All of these activities come under the control of the National Institutes of Health, specifically the Office of Science Policy (OSP) https://osp.od.nih.gov/about-us/ and supplemented technically, when needed by the Recombinant-DNA Advisory Committee (RAC).

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A place to start looking for the appropriate Biosafety Levels and Risk Groups is in the *NIH Guidelines,* found at:

https://osp.od.nih.gov/wp-content/uploads/2013/06/NIH_Guidelines.pdf

under the Section II and Appendices "B" and "G", based on how much of the original backbone organism exists. In the case where the gene or entire chromosome has been synthesized *de novo*, the closest organism should be selected, and the BSL and RG levels worked-out along with recommendations from OBA and RAC, under "Major Actions" procedures.

See Chapter 11 for information on the NIH Guidelines, and recombinant DNA

Chapter 3: Laboratory Biosafety Level Criteria

Part 1 Standard Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 1 of this section and discussed in Section 2. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1:

Laboratory Biosafety Level Criteria – Biosafety Level 1

A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control

access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

**** Excerpted entirely from the *BMBL*, 5th Edition Laboratory Biosafety Level Criteria**

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, andbroken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary

importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials

before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required. See Appendix G.

11. The laboratory supervisor must ensure that laboratory personnel receive

apropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.

Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Laboratory Biosafety Level Criteria – Biosafety Level 1

B. *Special Practices*

None required.

Laboratory Biosafety Level Criteria – Biosafety Level 1

C. *Safety Equipment* (Primary Barriers and Personal Protective Equipment)

1. Special containment devices or equipment, such as BSCs, are not generally required.

2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons

who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:

a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

Hand washing protocols must be rigorously followed.

Laboratory Biosafety Level Criteria – Biosafety Level 1

D. *Laboratory Facilities (Secondary Barriers)*

1. Laboratories should have doors for access control.

2. Laboratories must have a sink for hand washing.

3. The laboratory should be designed so that it can be easily cleaned.

Carpets and rugs in laboratories are not appropriate.

4. Laboratory furniture must be capable of supporting anticipated loads and uses.

Spaces between benches, cabinets, and equipment should be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratories windows that open to the exterior should be fitted with screens.

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is

restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in

BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

Laboratory Biosafety Level Criteria – Biosafety Level 2

A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control

   access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and

   storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required. See Appendix G.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Laboratory Biosafety Level Criteria – Biosafety Level 2

B. *Special Practices*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the

laboratory.

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

   a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

Laboratory Biosafety Level Criteria – Biosafety Level 2

C. *Safety Equipment* (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used

whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution.
   It is recommended that laboratory clothing not be taken home.

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or

containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:

    a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Biosafety Level Criteria – Biosafety Level 2

D. *Laboratory Facilities (Secondary Barriers)*

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

   a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

   b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with

appropriate disinfectant.

5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required. **See: Figure 2, Page 59.**

8. An eyewash station must be readily available.

9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations.

BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment.

A BSL-3 laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

Laboratory Biosafety Level Criteria – Biosafety Level 3

A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical,laboratory supervisors should adopt improved engineering and work practicecontrols that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped,

removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all

laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).Depending on where the decontamination will be performed, the following methods should be used prior to transport:

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required. See Appendix G.

11. The laboratory supervisor must ensure that laboratory personnel receive

appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Laboratory Biosafety Level Criteria – Biosafety Level 3

B. *Special Practices*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the

l aboratory.

3. Each institution must establish policies and procedures describing the

collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

    a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

    b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

Laboratory Biosafety Level Criteria – Biosafety Level 3

C. *Safety Equipment* (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC **(**preferably Class II or Class III), or other physical

containment devices.

2. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:

   a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

   b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

Laboratory Biosafety Level Criteria – Biosafety Level 3

D. *Laboratory Facilities (Secondary Barriers)*

1. Laboratory doors must be self closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self- closing doors.

2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.

3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation

openings should be capable of being sealed to facilitate space decontamination.

a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.

b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. All windows in the laboratory must be sealed.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

8. An eyewash station must be readily available in the laboratory.

9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.

a. Laboratory personnel must be able to verify directional air flow. A

visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

b. The laboratory exhaust air must not re-circulate to any other area of the building.

c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

12. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.

13.Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

14.Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas- tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level.

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Laboratory staff must have specific and thorough training in handling extremely

hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics.

All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. Access to the laboratory is controlled by the laboratory supervisor in accordance with institutional policies.

There are two models for BSL-4 laboratories:

(1) A *Cabinet Laboratory* where all handling of agents must be performed in a

Class III BSC.

(2) A *Suit Laboratory* where personnel must wear a positive pressure protective

suit. BSL-4 Cabinet and Suit Laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

Biosafety Level 4 is beyond the scope of current practice here at Mount Sinai School of Medicine. Refer to the BMBL for further information on BSL-4.

Part 2 *Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities*

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and is also useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, the institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care for laboratory animal. Laboratory animal facilities are a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

The animal room can present unique problems. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol driven risk assessment. These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., *Guide for the Care and Use of Laboratory Animals*1 and *Laboratory Animal Welfare Regulations*2) and that appropriate species have been selected for animal experiments.

In addition, the organization must have an occupational health and safety program that addresses potential hazards associated with the conduct of laboratory animal research. The following publication by the Institute for Laboratory Animal Research (ILAR), *Occupational Health and Safety in the Care of Research Animals*3 is most helpful in this regard. Additional safety guidance on working with non-human primates is available in the ILAR publication, *Occupational Health and Safety in the Care and Use of Nonhuman*

*Primates.*4

Facilities for laboratory animals used in studies of infectious or non-infectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities providing patient care. Traffic flow that will minimize the risk of cross contamination should be incorporated into the facility design.

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These four combinations, designated Animal Biosafety Levels (ABSL) 1-4,

provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices applicable to work with animals infected with agents assigned to

Biosafety Levels 1-4, respectively. Investigators that are inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

In addition to the animal biosafety levels described in this section the USDA has

developed facility parameters and work practices for handling agents of agriculture significance. Appendix D includes a discussion on Animal Biosafety Level-3 Agriculture (ABSL-3-Ag). USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. Appendix

D also describes some of the enhancements beyond BSL/ABSL-3 that may be required by USDA-APHIS when working in the laboratory or vivarium with certain veterinary agents of concern.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in this section. The reader is referred to Appendix E for more information on the Arthropod Containment Guidelines.

Animal Biosafety Level 1

Animal Biosafety Level 1 is suitable for work involving well characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment (See Section 2).

Personnel must have specific training in animal facility procedures and must be

supervised by an individual with adequate knowledge of potential hazards and

experimental animal procedures.

The following standard practices, safety equipment, and facility requirements apply to ABSL-1:

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 1

A. *Standard Microbiological Practices*

1. The animal facility director establishes and enforces policies, procedures, and

   protocols for institutional policies and emergency situations.

   Each institute must assure that worker safety and health concerns are

   addressed as part of the animal protocol review. Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC)5 and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in

consultation with the animal facility director and appropriate safety professionals. he safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices

and procedures.

3. Supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for

appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted

respiratory protection program.

5. A sign incorporating safety information must be posted at the entrance to the

areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. Security-sensitive agent information should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery

plans, as a contingency for man-made or natural disasters.1,3,4

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility.

All persons including facility personnel, service workers, and visitors are

advised of the potential hazards (natural or research pathogens, allergens, etc) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent

contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals. Gloves and personal protective

equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the

areas where infectious materials and/or animals are housed or are

manipulated. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and

storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.

9. All procedures are carefully performed to minimize the creation of aerosols or

splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions,

including those listed below, must always be taken with sharp items. These

include:

a. Needles and syringes or other sharp instruments are limited to use in

the animal facility when there is no alternative for such procedures as

parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

e. Equipment containing sharp edges and corners should be avoided.

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.

13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.

14. An effective integrated pest management program is required. See Appendix G.

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 1

B. *Special Practices*

None required.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 1

C. *Safety Equipment* (Primary Barriers and Personal Protective Equipment)

1. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

2. Special containment devices or equipment may not be required as determined by appropriate risk assessment. Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.

3. Protective eyewear is worn when conducting procedures that have the

potential to create splashes of microorganisms or other hazardous materials.

Persons who wear contact lenses should also wear eye protection when

entering areas with potentially high concentrations or airborne particulates.

Persons having contact with the NHP should assess risk of mucous membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, faceshields, etc.) as needed.

4. Gloves are worn to protect hands from exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for

the task and alternatives to latex gloves should be available. Change gloves when contaminated, integrity has been compromised, or when

otherwise necessary. Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the

areas where infectious materials and/or animals are housed or are

manipulated. Hand washing should occur after the removal of gloves.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 1

D. *Laboratory Facilities (Secondary Barriers)*

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted.

   Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. The animal facility must have a sink for hand washing.

   Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.

   It is recommended that penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control and proper cleaning.

   Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

   Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.1 No recirculation of exhaust air should occur. It is recommended that animal rooms have inward directional airflow.

   Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

8. If floor drains are provided, the traps are filled with water, and/or appropriate

disinfectant to prevent the migration of vermin and gases.

9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.

10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Animal Biosafety Level 2

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, should be

conducted in BSCs or Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee

occupational health programs should be considered.

The following standard and special practices, safety equipment, and facility requirements apply to ABSL-2:

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 2

A. *Standard Microbiological Practices*

1. The animal facility director establishes and enforces policies, procedures, and

   protocols for institutional policies and emergency situations. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study animal protocols must also be reviewed and approved by the IACUC5 and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in

   consultation with the animal facility director and appropriate safety

   professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.

   Consideration should be given to specific biohazards unique to the animal

   species and protocol in use.

3. Supervisor must ensure that animal care, laboratory and support personnel

   receive appropriate training regarding their duties, animal husbandry

procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

   Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

   Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted

respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the

entrance to areas where infectious materials and/or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other

responsible personnel), telephone number, and required procedures for

entering and exiting the animal areas. Identification of specific infectious

agents is recommended when more than one agent is being used within an

animal room.

Security-sensitive agent information and occupational health requirements

should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery

plans, as a contingency for man-made or natural disasters.1,3,4

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent

   contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.

   Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where

   infectious materials and/or animals are housed or are manipulated.

   Persons must wash their hands after removing gloves, and before leaving the

   areas where infectious materials and/or animals are housed or are

   manipulated. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and

   storing food for human use should only be done in designated areas and are

   not permitted in animal or procedure rooms.

9. All procedures are carefully performed to minimize the creation of aerosols or

   splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt

improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

e. Equipment containing sharp edges and corners should be avoided.

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.

13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.

14. An effective integrated pest management program is required See Appendix G.

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.
Decontaminate of all potentially infectious materials before disposal using an effective method.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 2

B. *Special Practices*

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and

   administered appropriate immunizations for agents handled or potentially

   present, before entry into animal rooms.

   When appropriate, a base line serum sample should be stored**.**

2. Procedures involving a high potential for generating aerosols should be

   conducted within a biosafety cabinet or other physical containment device.

   When a procedure cannot be performed within a biosafety cabinet, a

   combination of personal protective equipment and other containment devices

   must be used. Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc).

3. Decontamination is recommended for all potentially infectious materials and

   animal waste before movement outside the areas where infectious materials

   and/or animals are housed or are manipulated by an appropriate method (e.g.

   autoclave, chemical disinfection, or other approved decontamination

   methods). This includes potentially infectious animal tissues, carcasses,

   contaminated bedding, unused feed, sharps, and other refuse.

   Consideration should be given to means for decontaminating routine

   husbandry equipment, sensitive electronic and medical equipment.

Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must contain a universal biohazard label. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

4. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

   Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

5. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records

maintained.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 2

C. *Safety Equipment* (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab

   coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols or splashes. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

   When indicated by risk assessment, animals are housed in primary biosafety

   containment equipment appropriate for the animal species, such as solid wall

   and bottom cages covered with filter bonnets for rodents, or larger cages

   placed in inward flow ventilated enclosures or other equivalent primary

   containment systems for larger animal cages.

2. A risk assessment should determine the appropriate type of personal

   protective equipment to be utilized. Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for anticipated splashes/ sprays from infectious or other hazardous

materials and when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with the NHP should assess risk of mucous membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, faceshields, etc.) as needed. Respiratory protection is worn based upon risk assessment.

4. Gloves are worn to protect hands from exposure to hazardous materials. A

risk assessment should be performed to identify the appropriate glove for the

task and alternatives to latex gloves should be available. Gloves are changed when contaminated, integrity has been compromised, or when otherwise necessary. Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other

contaminated waste. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 2

D. *Laboratory Facilities (Secondary Barriers)*

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.

Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.

   Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings)

are water resistant.

Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control and proper cleaning. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

   Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated.

   Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, windows should be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.1 The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided.

Exhaust air is discharged to the outside without being recirculated to other rooms.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.

8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage/equipment cleaning process.

10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

11. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

    HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection.

    Provisions to assure proper safety cabinet performance and air system operation must be verified. Correct performance of the BSCs should be recertified at least once a year.

    All BSCs should be used according to manufacturer's recommendation, to protect the worker and avoid creating a hazardous environment from volatile chemical and gases.

12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.

13. An autoclave should be considered in the animal facility to facilitate

    decontamination of infectious materials and waste.

14. Emergency eyewash and shower are readily available; location is determined

    by risk assessment.

Animal Biosafety Level 3

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

ABSL-3 laboratory has special engineering and design features.

ABSL-3 requires that 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented.

The following standard and special safety practices, safety equipment, and facility requirements apply to ABSL-3

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 3

A. *Standard Microbiological Practices*

1. The animal facility director establishes and enforces policies, procedures, and

   protocols for institutional policies and emergency situations. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study animal protocols must also be reviewed and approved by the IACUC5 and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in

   consultation with the animal facility director and appropriate safety

   professionals.

   The safety manual must be available and accessible. Personnel are advised of potential and special hazards, and are required to read and follow instructions on practices and procedures. Consideration should be given to specific biohazards unique to the animal species and protocol in use.

3. Supervisor must ensure that animal care, laboratory and support personnel

   receive appropriate training regarding their duties, animal husbandry

   procedure, potential hazards, manipulations of infectious agents, necessary

   precautions to prevent hazard or exposures, and hazard/exposure evaluation

   procedures (physical hazards, splashes, aerosolization, etc.). Personnel must

   receive annual updates or additional training when procedures or policies

   change. Records are maintained for all hazard evaluations, employee training

sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

   Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel),

telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security-sensitive agent information and occupational health requirements

should be posted in accordance with the institutional policy. Advance consideration should be given to emergency and disaster recovery

plans, as a contingency for man-made or natural disasters.1,3,4

6. Access to the animal room is limited to the fewest number of individuals

possible. Only those persons required for program or support purposes are

authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated.

All persons including facility personnel, service workers, and visitors are

advised of the potential hazards (natural or research pathogens, allergens, etc) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent

contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious/ and hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment.

Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where

infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.

9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps**.** Plasticware should be substituted for glassware whenever possible.

e. Equipment containing sharp edges and corners should be avoided.

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any

spills, splashes, or other overt contamination.

13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.

14. An effective integrated pest management program is required. See Appendix G.

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontamination of all potentially infectious materials before disposal using an effective method.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 3

B. *Special Practices*

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. When appropriate, a base line serum sample should be stored.

2. All procedures involving the manipulation of infectious materials, handling

   infected animals or the generations of aerosols must be conducted within

   BSCs or other physical containment devices when practical. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

   Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical

   restraint devices, chemical restraint medications, etc).

3. The risk of infectious aerosols from infected animals or their bedding also can

   be reduced if animals are housed in containment caging systems (such as solid wall and bottom cages covered with filter bonnets, open cages placed in

   inward flow ventilated enclosures, HEPA-filter isolators and caging systems,

   or other equivalent primary containment systems).

4. Actively ventilated caging systems must be designed to prevent the escape of

   microorganisms from the cage. Exhaust plenums for these systems should be

   sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate when operational malfunctions occur.

5. A method for decontaminating all infectious materials must be available

within the facility, preferably within the areas where infectious materials and/or animals are housed or are manipulated (e.g. autoclave, chemical disinfection, or other approved decontamination methods).

Consideration should be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment.
Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) before removal from the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method.
It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and / or animals are housed or are manipulated, preferably within the caging system.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

6. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 3

C. *Safety Equipment* (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs, and other physical containment devices or equipment, should be used for all manipulations for infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

The risk of infectious aerosols from infected animals or bedding can be reduced through the use of primary barrier systems. These systems may

include solid wall and bottom cages covered with filter bonnets; ventilated cage rack systems; or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Protective clothing such as uniforms or scrub suits is worn by personnel within the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.

Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.

Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility.

Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Appropriate eye, face and respiratory protection are worn by all personnel entering areas where infectious materials and/or animals are housed or are manipulated. To prevent cross contamination boots, shoe covers, or other

protective footwear, are used where indicated. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

4. Gloves are worn to protect hands from exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for

the task and alternatives to latex gloves should be available.

Procedures may require the use of wearing two pairs of gloves (double-glove).

Gloves are changed when contaminated, integrity has been compromised, or

when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that

prohibits transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other

contaminated waste.

Persons must wash their hands after handling animals and before leaving the

areas where infectious materials and/or animals are housed or are

manipulated. Hand washing should occur after the removal of gloves.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 3

D. *Laboratory Facilities (Secondary Barriers)*

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted.

   Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

   Entry into the containment area is via a double-door entry which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.

2. A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for

hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated.

If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or are manipulated, a sink must also be available for hand washing at the exit from each segregated area.

Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control, proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases.

   Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.

4. Cabinets and bench tops must be impervious to water and resistant to heat,

organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation to the facility should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.1 The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.

   This system creates directional airflow which draws air into the animal room from "clean" areas and toward "contaminated" areas.

   Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

Filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areasand air intakes, or the exhaust must be HEPA-filtered.

Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under failure conditions the airflow will not be reversed. Audible alarms should be considered to notify personnel of ventilation and HVAC system failure.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.

8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space.

The cage wash facility should be designed and constructed to accommodate high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage cleaning process.

10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

11. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with its proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

    HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance.

    Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room.

All BSCs should be used according to manufacturers' recommendations. When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

12. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special practices should be developed for transport of infectious materials designated alternate location/s within the facility.

13. Emergency eyewash and shower are readily available; location is determined by risk assessment.

14. The ABSL-3 facility design and operational procedures must be documented.

The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.

15. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision or

effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.

Animal Biosafety Level 4

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring ABSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or to re-designate the level. Animal care staff must have specific and thorough training in handling extremely hazardous, infectious agents and infected animals.

Animal care staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All animal care staff and supervisors must be competent in handling animals, agents and procedures requiring ABSL-4 containment. Access to the animal facility within the ABSL-4 laboratory is controlled by the animal facility director and/or laboratory supervisor in accordance with institutional policies.

There are two models for ABSL-4 laboratories:

(1) A *Cabinet Laboratory* where all handling of agents, infected animals and housing of infected animals must be performed in Class III BSCs (See Appendix A).

(2) A *Suit Laboratory* where personnel must wear a positive pressure protective

suit (See Appendix A); infected animals must be housed in ventilated enclosures with inward directional airflow and HEPA filtered exhaust; infected animals

should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

ABSL-4 builds upon the standard practices, procedures, containment equipment,

and facility requirements of ABSL-3**.** However ABSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment and personnel.

The ABSL-4 cabinet laboratory is distinctly different from an ABSL-3 laboratory containing a Class III BSC.

Animal Biosafety Level 4 is beyond the scope of current practice here at Mount Sinai School of Medicine. Refer to the BMBL for further information on BSL-4.

Part Three:

A. Recombinant DNA Research

All pratices as recommended under the NIH "***Guidelines for Research Involving Recombinant DNA Molecules"*** are adopted by Icahn School of Medicine at Mount Sinai (See: **http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.** The School receives funding from NIH grants, and as a recipient**, must adhere** to these and other NIH regulations. The NIH asserts that all research, NIH-funded and Non-NIH funded must be conducted in accordance with the guidelines issued by the Office of Biotechnology Activities, and that this Office is the entity charged with oversight and regulation of all recombinant DNA activities in the United States.

The "***Guidelines"*** have specific sections addressing risk assessment with respect to the hazards associated with the manipulation of microorganisms. Appendices "A", "B", "G", "H", and

"K" should be reviewed and understood completely before designing experiments. Specific transgenic animal and human gene therapy protocols require ISM –MS Institutional Biosafety Committee review and approval. Gene Therapy protocols and product development (INDs) require both NIH and FDA review and approval ***before*** initiation of any activity or enrollment of human subjects.

B. Human Immunodeficiency Virus, Hepatitis B Virus, and Other Potentially Infectious Material (Bloodborne Pathogens)

The Occupational Safety and Health Administration (OSHA) have issued specific regulations governing the materials identified under this heading in ***29 CFR 1910.1030 the Bloodborne Pathogen Standard***. (See: **http://www.osha-slc.gov/OshStd_data/1910_1030.html**). ***This is an enforceable standard with very specific requirements for research involving these materials***. All research using human-derived tissues and body fluids requires full compliance with the Standard (Universal) Precautions at a minimum.

Projects utilizing HIV, HBV viruses must be conducted in accordance with section *29 CFR 1910.1030(e) (1)-(5).* It is to be noted, that although the specific CDC and NIH Guidelines referred to through out this manual do not have force of law the same as the OSHA Standards, OSHA can invoke the ***"General Duty Clause"*** to apply these guidelines as "consensus standards" and can issue citations and levy fines against the School for each infraction uncovered.

C. CULTURE PROCEDURES

Long-Term Tissue Culture

Concern has been expressed in the literature by several researchers over the possibility of acquiring infections from the manipulation of tissue cultures and established cell lines.

While there is still uncertainty as to whether such infections can occur, a common-sense approach to handling tissue cultures is to consider these materials as *other potentially infectious materials*, and to use biological safety cabinets and other aerosol prevention practices. Most often the cabinets are used to keep cultures uncontaminated, but in addition to this, a greater part of the hazards of aerosol generation are also reduced. All laboratory work should be performed using the practices specified

under **Biosafety Level 2, at *a minimum.*** If the cell line is developed from human cells, OSHA would regulate all manipulations under the ***Bloodborne Pathogen Standard,*** www.osha.gov

The standard microbiological practices concerning hygiene, pipette use, waste disposal etc., as outlined under Biosafety Level 2, ensure the safety of those workers handling moderately infectious agents. These procedures will also protect against transforming viruses, viruses that are shed in cultures, and the microorganisms found in contaminated cultures.

It is recommended that workers handling established cell lines identify the viruses that may be present in the cells, and practice the safety procedures specific for that agent listed in ***Chapter 5. B:, Classification of Biohazardous Agents*.** Repositories such as the American Type Culture Collection, ATCC, http://www.atcc.org/, can provide this information to you on request.

Reviewed / revised: 9/2018 Pgh:

PART FOUR Summary Tables and Sample Door Sign

Table 1: Summary of Recommended Biosafety Levels for Infectious Agents [ref.6]

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 plus: practice Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering Baseline serum	Primary barriers = Class I or II BCSs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: Physical separation from access corridors Self-closing, double-door access Exhausted air not recircul-ated Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: Clothing change before entering Shower on exit All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: Separate building or isolated zone; Dedicated supply and exhaust, vacuum, and decon systems; Other requirements outlined in the text

Table 2. Summary of Recommended Biosafety Levels for Activities in Which Experimentally or Naturally Infected Vertebrate Animals Are Used[ref.6)]

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy human adults.	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species.	Standard animal facility No recirculation of exhaust air Directional air flow recommended Handwashing sink recommended
2	Associated with human disease. Hazard: percutaneous exposure, ingestion, mucous membrane exposure.	ABSL-1 practices plus: Limited access Biohazard warning signs Sharps precautions Biosafety manual Decontamination of all infectious wastes and of animal cages prior to washing	ABSL-1 equipment plus primary barriers: containment equipment appropriate for animal species; PPES: laboratory coats, gloves, face and respiratory protection as needed.	ABSL-1 facility plus: Autoclave available Handwashing sink available in the animal room. Mechanical cage washer used
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects.	ABSL-2 practices plus: Controlled access Decontamination of clothing before laundering Cages decontaminated before bedding removed Disinfectant foot bath as needed	ABSL-2 equipment plus: Containment equipment for housing animals and cage dumping activities Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: appropriate respiratory protection	ABSL-2 facility plus: Physical separation from access corridors Self-closing, double-door access Sealed penetrations Sealed windows Autoclave available in facility
4	Dangerous/exotic agents that pose high risk of life threatening disease; aerosol transmission, or related agents with unknown risk of	ABSL-3 practices plus: Entrance through change room where personal clothing is removed and laboratory clothing	ABSL-3 equipment plus: Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel	ABSL-3 facility plus: Separate building or isolated zone

	transmission.	is put on; shower on exiting All wastes are decontaminated before removal from the facility	suit) used for all procedures and activities	Dedicated supply and exhaust, vacuum and decontamination systems Other requirements outlined in the text

BIOHAZARD

Admittance to Authorized Personnel Only

Principal Investigator_____

Biological

Agent _____

Risk Group ☐1 ☐2 ☐3 **Entry By:** ☐Lab Staff ☐Maintenance

Biosafety Level ☐1 ☐2 ☐3 ☐Emergency Personnel

Emergency Contact_____ **Phone**_____

Icahn School of Medicine at Mount Sinai

Figure 1. Sample Door Sign

Optional Flask

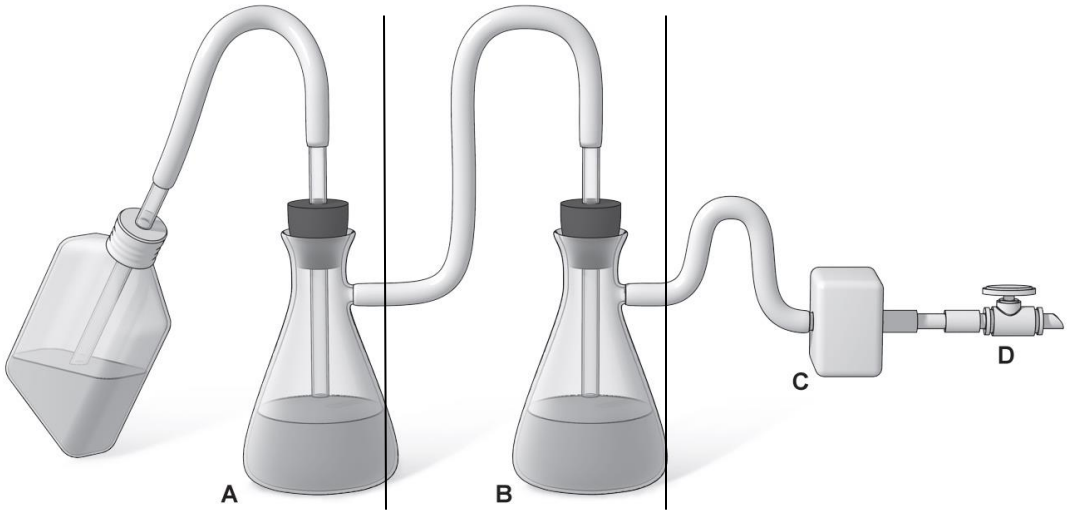

Figure 2 : The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution (undiluted bleach, Virkon or other disinfectant).; the right flask (B) serves as a fluid overflow collection vessel(Optional). An in-line HEPA filter (C) is used to protect the vacuum system (D) and is required by the NIH Guidelines from aerosolized microorganisms.

BSL-2.D.7 and BSL-3.D.7(above)

"*Vacuum lines should** be protected with High Efficiency Particulate Air*

(HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid

disinfectant traps may be required."

** NIH Guidelines***: **MUST be protected*** for rDNA work

Chapter 4: Biological Risk Assessment

(This Section is QUOTED Entirely from CDC-NIH *BMBL* 5th Edition)

A. BIOLOGICAL RISK ASSESSMENT

Risk assessment is an important responsibility for directors and principal investigators of microbiological and biomedical laboratories. Institutional biosafety committees (IBC), animal care and use committees, biological safety professionals, and laboratory animal veterinarians share in this responsibility. Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection.

The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs. Laboratory directors and principal investigators should use risk assessment to alert their staffs to the hazards of working with infectious agents and to the need for developing proficiency in the use of selected safe practices and containment equipment. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building, and the public.

Risk assessment requires careful judgment. Adverse consequences are more likely to occur if the risks are underestimated. By contrast, imposition of safeguards more rigorous than actually needed may result in additional expense and burden for the laboratory, with little safety enhancement. Unnecessary burden may result in circumvention of required safeguards. However, where there is insufficient information to make a clear determination of risk, it is prudent to consider the need for additional safeguards until more data are available.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. In addition, the capability of the laboratory staff to control hazards must be considered. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.

The agent summary statements contained in BMBL identify the primary agent and procedure hazards for specific pathogens and recommend precautions for their control. The guest editors and contributors of this and previous editions of BMBL based their recommendations on an assessment of the risks associated with the handling of pathogens using generally routine generic laboratory procedures. A review of the summary statement for a specific pathogen is a helpful starting point for assessment of the risks of working with that agent and those for a similar agent.

B. HAZARDOUS CHARACTERISTICS OF AN AGENT

The principal hazardous characteristics of an agent are: its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease. The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on these principal characteristics and the route of transmission of the natural disease.[1]

The four groups address the risk to both the laboratory worker and the community. The *NIH Guidelines* established a comparable classification and assigned human etiological agents into four risk groups on the basis of hazard.[2] The descriptions of the WHO and NIH risk group classifications are presented in Table 1. They correlate with but do not equate to biosafety levels. A risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level. See Section 3 for a further discussion of the differences and relatedness of risk groups and biosafety levels.

Other hazardous characteristics of an agent include probable routes of transmission of laboratory infection, infective dose, stability in the environment, host range, and its endemic nature. In addition, reports of LAIs are a clear indicator of hazard and often are sources of information helpful for identifying

agent and procedural hazards, and the precautions for their control. The absence of a report does not indicate minimal risk.

Reports seldom provide incidence data, making comparative judgments on risks among agents difficult. The number of infections reported for a single agent may be an indication of the frequency of use as well as risk. Nevertheless, reporting of LAIs by laboratory directors in the scientific and medical literature is encouraged. Reviews of such reports and analyses of LAIs identified through extensive surveys are a valuable resource for risk assessment and reinforcement of the biosafety principles.

The summary statements in BMBL include specific references to reports on LAIs. The predominant probable routes of transmission in the laboratory are:

1) direct skin, eye or mucosal membrane exposure to an agent;

2) parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors; 3) ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and

4) inhalation of infectious aerosols.

An awareness of the routes of transmission for the natural human disease is helpful in identifying probable routes of transmission in the laboratory and the potential for any risk to the public health. For example, transmission of infectious agents can occur by direct contact with discharges from respiratory mucous membranes of infected persons, which would be a clear indication that a laboratory worker is at risk of infection from mucosal membrane exposure to droplets generated while handling that agent. The American

Public Health Association publication *Control of Communicable Diseases Manual* is an excellent reference for identifying both natural and often noted laboratory modes of transmission.[3]

TABLE 3. CLASSIFICATION OF INFECTIOUS MICROORGANISMS BY RISK GROUP

RISK GROUP	CLASSIFICATION from *NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES* 2002(2)	WORLD HEALTH ORGANIZATION *LABORATORY BIOSAFETY MANUAL* 3RD EDITION 2004 (1)
Risk Group 1	Agents that are not associated with disease in healthy adult humans.	(No or low individual and community risk) A microorganism that is unlikely to cause human or animal disease.
Risk Group 2	*Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.*	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
	Agents that are associated with serious or lethal human disease for which preventive or	(High individual risk; low community risk) A pathogen that usually causes serious human or animal

Risk Group 3	therapeutic interventions *may be* available (high individual risk but low community risk).	disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents that 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).	(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.[3]

However, it is important to remember that the nature and severity of disease caused by a laboratory infection and the probable laboratory route of transmission of the infectious agent may differ from the route of transmission and severity associated with the naturally-acquired disease.[4]

An agent capable of transmitting disease through respiratory exposure to infectious aerosols is a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. This hazard requires special caution because infectious aerosols may not be a recognized route of transmission for the natural disease. Infective dose and agent stability are particularly important in establishing the risk of airborne transmission of disease. For example, the reports of multiple infections in laboratories associated with the use of *Coxiella burnetii* are explained by its low inhalation infective dose, which is estimated to be ten inhaled infectious particles, and its resistance to environmental stresses that enables the agent to survive outside of a living host or culture media long enough to become an aerosol hazard.[5]

When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents require careful consideration in risk assessment. Evidence that experimental animals can shed zoonotic agents and other infectious agents under study in saliva, urine, or feces is an important indicator of hazard. The death of a primate center laboratory worker from Cercopithecine herpesvirus 1 (CHV-1, also known as monkey B virus) infection following an ocular splash exposure to biologic material from a rhesus macaque emphasizes the seriousness of this hazard.[6] Lack of awareness for this potential hazard can make laboratory staff vulnerable to an unexpected outbreak involving multiple infections.[7]

Experiments that demonstrate transmission of disease from an infected animal to a normal animal housed in the same cage are reliable indicators of hazard. Experiments that do not demonstrate transmission, however, do not rule out hazard. For example, experimental animals infected with *Francisella tularensis*, *Coxiella burnetii*, *Coccidioides immitis*, or *Chlamydia psittaci*, agents that have caused many LAIs, rarely infect cagemates.[8]

The origin of the agent is also important in risk assessment. Non-indigenous agents are of special concern because of their potential to introduce risk of transmission, or spread of human and animal or infectious diseases from foreign countries into the United States.

Importation of etiological agents of human disease requires a permit from the CDC. Importation of many etiological agents of livestock, poultry and other animal diseases requires a permit from the USDA's Animal and Plant Health Inspection Service (APHIS). For additional details see Appendix F.

Genetically-modified agent hazards. The identification and assessment of hazardous characteristics of genetically modified agents involve consideration of the same factors used in risk assessment of the wild-type organism. It is particularly important to address the possibility that the genetic modification could increase an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments. The risk assessment can be difficult or incomplete, because important information may not be available for a newly engineered agent. Several investigators have reported that they observed unanticipated enhanced virulence in recent studies with engineered agents.[9-12] These observations give reason to remain alert to the possibility that experimental alteration of virulence genes may lead to

increased risk. It also suggests that risk assessment is a continuing process that requires updating as research progresses.

The *NIH Guidelines* are the key reference in assessing risk and establishing an

appropriate biosafety level for work involving recombinant DNA molecules.[2] The

purpose of the *NIH Guidelines* is to promote the safe conduct of research involving recombinant DNA. The guidelines specify appropriate practices and procedures for research involving constructing and handling both recombinant DNA molecules and organisms and viruses that contain recombinant DNA. They define recombinant DNA as a molecule constructed outside of a living cell with the capability to replicate in a living cell. The *NIH Guidelines* explicitly address experiments that involve introduction of recombinant DNA into Risk Groups 2, 3, and 4 agents, and experiments in which the DNA from Risk Groups 2, 3, and 4 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. Compliance with the *NIH Guidelines* is mandatory for investigators conducting recombinant DNA research funded by the NIH or performed at, or sponsored by, any public or private entity that receives any NIH funding for

recombinant DNA research. Many other institutions have adopted these guidelines as the best current practice.

The *NIH Guidelines* were first published in 1976 and are revised on an ongoing basis in response to scientific and policy developments. They outline the roles and responsibilities of various entities affiliated with recombinant DNA research, including institutions, investigators, and the NIH. Recombinant DNA research subject to the *NIH Guidelines* may require: 1) approval by the NIH Director, review by the NIH Recombinant DNA Advisory Committee (RAC), and approval by the IBC; or 2) review by the NIH Office of Biotechnology Activities (OBA) and approval by the IBC; or 3) review by the RAC and approvals by the IBC and Institutional Review Board; or 4) approval by the IBC prior to initiation of the research; or 5) notification of the IBC simultaneous with initiation of the work. It is important to note that review by an IBC is required for all non-exempt

experiments as defined by the *NIH Guidelines*.

The *NIH Guidelines* were the first documents to formulate the concept of an IBC as the responsible entity for biosafety issues stemming from recombinant DNA research. The NIH Guidelines outlines the membership, procedures, and functions of an IBC. The institution is ultimately responsible for the effectiveness of the IBC, and may define additional roles and responsibilities for the IBC apart from those specified in the NIH Guidelines. See Appendix J for more information about the *NIH Guidelines* and OBA.

Cell cultures. Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. This risk is well understood and illustrated by the reactivation of herpes viruses from latency,[13,14] the inadvertent transmission of disease to organ recipients,[15,16] and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) within infected individuals in the U.S. population.[17] There also is evidence of accidental transplantation of human tumor cells to healthy recipients which indicates that these cells are potentially hazardous to laboratory workers who handle them.[18] In addition, human and animal cell lines that are not well characterized or are obtained from secondary sources may introduce an infectious hazard to the laboratory. For example, the handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple LAIs.[19] The potential for human cell lines to harbor a bloodborne pathogen led the Occupational Health and Safety Administration (OSHA) to interpret that the occupational

exposure to bloodborne pathogens final rule would include human cell lines.[17]

C. HAZARDOUS CHARACTERISTICS OF LABORATORY PROCEDURES

Investigations of LAIs have identified five principal routes of laboratory transmission. These are parenteral inoculations with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, animal bites and scratches, and inhalation exposures to infectious aerosols.

The first four routes of laboratory transmission are easy to detect, but account for less than 20 percent of all reported LAIs. [20] Most reports of such infections do not include information sufficient to identify

the route of transmission of infection. Work has shown that the probable sources of infection—animal or ectoparasite, clinical specimen, agent, and aerosol—are apparent in approximately 50 percent of cases.[21]

Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are extremely pervasive, placing the laboratory worker carrying out the procedure and other persons in the laboratory at risk of infection.

There is general agreement among biosafety professionals, laboratory directors and principal investigators who have investigated LAIs that an aerosol generated by procedures and operations is the probable source of many LAIs, particularly in cases involving workers whose only known risk factor was that they worked with an agent or in an area where that work was done.

Procedures that impart energy to a microbial suspension will produce aerosols.

Procedures and equipment used routinely for handling infectious agents in laboratories, such as pipetting, blenders, non-self contained centrifuges, sonicators and vortex mixers are proven sources of aerosols. These procedures and equipment generate respirable-size particles that remain airborne for protracted periods.

When inhaled, these particles are retained in the lungs creating an exposure hazard for the person performing the operation, coworkers in the laboratory, and a potential hazard for persons occupying adjacent spaces open to air flow from the laboratory. A number of investigators have determined the aerosol output of common laboratory procedures. In addition, investigators have proposed a model for estimating inhalation dosage from a laboratory aerosol source. Parameters that characterize aerosol hazards include an agent's inhalation infective dose, its viability in an aerosol, aerosol concentration, and particle size.[22, 23, 24]

Procedures and equipment that generate respirable size particles also generate larger size droplets that can contain multiple copies of an infectious agent. The larger size droplets settle out of the air rapidly, contaminating the gloved hands and work surface and possibly the mucous membranes of the persons performing the procedure. An evaluation of the release of both respirable particles and droplets from laboratory operations determined that the respirable component is relatively small and does not vary widely; in contrast hand and surface contamination is substantial and varies widely.[25] The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

Technique can significantly impact aerosol output and dose. The worker who is careful and proficient will minimize the generation of aerosols. A careless and hurried worker will substantially increase the aerosol hazard. For example, the hurried worker may operate a sonic homogenizer with maximum aeration whereas the careful worker will consistently operate the device to assuring minimal aeration.

Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration.[22] Similar results were shown for pipetting with bubbles and with minimal bubbles. Containment and good laboratory practices also reduce this risk.

D. POTENTIAL HAZARDS ASSOCIATED WITH WORK PRACTICES, SAFETY EQUIPMENT AND FACILITY SAFEGUARDS

Workers are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous agents. Protection depends on the conscientious and proficient use of good microbiological practices and the correct use of safety equipment. A risk assessment should identify any potential deficiencies in the practices of the laboratory workers. Carelessness is the most serious concern, because it can compromise any safeguards of the laboratory and increase the risk for coworkers.

Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for a laboratory staff in order to reduce the inherent risks that attend work with hazardous agents. Not all workers who join a laboratory staff will have these prerequisite traits even though they may possess excellent scientific credentials. Laboratory directors or principal investigators should train and retrain new staff to the point where aseptic techniques and safety precautions become second nature.[26]

There may be hazards that require specialized personal protective equipment in addition to safety glasses, laboratory gowns, and gloves. For example, a procedure that presents a splash hazard may require the use of a mask and a face shield to provide adequate protection. Inadequate training in the proper use of personal protective equipment may reduce its effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker. For example, a respirator may impart a risk to the wearer independent of the agents being manipulated.

Safety equipment such as Biological Safety Cabinets (BSC), centrifuge safety cups, and sealed rotors are used to provide a high degree of protection for the laboratory worker from exposure to microbial aerosols and droplets. Safety equipment that is not working properly is hazardous, especially when the user is unaware of the malfunction. The containment capability of a BSC is compromised by poor location, room air currents, decreased airflow, leaking filters, raised sashes, crowded work surfaces, and poor user technique. The safety characteristics of modern centrifuges are only effective if the equipment is operated properly. Training in the correct use of equipment, proper procedure, routine inspections and potential malfunctions, and periodic re-certification of equipment, as needed, is essential.

Facility safeguards help prevent the accidental release of an agent from the laboratory. Their use is particularly important at BSL-3 and BSL-4 because the agents assigned to those levels can transmit disease by the inhalation route or can cause life-threatening disease. For example, one facility safeguard is directional airflow. This safeguard helps to prevent aerosol transmission from a laboratory into other areas of the building.

Directional airflow is dependent on the operational integrity of the laboratory's heating, ventilation, and air conditioning (HVAC) system. HVAC systems require careful monitoring and periodic maintenance to sustain operational integrity. Loss of directional airflow compromises safe laboratory operation. BSL-4 containment facilities provide more complex safeguards that require significant expertise to design and operate.

Consideration of facility safeguards is an integral part of the risk assessments. A

biological safety professional, building and facilities staff, and the IBC should help assess the facility's capability to provide appropriate protection for the planned work, and recommend changes as necessary. Risk assessment may support the need to include additional facility safeguards in the construction of new or renovation of old BSL-3 facilities.

E. AN APPROACH TO ASSESS RISKS AND SELECT APPROPRIATE SAFEGUARDS

Biological risk assessment is a subjective process requiring consideration of many hazardous characteristics of agents and procedures, with judgments based often on incomplete information. There is no standard approach for conducting a biological risk assessment, but some structure can be helpful in guiding the process. This section describes a five-step approach that gives structure to the risk assessment process.

First, identify agent hazards and perform an initial assessment of risk. Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments.

There are several excellent resources that provide information and guidance for making an initial risk assessment. The BMBL provides agent summary statements for some agent statements also identify known and suspected routes of transmission of laboratory infection and, when available, information on

infective dose, host range, agent stability in the environment, protective immunizations, and attenuated strains of the agent.

A thorough examination of the agent hazards is necessary when the intended use of an agent does not correspond with the general conditions described in the Summary Statement or when an agent summary statement is not available.

Although a summary statement for one agent may provide helpful information for assessing the risk of a similar agent, it should not serve as the primary resource for making the risk determination for that agent. Refer to other resources for guidance in identifying the agent hazards.

The *Control of Communicable Diseases Manual* (APHA Publication) provides information on communicable diseases including concise summaries on severity, mode of transmission, and the susceptibility and resistance of humans to disease.[3] In addition, it is always helpful to seek guidance from colleagues with experience in handling the agent and from biological safety professionals.

Often there is not sufficient information to make an appropriate assessment of risk. For example, the hazard of an unknown agent that may be present in a diagnostic specimen will be unknown until after completing agent identification and typing procedures.

It would be prudent in this case to assume the specimen contains an agent presenting the hazardous classification that correlates with BSL-2 unless additional information suggests the presence of an agent of higher risk. Identification of agent hazards associated with newly emergent pathogens also requires judgments based on incomplete information. Consult interim biosafety guidelines prepared by the CDC and the WHO for risk assessment guidance. When assessing the hazards of a newly attenuated pathogen, experimental data should support a judgment that the attenuated pathogen is less hazardous than the wild-type parent pathogen before making any reduction in the containment recommended for that pathogen.

Make a preliminary determination of the biosafety level that best correlates with the initial risk assessment based on the identification and evaluation of the agent hazards. Remember that aerosol and droplet routes of agent transmission also are important considerations in specification of safety equipment and facility design that result in a given BSL level.

Second, identify laboratory procedure hazards. The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

The complexity of a laboratory procedure can also present a hazard. The agent summary statement provides information on the primary laboratory hazards associated with typically routine procedures used in handling an agent. In proposed laboratory procedures where the procedure hazards differ from the general conditions of the agent summary statement or where an agent summary statement is not available, the risk assessment should identify specific hazards associated with the procedures.

Third, make a final determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment. The final selection of the appropriate biosafety level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards described in Sections 3, 4 and 5 of this

publication. There will be situations where the intended use of an agent requires greater precautions than those described in the agent's Summary Statement. These situations will require the careful selection of additional precautions. An obvious example would be a procedure for exposing animals to experimentally generated infectious aerosols. It is unlikely that a risk assessment would indicate a need to alter the recommended facility safeguards specified for the selected biosafety level. If this does occur, however, it is important that a biological safety professional validate this judgment independently before augmenting any facility secondary barrier.

It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Preexisting diseases, medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure to infants to certain agents, are some of the conditions that may increase

the risk of an individual for acquiring a LAI. Consultation with an occupational physician knowledgeable in infectious diseases is advisable in these circumstances.

Fourth, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment. The protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. In conducting a risk assessment, the laboratory director or principal investigator should ensure that laboratory workers have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of the agent, and have developed good habits that sustain excellence in the performance of those

practices. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and BSCs, ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is important insurance that a laboratory worker is capable of working safely.

The laboratory director or principal investigator should also ensure that the necessary safety equipment is available and operating properly. For example, a BSC that is not certified represents a potentially serious hazard to the laboratory worker using it and to others in the laboratory. The director should have all equipment deficiencies corrected before starting work with an agent.

Fifth, review the risk assessment with a biosafety professional, subject matter expert, and the IBC. A review of the risk assessment and selected safeguards by knowledgeable individuals is always beneficial and sometimes required by regulatory or funding agencies, as is the case with the *NIH Guidelines*.[2] Review of potentially high risk protocols by the local IBC should become standard practice. Adopting this step voluntarily will promote the use of safe practices in work with hazardous agents in microbiological and biomedical laboratories.

F. CONCLUSION

Risk assessment is the basis for the safeguards developed by the CDC, the NIH, and the microbiological and biomedical community to protect the health of laboratory workers and the public from the risks associated with the use of hazardous biological agents in laboratories. Experience shows that these established safe practices, equipment, and facility safeguards work.

New knowledge and experiences may justify altering these safeguards. Risk assessment, however, must be the basis for recommended change. Assessments conducted by laboratory directors and principal investigators for the use of emergent agents and the conduct of novel experiments will contribute to our understanding of the risks these endeavors may present and the means for their control. Those risk assessments will likely mirror progress in science and technology and serve as the basis for future revisions of BMBL.

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Chapter 5: Classification of Biohazardous Agents

This is an abbreviated section. Refer to the BMBL and NIH Guidelines for more detail.

A. Risk Assessment (ref. 6, 10, 13)*

Selection of an appropriate biosafety level for work with a particular agent or animal study depends upon a number of factors. Some of the most important are: the virulence, pathogenicity, biological stability, route of spread, and communicability of the agent; the nature or function of the laboratory; the procedures and manipulations involving the agent; the endemicity of the agent; and the availability of effective vaccines or therapeutic measures.

Agent Summary Statements are found in the *BMBL,* and provide guidance for the selection of appropriate biosafety levels. Specific information on laboratory hazards associated with a particular agent, and recommendations regarding practical safeguards that can significantly reduce the risk of laboratory-associated diseases, are included. Agent summary statements are presented for agents which meet one or more of the following criteria: the agent is a proven hazard to laboratory personnel working with infectious materials (e.g., hepatitis B virus, M. tuberculosis); the potential for laboratory associated infections is high, even in the absence of previously documented laboratory-associated infections (e.g., exotic arboviruses); or, the consequences of infection are grave.

Recommendations for the use of vaccines and toxoids are included in agent summary statements when such products are available, either as licensed or Investigational New Drug (IND) products. When applicable, recommendations for the use of these products are based on current recommendations of the Public Health Service Advisory Committee on Immunization Practice, and are specifically targeted to at-risk laboratory personnel and others who must work in or enter laboratory areas. These specific recommendations should in no way preclude the routine use of such products as diphtheria-tetanus toxoids, poliovirus vaccine, influenza vaccine and others, because of the potential risk of community exposures irrespective of any laboratory risks. Appropriate precautions should be taken in the administration of live attenuated virus vaccines in individuals with altered immunocompetence, or other medical condition (e.g., pregnancy), in which a viral infection could result in adverse consequences.

Risk assessments (*NIH Guidelines*) and biosafety levels recommended in the agent summary statements (BMBL) presuppose a population of immunocompetent individuals. Persons with altered immunocompetence may be at an increased risk when exposed to infectious agents. Immunodeficiency may be hereditary, congenital, or induced by a number of neoplastic or infectious diseases, by therapy, or by radiation. Refer to the following:

- http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
- http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

The risk of becoming infected or the consequence of infection may also be influenced by such factors as age, sex, race, pregnancy, surgery (e.g., splenectomy, gastrectomy), predisposing diseases (e.g., diabetes, lupus erythematosus) or altered physiological function. These and other variables must be considered in applying the generic risk assessments of the agent summary statements to specific activities of selected individuals.

The biosafety level assigned to an agent is based on the activities typically associated with the growth and manipulation of the quantities and concentrations of infectious agents required to accomplish identification or typing. If activities with clinical materials pose a lesser risk to personnel than those activities associated with manipulation of cultures, a lower biosafety level is recommended. On the other hand, if the activities involve large volumes and/or concentrated preparations ("production quantities"), or manipulations which are likely to produce aerosols or which are otherwise intrinsically hazardous, additional personnel precautions and increased levels of primary and secondary containment may be indicated.

"Production quantities" refers to large volumes or concentrations of infectious agents considerably in excess of those typically used for identification and typing activities. Propagation and concentration of infectious agents as occurs in large-scale fermentations, antigen and vaccine production, and a variety of other commercial and research activities, clearly deal with significant masses of infectious agents that are reasonably considered "production quantities". However, in terms of potentially increased risk as a function of the mass of infectious agents, it is not possible to define "production quantities" in finite volumes or concentrations for any given agent. Therefore, the laboratory director must make an assessment of the activities conducted and select practices, containment equipment, and facilities appropriate to the risk, irrespective of the volume or concentration of agent involved.

Occasions will arise when the laboratory director should select a biosafety level higher than that recommended. For example, a higher biosafety level may be indicated by the unique nature of the proposed activity (e.g., the need for special containment for experimentally generated aerosols for inhalation studies) or by the proximity of the laboratory to areas of special concern (e.g., a diagnostic laboratory located near patient care areas). Similarly, a recommended biosafety level may be adapted to compensate for the absence of certain recommended safeguards. For example, in those situations where Biosafety Level 3 is recommended, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for

identification, typing and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations, provided the recommended

Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 are rigorously followed.

One example involves work with the Human Immunodeficiency Viruses (HIVs). Routine diagnostic work with clinical specimens can be done safely at Biosafety Level 2, using Biosafety Level 2 practices and procedures. Research work (including co-cultivation, virus replication studies, or manipulations involving concentrated virus) can be done in a BSL-2 facility, using BSL-3 practices and procedures. Virus production activities, including virus concentrations, require a BSL-3 facility and use of BSL-3 practices and procedures (see Agent Summary Statement).

The decision to adapt Biosafety Level 3 recommendations in this manner should be made only by the laboratory director. This adaptation, however, is not suggested for agent production operations or activities where procedures are frequently changing. The laboratory director should also give special consideration to selecting appropriate safeguards for materials that may contain a suspected agent. For example, sera of human origin may contain hepatitis B virus, and therefore, all blood or blood-derived fluids should be handled under conditions which reasonably preclude cutaneous, mucous membrane or parenteral exposure of personnel. Sputa submitted to the laboratory for tubercle bacilli assay should be handled under conditions which reasonably preclude the generation of aerosols during the manipulation of clinical materials or cultures.

The infectious agents which meet the previously stated criteria are listed by category of agent in Section VII. To use these summaries, first locate the agent in the listing under the appropriate category of agent. Second, utilize the practices, safety equipment, and type of facilities recommended in the agent summary statement as described in Section VII for working with clinical materials, cultures or infectious agents, or infected animals.

The laboratory director is also responsible for appropriate risk assessment and for utilization of appropriate practices, containment equipment, and facilities for agents not included in the agent summary statements.

B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

This appendix (Section excerpted from the *NIH Guidelines)* includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of

representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all inclusive. Information on agent risk assessment may be found in the *Agent Summary Statements* of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories* (see Sections V-C, V-D, V-E, and V-F, *Footnotes and References of Sections I through IV*. Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the *NIH Guidelines*.

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

1. Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions), *Escherichia coli* K-12 (see Appendix C-II-A, *Escherichia coli* K-12 Host Vector Systems, Exceptions), adeno-associated virus (AAV) types 1 through 4, and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed. See: Pages 37 - 43 of the *NIH Guidelines* for specific agents and corresponding risk groups. See: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

2. Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

3. Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

4. Risk Group 4 (RG4) Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

5. Animal Viral Etiologic Agents in Common Use

The list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

6. Animal Viral Etiologic Agents in Common Use

--Feline sarcoma virus

--Gibbon leukemia virus

--Mason-Pfizer monkey virus

--Mouse mammary tumor virus

--Murine leukemia virus

--Murine sarcoma virus

--Rat leukemia virus

7. Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

8. Arboviruses

The American Committee on Arthropod-borne Viruses (ACAV) registered 535 arboviruses as of December 1991. In 1979, the ACAV's Subcommittee on Arbovirus Laboratory Safety (SALS) categorized each of 424 viruses then registered in the *Catalogue of Arboviruses and Certain Other Viruses of Vertebrates* into one of four recommended practices, safety equipment, and facilities described in this publication as Biosafety Levels 1-4 (ref. 6). Since 1980, SALS has periodically updated the 1980 publication by providing a supplemental listing and recommended levels of practice and containment for arboviruses registered since 1979. SALS recommended that the work with the majority of these agents should be conducted at the equivalent of Biosafety Level 2. SALS also recognizes five commonly used vaccine strains for which attenuation is firmly established, which may be handled at BSL-2, provided that personnel working with these strain are immunized. SALS has classified all registered viruses for which insufficient laboratory experience exists as BSL-3, and reevaluates the classification whenever additional experience is reported.

Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that 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)

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Chapter 6: Disinfectants and Sterilization

A. Disinfectants[ref. 4, 11, 13, 14, 15, 16, 17, 25]

The information presented in this section will provide a general guideline for selecting a particular disinfectant for use with a given agent.

The best way of ascertaining the suitability of a disinfectant against a particular agent is to challenge that agent with the disinfectant **at the manufacturer's recommended concentration,** or several concentrations in order to establish a kill curve. In general this is not necessary due to a large body of literature available on many disinfectants, and the manufacturer's own test results which can be obtained in many cases. (See: https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants EPA Registered Disinfectants). A brief description of the mode of action of each class of disinfectant is given below.

Although physical methods are often superior to chemical disinfection / sterilization, it is not practical to autoclave or subject many items to high heat, especially if the items can be damaged through repeated exposure to heat. Treatment of inert surfaces and heat labile materials can be accomplished through the use of disinfectants, provided that the following factors are considered: concentration of active ingredient, duration of contact between disinfectant and item to be disinfected, pH, temperature, humidity, and the presence of organic matter or soil load. The interplay of these factors will determine the degree of success in accomplishing either disinfection or sterilization. In all situations, review the manufacturer's recommendations for correct formulation and use. Do not attempt to use a chemical disinfectant for a purpose it was not designed for.

B. Disinfectant Groups

1. Aldehydes: (Formaldehyde, Paraformaldehyde, Glutaraldehyde)

Formaldehyde and its polymerized solid form, paraformaldehyde, have broad-spectrum biocidal activity and are both effective for surface and space decontamination. Formaldehyde gas is used to decontaminate large spaces and biological safety cabinets, and when used with superheated steam, can disinfect a terminal filtration bank. As a

liquid (5% concentration) it is an effective liquid decontaminant. Formaldehyde's drawbacks are reduction in efficacy at refrigeration temperature, its pungent, irritating odor, and several safety concerns. Formaldehyde is presently considered to be a carcinogen or a cancer-suspect agent according to several regulatory agencies.

The gas is explosive over a wide range of percentages **(7.0 – 73% v/v in air)** and has to be used with extreme caution since it has a high vapor pressure. Its biocidal action is through alkylation of carboxyl, hydroxyl and sulfhydryl groups on proteins. Cidex, a commercially prepared buffered glutaraldehyde disinfectant is used routinely for cold surface sterilization of clinical instruments.

2. Halogen-Based Biocides: (Iodine, Chlorine)

Both Chlorine and Iodine behave similarly with respect to biocidal activity, by binding to proteins and modifying sulfhydryl, amino, indole and phenolic groups, and generally acting as oxidizing agents. Sodium hypochlorite (household bleach) has an available chlorine content of 5.25%, or **52,500 ppm**. Because of its oxidizing power, it loses potency quickly and **must be made fresh** and used within the same day it is prepared. Care must be exercised in using chlorine-based disinfectants which can corrode or damage metal, rubber, and other susceptible surfaces.

Free organic matter such as protein, will compete for the chlorine ion against the microbial agent, thereby reducing the biocidal activity and making the disinfectant organic load dependent. Bleached articles should never be autoclaved without reducing the bleach with sodium thiosulfate or sodium bisulfate. Chloramine T which is prepared from sodium hypochlorite and p-toluenesulfonamide is a more stable, odorless, less corrosive form of chlorine but has decreased biocidal activity in comparison to bleach.

Wescodyne, *Betadyne*, Povidone-Iodine and other iodophors are commercially available Iodine-based disinfectants, which give good control when the manufacturer's instructions for formulation and application are followed. ***Both bleach and iodophors should be made up in cold water in order to prevent breakdown of the disinfectant.***

3. Quaternary Ammonium Compounds (Zephirin, CDQ, A-3)

These compounds are considered to be cationic detergents and in general are more effective against gram positive bacteria. The "quarts" are considered to be more biostatic than biocidal, and are generally ineffective against unenveloped viruses, bacterial spores and *Mycobacterium tuberculosis.* The activity is reduced when mixed with soaps, detergents, acids and when in the presence of heavy organic matter loads. Basically these compounds are not suitable for any type of terminal disinfection.

The mode of action of the "quats" is through membrane damage and leakage, followed by protein denaturation Many of these compounds are better used in water baths, incubators, and other applications where halide or phenolic residues are not desired.

The quaternary ammonium compounds are widely used as surface disinfectants. There have been some reports of health care–associated infections associated with contaminated quaternary ammonium compounds used to disinfect patient care supplies or equipment such as cystoscopes or cardiac catheters. As with several other dis-infectants (e.g., phenolics, iodophors), gram-negative bacteria have been found to survive or grow in them.

Results from manufacturers' data sheets and from published scientific literature indicate that the quaternaries sold as hospital disinfectants are generally fungicidal, bactericidal, and virucidal against lipophilic (enveloped) viruses; they are not sporicidal and generally not tuberculocidal or virucidal against hydrophilic (nonenveloped) viruses. Poor mycobactericidal activities of quaternary ammonium compounds have been reported.
The quaternaries are commonly used in ordinary environmental sanitation of noncritical surfaces such as floors, furniture, and walls. EPA-registered quaternary ammonium compounds are appropriate to use when disinfecting medical equipment that comes into contact with intact skin (e.g., blood pressure cuffs).

Source:

William A. Rutala, David J. Weber, in Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition), 2015

4. Phenolics: (O-phenophenoate-base Compounds)

These biocides act through membrane damage and are effective against enveloped viruses, rickettsiae, fungi and vegetative bacteria. They are not as adversely affected by organic loads as other disinfectants. Cresols, hexachlorophene, alkyl- and chloro-derivatives and diphenyls are more active than phenol itself. Available commercial products are *Amphyl, O-syl, Tergisyl, Lysol, Vesphene, L-Phase and Expose.*

5. Acids / Alkalis:

Strong mineral acids and alkalis have disinfectant properties proportional to the extent of their dissociation in solution. Some hydroxides are more effective than would be predicted from their values. In general acids are better disinfectants than alkalis. Mode of action is attributed to an increase of H^+ and OH^- species in solutions which interfere with certain microbial functions, however the total effect is not only dependent on pH alone.

Weak organic acids are more potent than inorganic acids despite low dissociation rates in solution. Action is attributed to the disruption of 2^o and 3^o conformation of enzymes and structural proteins.

6. Heavy Metals:

Soluble salts of mercury, silver lactate, mercuric chloride and miraculous chloride are efficient bactericidal agents. Silver nitrate and mercuric chloride are commonly used as 1:1000 aqueous solutions. Action is through attack on protein sulfhydryl groups and

disruption of enzyme functions. Organic matter can reverse the disinfectant properties of mercurials.

7. Alcohols:

Alcohols work through the disruption of cellular membranes, solubilization of lipids, and denaturation of proteins by acting directly on S-H functional groups. The compounds are effective against lipid-containing viruses and a broad spectrum of bacterial species, but ineffective against spore-forming bacteria. They can be combined with phenolics and iodine to enhance activity. They evaporate quickly, and leave no residue, but evaporation interferes with contact time unless the article is immersed in the alcohol. Alcohols are generally regarded as being non-corrosive.

Higher molecular weight alcohols are more effective but are less miscible with water, which is required for adequate effectiveness. Ethanol and isopropanol are used as 70-80% aqueous solutions. Absolute alcohols are not as effective indicating that some water is required in the disinfection process.

8. EPA Listed Disinfectants

EPA regulates pesticides under the statutory authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The registration requirements for antimicrobial pesticides differ somewhat from those of other pesticides. EPA maintains a list of approved disinfectants, and specifically indicates what microorganisms the disinfectant is effective against. Each disinfectant must have an EPA registration number posted on the container and specify what microbes it is effective against.

The following lists of antimicrobial products registered by the EPA for healthcare use are effective against the most common emerging pathogens, as indicated in the list titles. EPA-registered antimicrobial products may not make efficacy claims against these pathogens unless the agency has reviewed data to support the claim and approved the claim on the label.

Use of the listed EPA-registered products must be consistent with the product labeling and complies with the Occupational Safety and Health Administration's requirements for Occupational Exposure to blood borne Pathogens (29 CFR 1910). The following website has listed agents by categories as of October, 2014:

http://www.epa.gov/oppad001/chemregindex.htm .

C. Sterilization

1. Autoclave:

Autoclaving at a temperature of 121^{o} C (steam under pressure) at 20 psi is one of the most convenient and effective means of sterilization available. Care must be taken to ensure that the steam can circulate around articles in order to provide even heat distribution. The success of the sterilization is very time-dependent in liquid media, with large volumes requiring longer periods of time to reach the effective temperature within the media itself. Additionally, there should be no void spaces in the load that could insulate against the steam--this condition could prevent the transference of heat to the vessels resulting in no sterilization of the contents.

In dry loads small amounts of water (50-75 cc's per 30 gallon bag) should be included inside the autoclave bag to ensure sufficient moisture content within the load to allow for heat transference and distribution.

It is recommended that a Diack, or commercially available ***Geobacillus stearothermophilis*** or *Bacillus subtilis* var. niger test strips be used periodically to validate and document the killing efficiency of the autoclave. This is critically important if the autoclave is used for the sterilization of pathogenic cultures. Autoclave tape can be used for routine runs where glassware or sterile media are prepared before use.

2. Dry Heat:

Ovens operating at 160^{o} – 170^{o} C for periods of 2-4 hours are efficient for sterilizing glassware, or other non-porous heat conductive materials. It is unsatisfactory for organic and inorganic materials that can act as insulation and is also unsuitable for heat labile materials.

Incineration is a very effective means of final sterilization and disposal, and is also used for “spot” sterilization of inoculating needles and loops as well as flaming glassware during microbiological culturing procedures. Care has to be exercised when flaming “charged” items, since this practice can release infectious microaerosols through spattering.

3. Radiation:

Ionizing radiation is not used for general laboratory sterilization, however ultraviolet radiation (U.V.) is used to control airborne microorganisms and environmental surface decontamination. Ultraviolet sources are used in biological safety cabinets for *partial* contamination control. This form of control is extremely limited due its poor penetrating power, susceptibility to air movement, requirement for long contact time periods, and has not been documented as an effective control method.

4. Vapors and Gases:

From a practical point of view, formaldehyde, beta-propiolactone and ethylene oxide are not routinely used in laboratory sterilization practices. These sterilants are used in hospitals and commercial facilities where closed systems controlling temperature, humidity, and concentration are required to achieve sterilization using these agents.

Biological safety cabinets are decontaminated using paraformaldehyde heated to decomposition in order to release formaldehyde gas. This procedure should be performed only by personnel trained in this procedure due to the explosive nature of Formaldehyde.

Of the sterilants listed above, Ethylene Oxide (ETO) has wide use as an alkyl ting agent with very broad biocidal activity including spores and viruses. Because of its toxicity and potential carcinogenicity, it is not used today.

Instruments and optics that may be damaged by other sterilization methods, rooms, buildings and air-handling systems in particular are also sterilized using these sterilants. All of these sterilants are extremely toxic, and are regulated under OSHA and EPA regulations.

As a final note, the desired result for any treatment is to arrive at a significant reduction in the numbers of infectious entities (several orders of magnitude or logs of reduction), with the final result that there is no longer a risk of acquiring an infection while handling the materials after treatment. Complete sterility with the exception of media preparation and the disposal of some highly infectious agents, is not required for the disposal of infectious waste.

D. Useful Dilutions of *Wescodyne* and Common Household Bleach

1. Standard Wescodyne Solution:

3 ounces = 90 cc; 1.2 cc / 5 gallons = 1 ppm solution

90cc	=	36cc	=	18cc	=	2.37cc	= 75 ppm available iodine
5 gal		2 gal		1 gal		500ml	

180cc =	72cc =	36cc =	4.8cc =	150 ppm available iodine
5 gal	2 gal	1 gal	500ml	

1800cc =	720cc =	360cc =	48cc =	1500 ppm available iodine
5 gal	2 gal	1 gal	500ml	

2. Bleach Solutions:

1/100 dilution of 5.25% bleach ≈ 525 ppm

1/10 dilution of 5.25% bleach ≈ 5,250 ppm

1.0 straight 5.25% bleach ≈ 52,500 ppm

1/8 dilution of 5.25% bleach (Dakin Solution) ≈ 562.5 ppm

500 ppm is the lowest recommended dilution of bleach to use for disinfection

3. Phenolics, Quarternary Disinfectants:

Follow the directions of the manufacturer for proper dilution and use of the concentrate. Any deviation from the concentrations recommended will result in less than satisfactory results. The preparation as formulated at a given concentration was tested using AOAC test methods, and has been documented as effective at that concentration against the microorganisms used in the assay.

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Chapter 7: The Biological Safety Cabinet

The Biological Safety Cabinet (BSC) [ref. 5, 6, 11, 12, 20, 21, 22]

Biological Safety Cabinets (BSCs) are among the most effective, as well as most commonly used primary containment devices in laboratories working with infectious agents. The three general types available (Classes I, II, and III) have different performance characteristics as described below. The type of cabinet to use will depend on application, type of agents to be used in the lab, and whether product sterility, personal protection, or both are critical considerations in the research environment.

Properly maintained Class I and II BSCs when used in conjunction with good microbiological techniques, provide a very effective containment system for the safe manipulation of low to moderate risk microorganisms (Risk Groups 1 and 2).

Both Class I and II BSCs have inward face velocities (75-100 linear feet per minute*) that provide comparable levels of containment for laboratory workers and the immediate environment from infectious aerosols generated within the cabinet.

Class II BSCs have the additional advantage of providing protection to the research material by HEPA (high efficiency particulate air) – filtration of the air flowing down over and across the work surface (vertical laminar flow). Class III cabinets offer the maximum protection to laboratory personnel, the surrounding community and the environment because all hazardous materials are contained in a totally enclosed, ventilated cabinet.

A. Biological Safety Cabinet Classes

1. Class I BSCs (Figure 2)

Note: Class I BSCs are no longer manufactured on a regular basis; many are being replaced by the Class II BSC's.

The Class 1 biological Safety Cabinet is an open-fronted negative-pressure ventilated cabinet with a minimum inward face velocity of at least 75 lf/m*. All of the air exhausted from the cabinet is discharged through a high efficiency particulate air (HEPA) filter either into the laboratory or directly outside. This cabinet is designed for general microbiological research **with *low and moderate risk*** agents, and is useful containment of mixers, blenders, and other equipment. The Class I BSCs are not appropriate for handling research materials that are vulnerable to airborne contamination, since the inward flow of unfiltered air from the laboratory can carry microbial contaminants into the cabinet.

Material in this chapter is developed principally from ref.6 and
http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm

2. Class II BSCs (Figures 3, 5a, 5b, 6, 7)

The Class II BSC is designed with an inward airflow at a velocity of between 75 – 100 lf/m, and a downward, vertical laminar flow in order to provide an air curtain barrier to protect the user from materials within and to protect materials within the cabinet from contamination originating outside the cabinet. All internal air is cleaned by flowing through HEPA filters prior to discharge within the laboratory, or through a duct system to the external environment. Design, construction and performance standards for Class II BSCs as well as a list of products that meet the NSF 49 Standard are available from the National Sanitation Foundation, International, Ann Arbor, Michigan. Utilization for this standard and the corresponding list of approved models should be a first step in selecting and purchasing a Class II BSC.

Class II BSCs are classified into two sub categories, A and B respectively, based on design configurations, construction, air flow velocities and exhaust systems used.

Basically, a Class II Type A cabinet is suitable for work with microbiological research in the absence of volatile or toxic chemicals and radionuclides, since the exhaust air is recirculated within the work area.

Type A cabinets may be exhausted through HEPA filters into the laboratory, or more preferably to the outside air by use of a "thimble"♦ connection to an exhaust duct system.

Type B cabinets are further divided into 3 subtypes- B1, B2 and B3. A comparison of their design features and applications are found in Table 5 of this chapter. Type B cabinets are often hard-ducted to an exhaust system and usually contain all negative pressure plena within (no internal plena are under positive pressure). These features, plus an increased face velocity of100 lf/m allow work to be done with toxic chemicals, carcinogens, radionuclides, and moderate-to -high risk microorganisms. Some manufacturers are producing a hybrid category, the Class II Type A / B3 which will assume the characteristics of a B3 when connected to a duct, and otherwise is operated as a Type A when standing alone.

♦ (A thimble is a canopy hood with design dimensions that allow the discharge from the BSC to be captured efficiently and transported through a duct system, while allow sufficient make-up air from the laboratory to enter simultaneously so that the system is not starved for air. This duct system has a terminal fan and usually an additional HEPA filter before the fan –see Figure 4).

3. Class III BSCs (Figure 8)

The Class III cabinet is a totally enclosed ventilated cabinet of gas-tight construction and offers the highest degree of personal and environmental protection from infectious aerosols as well as protection of research materials from microbiological contaminants. Class III cabinets are most suitable for work with hazardous agents that require Biosafety Level 3 or 4.containment.

All operations within the work area of the Class III cabinet are conducted through attached rubber gloves. When in use the Class III BSC is maintained under negative air pressure of at least 0.5 inches water gauge. Supply air is HEPA-filtered and the exhausted cabinet air is filtered by two HEPA filters installed in series, or HEPA filtration followed by incineration before discharge outside of the facility. Usually several BSCs are connected together to form a cabinet line.

All equipment required by the laboratory activity such as incubators, refrigerators and centrifuges must be an integral part of the cabinet system and included within the cabinet line. The Class III BSC must be connected to double door autoclaves and chemical dunk tanks in order to sterilize or disinfect all materials exiting the cabinet and to allow supplies to enter the cabinet line.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the BSC. Of particular note are those activities which may disrupt the inward directional flow through the work opening (face) of the Class I and II cabinet. Repeated insertion of the worker's arms into the work area, or briskly walking past a BSC while it is in use, are demonstrated causes of the release of aerosolized from within the cabinet. Class I and II BSCs should be located away from traffic patterns and doorways to the lab. Fans, heating and air conditioning registers and other air handling devices can also disrupt airflow patterns if located too close to the cabinet. Strict adherence to the recommended practices for the use of the BSC and proper placement of the BSC within the laboratory are important in attaining the maximum containment available from cabinet.

It is imperative that the Class I and II BSCs have periodic testing of the cabinet, motor and flow dynamics to ensure the continued safe operation of the BSC. Cabinets are usually evaluated when first installed in the lab, and at least annually in conformance to the OSHA Bloodborne Pathogens Standard. Cabinets should also be tested when moved or relocated to another laboratory.

B. Recommendations for Effective use of BSCs (ref.11)

1. Even though the cabinet is tested prior to shipping by the manufacturer, upon arrival in the lab the cabinet should be tested to ensure that no damage to the filter system resulted in shipment.

2. The overall integrity and function of the unit should be checked on a yearly basis, especially when pathogens requiring Biosafety Level 2 containment (or higher) are in use. ***This is currently required by OSHA in the Bloodborne Pathogen Standard, 29 CFR 1910.1030. (e)(2)(iii)(A).***
   http://www.osha-slc.gov/OshStd_data/1910_1030.html

3. Adequate space use of the cabinet should be planned to prevent over-crowding or restriction of movement in the cabinet.

4. The BSC should be allowed to operate five minutes before manipulations are initiated. This allows removal of any possible contaminated air that may have entered while breeching the air-barrier. Insertion of hands and equipment causes turbulence at point of entry, mixing clean air with dirty air.

5. Interior surfaces should be wiped down with 70% alcohol at the beginning end and at the end of the day. If equipped with a UV light, this should be turned on at the end of the day, and turned off while operating.

6. All equipment to be used should be brought inside the cabinet before starting up the cabinet's internal barrier.

7. Do not place anything over the front grill, especially if sterility is necessary. A substantial portion of the air is contaminated (make-up air for the exhaust), and therefore this practice defeats one of the features of the cabinet.

8. Learn to work deep in the interior of the cabinet at least four inches from the intake grill. This prevents contamination of the work, and eliminates spillage of liquids into interior surfaces of the cabinet through the grills.

9. Personnel movement near the cabinet front should be kept to a minimum. Ideally, a separate room with a door will reduce the chances of disturbing air-barrier flow. Movement at the hood face should be minimal, with all movements made slowly so that the airflow at the face of the BSC is not disturbed.

10. The use of centrifuges, open flames, and shakers should be performed with care, since these activities disturb air-flow in the cabinet, and can breech the air-barrier. **Note: Open flames can damage HEPA filters through heat build-up within the cabinet.**

11. The Biological Safety Cabinet is ***not*** a substitute for good microbiological technique. Proper practice of aseptic technique and tissue culture techniques is essential to ensure proper safety when using the BSC.

Table 5. Comparison of Biological Safety Cabinets (ref. 6)

CabinetType	Face Velocity lf/m	Airflow Pattern	Radionuclides / Toxic Chemicals	Biosafety Level(s)	Product Protection
Class I*	75	In at front; out rear and top through HEPA filter	NO	2,3	NO
Class II Type A1	75	70% recirculated through HEPA; Exhaust through HEPA	NO	2,3	YES
Class II Type B1	100	30% recirculated through HEPA; Exhaust through HEPA +/- hard ducted	YES Low levels / low volatility	2,3	YES

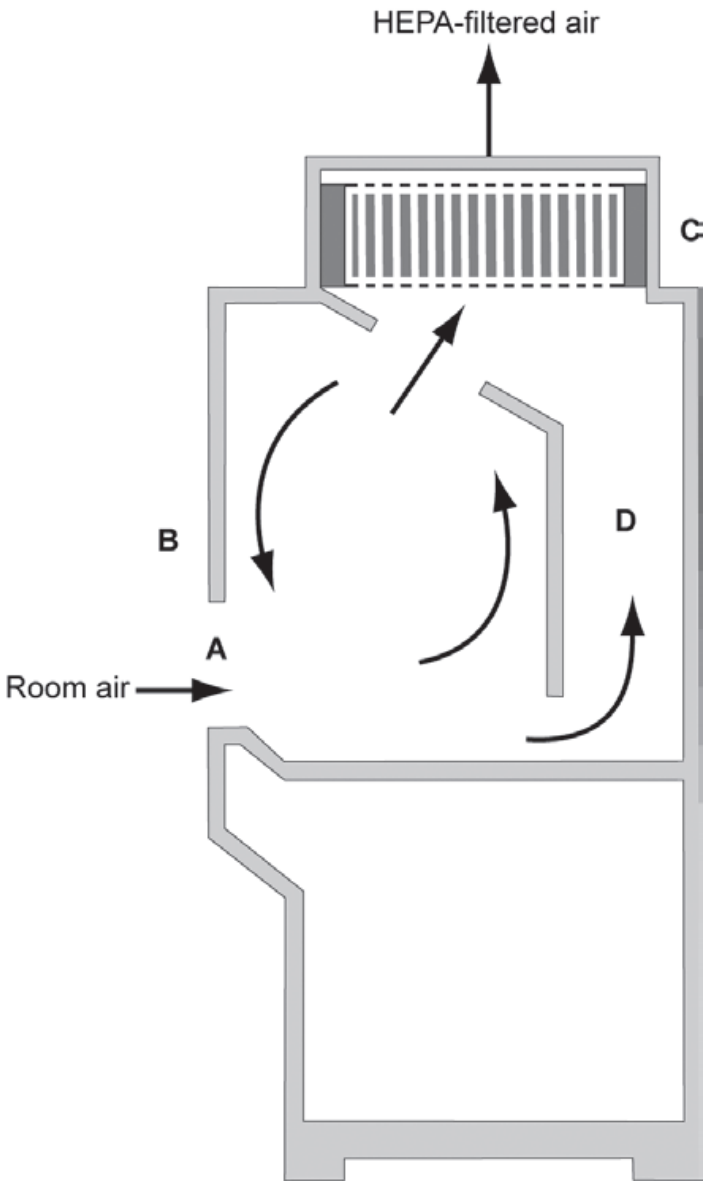
Class II **Type B2**	**100**	**30% recirculated through HEPA; 100%Exhaust through HEPA and hard ducted**	**YES**	**2,3**	**YES**
Class II **Type A2** **(Old B3)**	**100**	**30% recirculated through HEPA; 100%Exhaust through HEPA and hard ducted**	**YES**	**2,3**	**YES**
Class III	**NA**	**Supply and exhaust through 2 HEPA filters; 100%Exhaust through HEPA and hard ducted**	**YES**	**3,4**	**YES**

*** glove panels will increase face velocity to 150 lf/m and aloe work with chemicals / radionuclides of low volatility**

(Ref. 6): http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

Figure 1. The Class I BSC



The Class I BSC

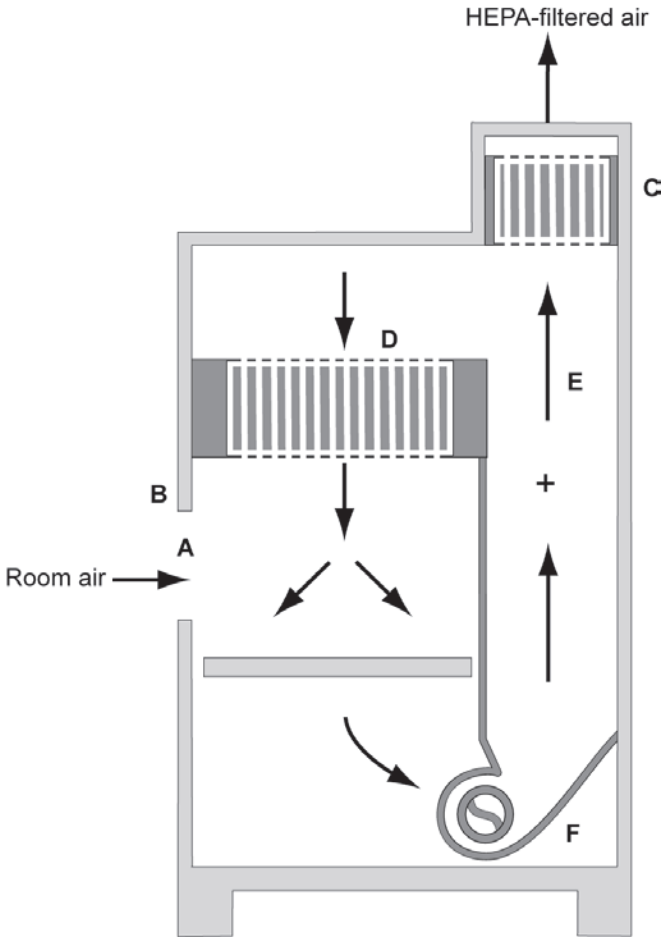
(A) front opening;
(B) sash;

(C) exhaust HEPA filter;

(D) exhaust plenum.

Source:
http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

`Figure 2.The Class II, Type A BSC.



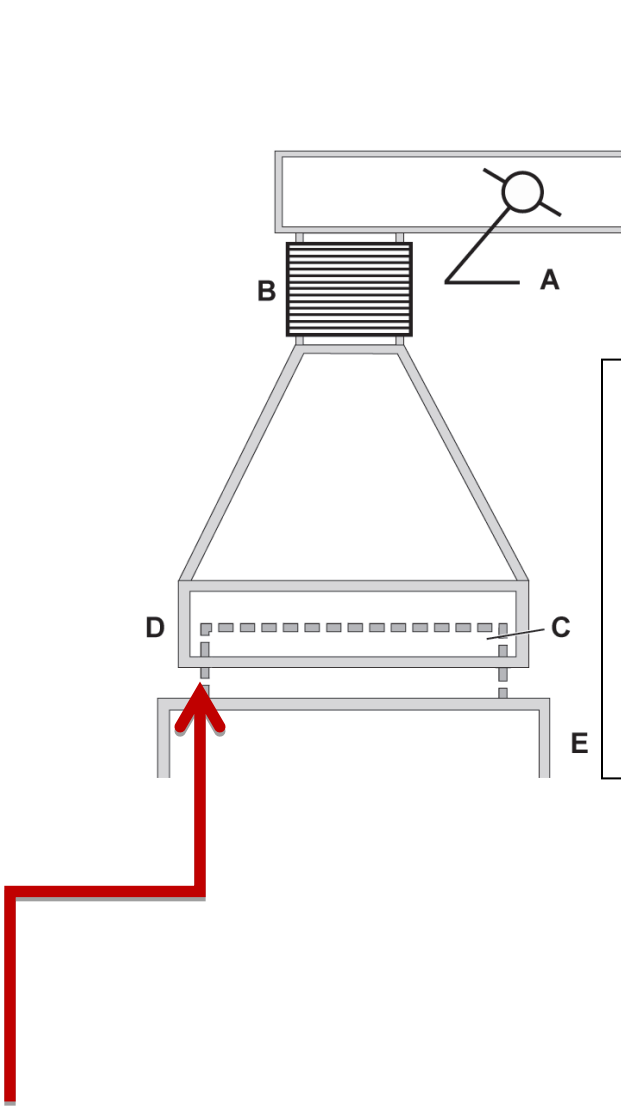
The Class II, Type A1 BSC

(A) front opening;
(B) sash;
(C) exhaust HEPA filter;
(D) supply HEPA filter;
(E) Common (+) pressure plenum;
(F) blower.

Source: CDC / ***Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets; 2nd Edition***

http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

Figure 3. Thimble unit
(for ducting a Class II, Type A BSC)



Canopy (thimble) unit for ducting a Class II, Type A2 BSC

(A)balancing damper;

(B) flexible connector to exhaust system;

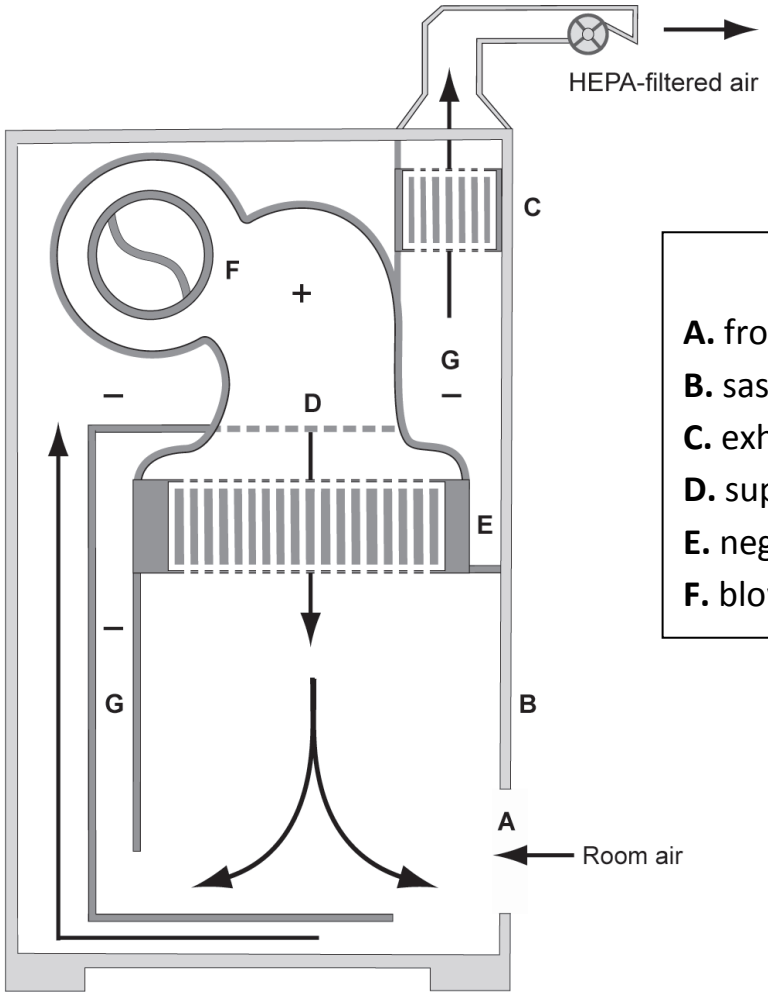
(C) cabinet exhaust HEPA filter housing;

Note: There is a 1" gap between the thimble unit (D) and the exhaust filter housing (C), through which room air is exhausted.

http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

Figure 4a. The Class II, Type B1 BSC (classic design).

Connection to building exhaust system required.



The Class II, Type B1 BSC

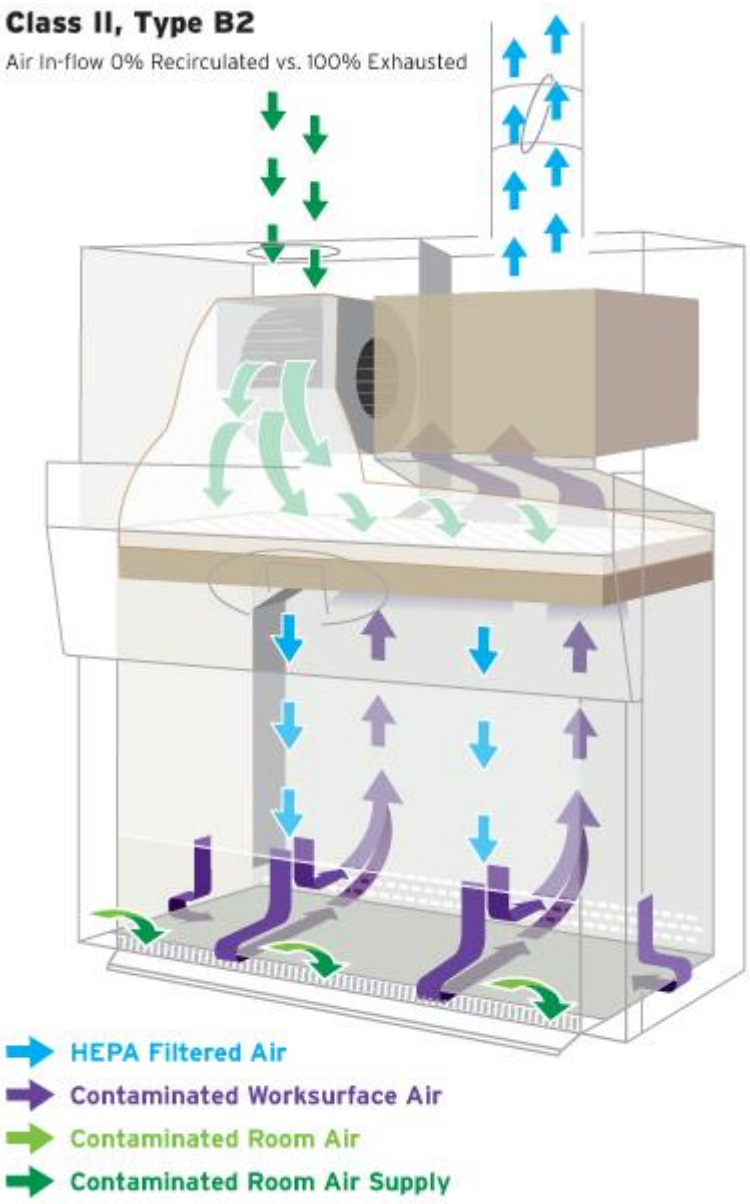
A. front opening
B. sash
C. exhaust HEPA filter
D. supply HEPA filter
E. negative pressure exhaust plenum
F. blower

Note: *The cabinet exhaust needs to be connected to the building exhaust*

Source: CDC / *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets; 2nd Edition*

http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

Figure 4b. The Class II, Type B1 BSC (Flow Patterns).
Connection to building exhaust system is required.

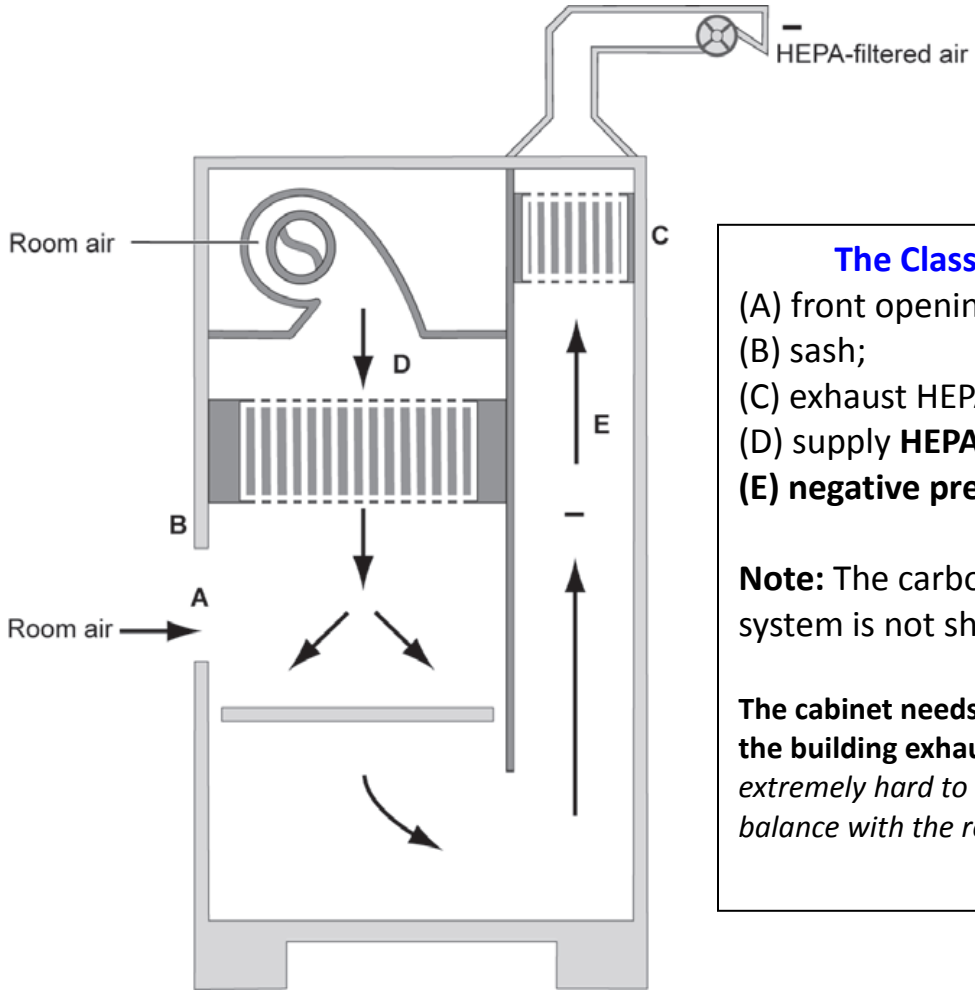


Note: The cabinet exhaust needs to be connected to the building exhaust.**Source: CDC** / ***Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets; 2nd Edition***

http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

Figure 5.The Class II, Type B2 BSC

Connection to building exhaust system required.



The Class II, Type B2 BSC

(A) front opening;
(B) sash;
(C) exhaust HEPA filter;
(D) supply **HEPA filter;**
(E) negative pressure exhaust plenum.

Note: The carbon filter in the exhaust system is not shown.

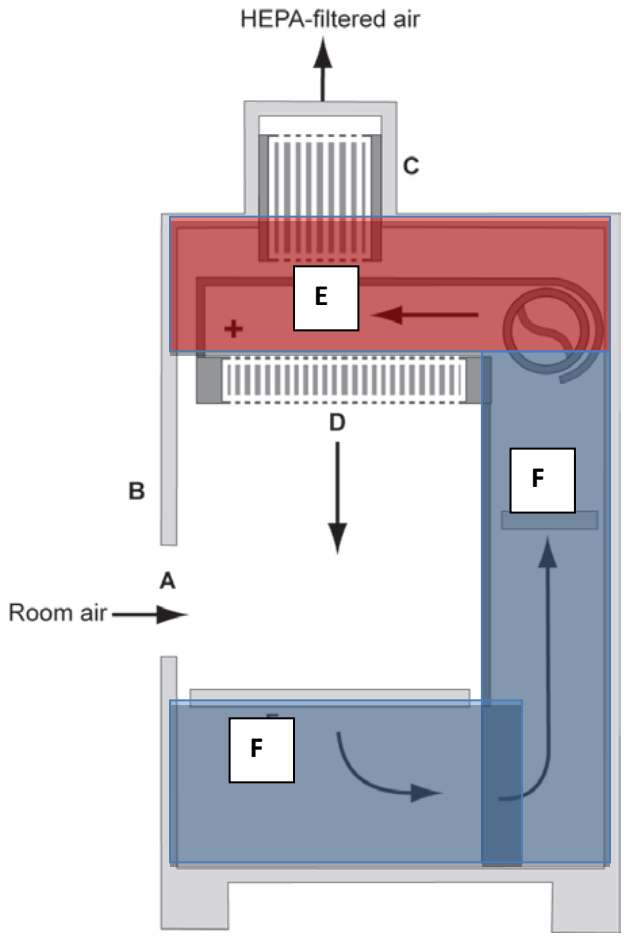
The cabinet needs to be hard connected to the building exhaust system. *These BSCs are extremely hard to balance and maintain in balance with the room's general ventilation*

Source: CDC / *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets; 2nd Edition*

http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

Figure 6. Class II, TypeA2 / B3 BSC (tabletop model)

Connection to building duct system is required.



Class II, Type A2/ B3 BSC

(The tabletop model of a Class II, Type A2 BSC

(A) front opening;

(B) sash;

(C) exhaust HEPA filter;

(D) supply HEPA filter;

(E) positive pressure common plenum;

(F) negative pressure plenum.

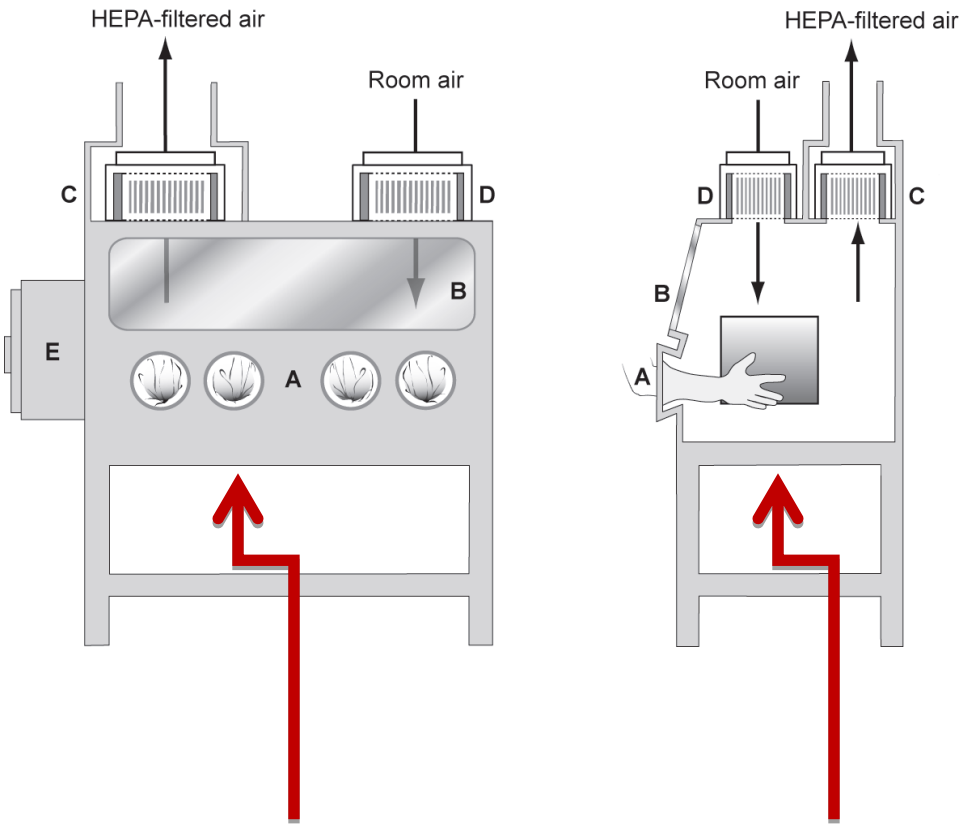
Note: ***The Class II Type A2 BSC is*** **not equivalent** ***to what was formerly called a Class II Type B3 unless it is connected to the laboratory exhaust system. The A2 BSC should be canopy connected to the exhaust system.***

Note: *The cabinet exhaust needs to be connected to the building exhaust system*

Source: CDC / *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets; 2nd Edition*

http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

Figure 7. The Class III BSC.
Connection to building exhaust system required.



The Class III BSC
(A) glove ports with O-ring for attaching arm-length gloves to cabinet;
(B) sash;
(C) exhaust HEPA filter;
(D) supply HEPA filter;
(E) double-ended autoclave or pass-through box.

Note: A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above The cabinet exhaust needs to be hard connected to an exhaust system . The exhaust air **must be**

Note: *A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be connected to the building exhaust system.*

Source: CDC / *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets; 2nd Edition*

A more detailed discussion of biosafety Cabinets is available at:
https://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf#x2013;%20Primary%20Containment%20for%20Biohazards:%20Selection,%20Installation%20and%20Use%20of%20Biological%20Safety%20Cabinets%20%5BPDF%20-%201.5%20MB%5D%3C/a%3E%20

Reviewed 9/2018:Pgh:

Chapter 8: Carcinogen Safety

Carcinogens

The effects of an accidental exposure to a carcinogen cannot be predicted, and may take upwards of thirty years to appear. This not only applies to chemicals, but to viral agents as well, some of which have been linked to oncogenic activity in the literature. Special care has to be exercised in the use of known or suspect carcinogens and mutagens that may be used in research procedures.

Due to many intricate interactions involving dose, route of exposure, metabolic uptake and processing, presence of promoters, etc. it is nearly impossible to accurately predict what the outcome of an exposure may be.

Much attention has been given to the dangers of carcinogenic agents in the popular media, causing exaggerated concern in some individuals, and a lack of concern in others. Many of these accounts are based on preliminary evidence or on retrospective case studies of human exposures. A cautious attitude and the use of appropriate personal protective equipment and engineering controls is highly recommended in all manipulations of known carcinogens and cancer –suspect agents.

An understanding of the hazards, risks and the protective equipment and practices available will serve the researcher well in reducing or eliminating potential exposures in laboratory procedures. The following guidelines, which were adapted from two sources available from the NIH, are given in order to provide an informed approach to the safe handling of carcinogens.

Currently, OSHA regulates the laboratory use of any chemical, including carcinogens under **29 CFR 1910.1450** ***The Laboratory Standard***, and requires the development of a *written* Standard Operating Procedure that outlines the use, storage and disposal steps to be used for each carcinogen in each project.

A definition of a standard operating procedure (SOP) is as follows:

standard operating procedure *n.*

1. Established procedure to be followed in carrying out a given operation or in a given situation.

2. A specific procedure or set of procedures so established.

To put it into laboratory terms, an SOP is ***your Materials and Methods*** practices for each protocol / project you participate in, with all of the safety and personal protective equipment and engineering controls you will need to do your job safely specified out. All steps in handling a microorganism, chemical or toxin are written out so as to track the material "from cradle to grave", eliminating potential miscommunications and utilizing forethought of how to dispose of the material well in advance of actually needing disposal.

A. Oncogenic Virus Guidelines

The general precautions recommended for Biosafety Level 2 are also adequate for work involving oncogenic viruses. No guidelines for high risk viruses are given since no agents in this category have been identified to date. Notification of a change in risk evaluation for a virus would be reported by the NIH on its Office of Safety Policy web site, **https://osp.od.nih.gov/** , and would appear in the publication ***NIH Guidelines* for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (*NIH Guidelines*)**

B. Chemical Carcinogens

a. Personnel Practices

1. Laboratory clothing that protects street clothing such as fully fastened laboratory coats or disposable jumpsuits are to be worn. Gloves, suitable to the hazard (i.e. chemically resistant) should be worn.

2. Laboratory clothing dedicated to carcinogen use is to be worn only in handling areas, and is not to be worn outside these areas at any time.

3. Laboratory clothing is to be decontaminated prior to disposal or laundering. Disposable gloves should also be decontaminated before discarding. If the decontamination procedure for a given chemical is not known, all protective equipment should be of the disposable type, and should be discarded at the end of the procedure. Contact the Biosafety Officer for assistance in arranging discard of these materials.

4. Eating, drinking, and smoking are not permitted in any laboratories where carcinogens are in use. In addition, gum chewing, tobacco chewing, application of cosmetics, storage of utensils, food and food containers, or any activity that promotes hand to mouth / face contact is not permitted. It is recommended that glasses be substituted for contact lenses while working with carcinogens.

5. All personnel should wash their hands immediately after completion of handling chemical carcinogens, and at the conclusion of the work day activities.

b. Laboratory Practices

1. For laboratories using the carcinogens listed in Table 4 intermittently and within the specified amounts as indicated, each storage container of chemical carcinogens should be clearly marked **"CAUTION-CARCINOGENIC AGENT"**. Laboratories using any of the carcinogens listed or any other cancer-suspect or toxic chemical should contact the Biosafety Officer in order to determine what other signs or labels may be required for the laboratory.

2. Access to the laboratory and storage areas is strictly restricted to the Principal Investigator and his/her research and support staff.

3. Maintenance and other responders such as Security Officers must be advised of any potential hazards they may encounter in the laboratory before making entry.

4. Doors to work areas and storage areas of chemical carcinogens are to be closed at all times while experiments are in progress.

5. All work surfaces on which chemical carcinogens are used are to be covered with stainless steel trays, plastic trays, dry absorbent, plastic-backed paper or other similarly impervious material in order to prevent permanent contamination of the work sites.

6. Depending on the material used, decontamination or disposal of coverings should be performed at the end of procedures or at the end of the day's activities.

7. Chemical carcinogens should not be used on the open bench top, but are to be used only in glove boxes; sealed systems within a single pass ducted chemical fume hood, Class II B2 Biological Safety Cabinets (BSC), or other enclosed systems. Aerosol prevention measures are to be practiced at all times.

8. Tissue culture work involving chemical carcinogens may be conducted in a Class II Type B BSC, or in a Class II A / B3 BSC provided that the BSC's are thimble connected into a single-pass, ducted ventilation system that discharges directly to the external environment.

9. Vapors or aerosols produced by analytical instruments when used with chemical carcinogens should be captured by a local exhaust system at the work site, or by using such instruments in chemical hoods or Biological Safety cabinets vented as described above.

10. Any use of respirators by personnel must conform to the OSHA Standards regulating training, proper wearing, fit-testing, medical evaluation and surveillance, proper storage and use of respirators, (29 CFR 1910.134 at www.osha.gov.)

Refer to the Table (Table 4) given below.

Table 4. NIH Approved Levels for the Laboratory Use of Chemical Carcinogens

Compound	Use Condition	Principal Investigator Approval Level	Laboratory or Branch Chief Approval Level	Occupational Health and Safety Committee Approval Level
Benzene; Carbon Tetra-chloride;Chloroform;1,2,Dibromo-3-chloropropane;1,1 Dimethyl-ethylenimene; p-Dioxane; Ethylene dibromide; Propy-leneimine	Storage Normal Operation[1] Complex Operation[2]	<10liters(=)* < I liter(=) < 0.1 liter	> 10 liters > I liter 0.1 to1.0 liter	— — > 1.0 liter
Bromoethyl methanesulfonat; Chlorormethyl methylether;Di-epoxybutane; 1,1-Dimethyl-hydrazine;1,2-Dimethylhydrazine; Ethylenimine;Ethyl methanesulfonate; Hydrazine; Methylhydrazine; Methyl meth-anesulfonate; N-Nitrosodiethy-lamine; N-Nitrosodimethylamine; N-Nitrosodi-n-butylamine; N-Nitrosodi-n-propylamine; N-Nitroso-N-ethylurethane; N-Nitrosopiperidine; Polychlorinated biphenyls; β-Propiolactone;	Storage Normal Operation Complex Operation	<1000 g(=) < 100 g(=) < 10 g	>1000 g > 100 g 10 g to 100 g	— — > 100 g
N-Acetoxy-2-acetylaminofluorene; 2-Acetylaminofluorene; Afla-toxins; o-Aminoazotoluene; 2-Aminofluorene; Benz(a)an-thracene; Benzo(a)pyrene; Chlorambucil; Cycasin; Diazo-methane; Dibenz[a,h]anthracene; 7,12-Dimethylbenz(a)anthracene; 4-Dimethylamino-azobenzene; 3-3'-Dimethyl-benzidine; 1,4-Dinitrosopiperazine; N-Hydroxy-2-acetyl-aminofluorene; 3-Methylcholanthrene; 4,4'-Methylene bis(2-chloroaniline); 1-	Storage Normal Operation Complex Operation	< 100 g < 10 g < 1 g	100gto1000g 10g to 100 g 1 g to 10 g	> 1000 g > 100 g > 10 g

Mehtyl-3-nitro-1-nitrosoguanidine; N-[4-(5-Nitro-2-furyl)-2-thiazoyl]-formamide; N-Nitroso-N-ethylurea; N-Nitroso-N-methylurea; 4-Nitro-quinoline-1-oxide; Procarbazine; 1,3-Propane sultone; m-Toluenediamine; Uracil mustard; Vinyl Chloride				
Bis(chloromethyl) ether	Storage Normal Operation Complex Operation	– – –	< 1 liter (=)* <0.01 liter(=) –	> 1 liter > 0.01 liter Any Quantity

(1) Note: Approval levels apply to principal investigators and laboratory/branch chiefs who have successfully completed the NIH course in the recognition and control of chemical hazards in the laboratory. The ISM-MS is the PEAK training offered at Sinaicentral.

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(2) **Normal Operation**: Any operation involving simple manipulations or reactions where the potential for release of the material is remote (e.g. dilutions; qualitative, controlled transfer of test materials; use of analytical standards).

(3) **Complex Operation**: Any operation involving the handling, manipulation or reaction of materials where the potential for release of the material is significant (e.g. rapid exothermic reactions; imparting of sufficient energy to a test system (heating, mixing, delivery under pressure) so that uncontrolled release of material could occur; transfer of electrostatic powders).

(4) Notation * < 10 liters (=); quantity less than or equals 10 liters

9/2018 Revision Pgh:

Chapter 9: Regulated Waste Management

REGULATED WASTE MANAGEMENT

All Medical School Employees and Students should know the definition of regulated (RMW), nonregulated waste (NRMW), chemo waste and the proper means for waste disposal. Approximately, 88% of total waste in the hospital is NRMW (clear bag waste), 11% is RMW (red bag waste) and 1% is (chemo waste). Refer to the MSMC Infection Control Manual. http://intranet1.online.mssm.edu/mount_sinai/.

A. DEFINING REGULATED MEDICAL WASTE (RMW)

The current definition of Regulated Medical Waste may be found in the New York State Department of Health Interpretive Guidelines for Implementing Revisions to Public Health law. There are presently five subcategories of **RMW:**

- Cultures and Stocks
- Human Pathological Waste.
- Human Blood and Blood Products.
- Sharps
- Animal Waste

B. EPA MEDICAL INFECTIOUS WASTE:

The EPA definition states:

> "Medical/infectious waste means any waste generated in the diagnosis, treatment or immunization of human beings, or animals, in research pertaining thereto, or in the production or testing of biologicals that is listed in paragraphs [2.1] through [2.7] of this definition. The definition does not include hazardous waste identified or listed under the regulations in part 261 of this chapter; household waste, as defined in 261.4 (b) (1) of this chapter; ash from incineration of medical / infectious waste, once the incineration process has been completed; human corpses, remains and anatomical parts that are intended for internment; and domestic sewage materials identified in 261.4 (a) (1) of this chapter.

1. **Cultures and stocks** of infectious agents and associated biologicals, including: cultures from pathological laboratories; cultures and stocks of infectious agents from research and industrial laboratories; wastes from the production of biologicals; discarded live and attenuated vaccines; and culture dishes and devices used to transfer, inoculate, and mix cultures.

2. **Human pathological waste***, including tissues, organs, and body fluids that are removed during surgery of autopsy, or other medical procedures, and specimens of body fluids and their containers.

3. **Human blood and blood products*** including:

    a. Liquid waste human blood;

    b. Products of blood;

    c. Items saturated and/or dripping with human blood; or

    d. Items that were saturated and/or dripping with human blood that are now caked with dried human blood; including serum, plasma, and other blood components and their containers, which were used or intended for use in either patient care, testing and laboratory analysis or the development of pharmaceuticals. Intravenous bags are also included in this category.

4. **Sharps** that have been used in animal or human patient care or treatment or in medical, research, or industrial laboratories, including hypodermic needles, syringes (with or without the attached needle), Pasteur pipettes, scalpel blades, blood vials, needles with attached tubing, glass slides and cover slips and culture dishes (regardless of presence of infectious agents). Also included are other types of broken or unbroken glassware that were in contact with infectious

agents, such as used slides and cover slips. **SESIPs -(Sharps with Engineered Sharps Injury Protection) are included, since they are "medical" in appearance and still look like needles-which they are.**

5. **Animal waste** including contaminated animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research (including research in veterinary hospitals), production of biologicals or testing or pharmaceuticals.

6. **Isolation wastes*** including biological waste and discarded materials contaminated with blood, excretions, exudates, or secretions from humans who are isolated to protect others from certain highly communicable diseases, or isolated animals known to be infected with highly communicable diseases.

7. **Unused sharps** including the following unused discarded sharps; hypodermic needles, suture needles, syringes and scalpel blades. "

C. WASTE COLLECTION

Areas used to stage RMW, NRMW, chemo and recyclables must be kept clean and free of clutter and allow easy access to all waste containers.

RMW and chemo containers must be kept in restricted areas which limit exposure to the public.

All radioactive waste (RMW, NRMW, and linen) must be stored in the proper containers and stored consistent with Department of Radiation Safety requirements. The Department of Radiation Safety must be notified to remove these items.

Containers used to store RMW **must be red in color or be labeled with the words Regulated Medical Waste or Infectious Waste or use the Universal BioHazardous symbol.**

These containers must be kept covered and free of visible soil.

Containers used to store chemo waste must be labeled to identify chemo waste. Chemo containers must be kept covered and free of visible soil.

RMW and NRMW waste will be removed from the unit's waste staging area on a daily basis by the Department of Waste Management (ext. 45015). The Department of Waste Management will line all red bag waste containers with red bags provided on the unit and replace all chemo containers removed from the unit.

D. RECYCLABLES

Cardboard and white paper are the two items that the hospital recycles. The Building Services support staff are responsible for flattening all cardboard and removing all white papers from their recycling bins on a daily basis and placing them in the unit's waste staging area. They are not to be thrown down the trash chutes. The Department of Waste Management will remove these two items from the unit's waste staging area on a daily basis.

E. SHARPS

All sharps (See: B.4 above) (needles and glass) are to be placed in a sharps container after use. Sharps containers are either wall mounted or free standing and are labeled Bio-hazardous material. These sharps containers are emptied several times per week by Steri-Cycle, an outside company under contract with the hospital. All employees should contact the technicians at pager 41300 then 4640# or 4639# if a sharps container is filled, missing or dismantled from the wall.

For the Medical School's use of the term ***"SHARP":*** any glass, metal, plastic instrument or item that can cut or has the potential to cut, puncture, scratch or abrade skin, *whether it is contaminated or not,* is to be handled as a ***SHARP*** and disposed of in the provided rigid puncture resistant box. **If you have any doubt, place it in the sharps box. If it hard….put it in the sharps box; if soft, put it in a red bag.**

If an infectious agent has been manipulated with the items under consideration, they must be autoclaved or disinfected *prior* to being placed into the **sharps** boxes. Research quantities of microbial agents can present numbers of organisms several times over a potentially infectious dose to an individual coming into contact with the needle box or its contents.

- If these items are being used in clinical-type applications they may be disposed of in the **sharps** needle boxes *without treatment.*

- Consideration should be given to the fact that the **sharps** needle boxes receive no treatment until reaching the service company's facilities and therefore infectious material may remain viable within these boxes. Autoclaving or disinfecting infectious materials, prior to disposal, and arranging for the timely removal of boxes from the laboratory will provide for a safer work environment.

All medical school employIees handling human blood, tissues and body fluids should be thoroughly familiar with the ISM-MS "**Bloodborne Pathogens Exposure Control Plan"**. http://intranet1.mountsinai.org/msmc/home.asp as required by OSHA's 29 CFR.1910.10. at https://www.osha.gov/SLTC/bloodbornepathogens/index.html and should dispose of related items as described in the entire **Section (d) (4) (iii) "Regulated Waste**" procedures.

Employees handling HIV, HBV, or other pathogenic agents must follow the procedures outlined in the **"Bloodborne Pathogens Exposure Control Plan"** and in **Biosafety in Microbiological and Biomedical Laboratories"** which is located on the Centers for Disease Control Website:

https://www.cdc.gov/biosafety/publications/bmbl5/index.htm

All employees handling these materials must take BBP training sessions annually on the PEAK site..

F. DISPOSAL PRACTICES

In order to standardize practices of disposal within the Medical Center, the information contained in the Infection Control Manual Sec I-6.1 (Reviewed 12 / 08) is directly incorporated here.

a. Discard in "Regulated Waste-Liquid Waste" Containers (with sliding lid) lined with red plastic bags:

1. All of the following body fluids in quantities equal to or *greater than* 20 cc:

Blood	*Pleural fluid*	*Amniotic fluid*
Any bloody body fluid	*Pericardial fluid*	
Cerebrospinal fluid	*Semen*	
Peritoneal fluid	*Vaginal secretions*	

Containers must be tightly stoppered. Note this does not include urine or stools unless visibly bloody (see below).

Note: all rigid containers (i.e. pleurovacs, hemovacs) regardless of fluid amount (even if *under* 20 cc) are to be disposed of in Regulated Waste-Liquid Waste Containers.

2. All blood bags and blood tubing.

3. Items dripping with blood except for tampons and sanitary napkins.

4. All waste generated from patients in the process of undergoing peritoneal hemodialysis.

b. Discard in "Regulated Waste-(Non) Liquid Waste" Containers with closing lid lined with red plastic:

1. Drained urinary bags / tubing / urinary catheters that have remnants of blood but with *less than* 20 cc visible blood.

2. All waste resulting from the care and treatment of patients with "highly communicable" diseases: Marburg, Lassa, Ebola, and similar viruses (for additional information contact Infection Control).

3. Dressings with saturated or dried body fluids i.e. gauze and chux.

c. Discard all used and unused sharps into the Steri-Cycle Reuseable Containers:

Sharps that have been used (or opened and not used) in human patient or animal care or in medical or research laboratories. These items include: needles, syringes, scalpel blades, lancets, Pasteur pipettes, blood vials, test tubes, slides, cover slips, and broken or unbroken glassware and other sharps. ***NOTE: after use, needles should not be recapped, bent, clipped, or broken by hand.***

d. The following are considered non-regulated waste and may be disposed of in clear bag trash:

1. Drained urinary bags / tubing / urinary catheters (without visible blood).
2. IV tubing and / or bags without visible blood.
3. Any material with drops of blood.
4. Glucose meter strips.
5. Sanitary napkins or tampons.

Special note: although many of the procedures mentioned above are more commonly associated with patient-related activities, in many cases similar activities are encountered in performing animal research. Articles that are contaminated with blood and body fluids can be indistinguishable from human-source material. Therefore disposal practices should be consistent whether the source is human or not; the regulatory agencies having jurisdiction over waste will not inquire as to the source of the material if an infraction has occurred.

e. The color of bags used for disposal of waste has been standardized:

1. **Red** bags are to be used for disposal of all potentially infectious waste regulated medical waste which requires off-site incineration with the exception of liquid waste as explained earlier.

   a. **Solids:** Are placed into red bags for disposal by the MSMC Waste Management personnel. Red Bags are to remain in the laboratory until picked-up by Waste Management. Storage in hallways, elevator alcoves and stair landings **is prohibited.**

   b. **Liquids:** May be disposed of by toilets or sanitary waste lines, **after autoclaving** or treatment with a suitable disinfectant**.** Chemicals may **not** be disposed of in this manner.

2. **White*** bags are to be used for autoclaving potentially infectious waste/regulated medical waste.

3. **Clear** bags are to be used for all other waste.

G . BIOHAZARD WASTE STREAM:

Compared to domestic waste, chemical waste, this stream poses the hazard to all Mount Sinai employees of infection with a pathogenic agent that is either directly associated with research activities, or may be coincidentally associated with the materials in use. Special procedures must be employed to ensure that there will be no exposure to the pathogens once the material is offered for disposal.

Both **"Universal Precautions"** materials (all human blood, body fluids, and tissues) and materials known to contain infectious agents, must be autoclaved, or treated in such a way that the material is rendered virtually non-infectious. Autoclaving is the preferred method. If autoclaving is not practical, a chemical disinfectant can be used.

Chapter VI of this booklet has a section summarizing the various disinfectants, their use and limitations.

For **"Universal Precautions"** materials and materials containing known infectious agents, many of the commercial disinfectants that have been proven to be tuberculocidal, and / or effective against Hepatitis B, polio virus types I, II, III, *Pseudomonas aeruginosa* or other bacteria or viruses using the U.S. EPA test protocols, are acceptable for surface decontamination of empty containers and work surfaces. Household bleach is acceptable for decontamination provided that **daily, fresh preparations** are made and used. After the material has been rendered non-infectious, disposal is effected depending on the physical form of the material, as noted below.

All Liquids that have been autoclaved or treated with a chemical disinfectant can be flushed down a toilet or sink with copious amounts of water afterward. All pathological and anatomical human tissues and animal tissues and carcasses are interred or incinerated off-site. If the specimens are derived from *ANIMAL* sources, arrangements to dispose of animal tissues and carcasses must be made individually with the Department of Veterinary Sciences, ext. 46683. If the specimens are derived from *HUMAN* sources, contact the BioSafety Officer at ext. 45169 to arrange for disposal.

H. Conclusion:

All waste should be disposed of according to the procedures described in detail above.

When known infectious agents or chemical reagents which are of moderate-to-high acute toxicity, moderate-to-high chronic toxicity, or known carcinogens are used, OSHA regulations 29 CFR 1910.1030 and .1450 require the development of *written standard operating procedures* which include waste disposal. SOP's must be available to all staff, or in a central location in the laboratory. Contact the BioSafety Officer to assist in the development of SOP's when necessary or if you have any questions regarding waste disposal procedures at extension 241-5169.

Chapter 10: Importation, Exportation (International) and Interstate Shipments of Human Pathogens, Zoonotics and Genetically – Modified Organisms

Importation, Exportation and Interstate Shipments of Human Pathogens, Zoonotics and Genetically – Modified Organisms

Source: http://www.cdc.gov/od/eaipp/regulations.htm

A. Introduction

Etiologic agents are those microorganisms and microbial toxins that cause disease in humans and include bacteria, bacterial toxins, viruses, fungi, rickettsiae, protozoans, and parasites. These disease-causing microorganisms may also be referred to as infectious agents. Arthropods and other organisms that transmit pathogens to animals (including humans) are called vectors.

Etiologic agents, vectors, and materials containing etiologic agents are recognized as hazardous materials. Materials containing etiologic agents are regularly transported from one location to another by common land and air carriers. Materials containing etiologic agents must be appropriately packaged to prevent breakage or leakage in order to avoid exposure of the package handlers, transporters, and the general public to the package contents. Materials containing etiologic agents must be packaged, labeled, and transported in accordance with all applicable regulations. Material containing etiologic agents being imported into the United States must be accompanied by a U.S. Public Health Service importation permit.

B. CDC Importation Permits

§71.54 Import regulations for infectious biological agents, infectious substances, and vectors.

(a) The following definitions apply to this section:

Animal. Any member of the animal kingdom except a human including an animal product (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws).

Diagnostic specimen. Specimens of human and animal matter (including tissue, blood, body discharges, fluids, excretions or similar material), or environmental samples.

Genomic material. Deoxyribonucleic acid (DNA) or Ribonucleic acid (RNA) comprising the genome or organism's hereditary information, that may be single-stranded or double-stranded, and in a linear, circular, or segmented configuration and may be positive sense (same polarity as mRNA), negative sense, or ambisense (mixture of the two).

Infectious biological agent. A microorganism (including, but not limited to, bacteria (including rickettsiae), viruses, fungi, or protozoa) or prion, whether naturally occurring, bioengineered, or

artificial, or a component of such microorganism or prion that is capable of causing communicable disease in a human.

Infectious substance. Any material that is known or reasonably expected to contain an infectious biological agent.

Select agents and toxins. Biological agents and toxins that could pose a severe threat to public health and safety as listed in 42 CFR 73.3 and 73.4.

Vector. Any animals (vertebrate or invertebrate) including arthropods or any noninfectious self-replicating system (e.g., plasmids or other molecular vector) or animal products (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws of an animal) that are known to transfer or are capable of transferring an infectious biological agent to a human.

(b) Unless excluded pursuant to paragraph (f) of this section, a person may not import into the United States any infectious biological agent, infectious substance, or vector unless:

(1) It is accompanied by a permit issued by the Centers for Disease Control and Prevention (CDC). The possession of a permit issued by the CDC does not satisfy permitting requirements placed on materials by the U.S. Department of Agriculture that may pose hazards to agriculture or agricultural production in addition to hazards to human health.

(2) The importer is in compliance with all of the permit requirements and conditions that are outlined in the permit issued by the CDC.

(3) The importer has implemented biosafety measures commensurate with the hazard posed by the infectious biological agent, infectious substance, and/or vector to be imported, and the level of risk given its intended use.

(4) The importer takes measures to help ensure that the shipper complies with all applicable legal requirements concerning the packaging, labeling, and shipment of infectious substances.

(c) If noted as a condition of the issued permit, subsequent transfers of any infectious biological agent, infectious substance or vector within the United States will require an additional permit issued by the CDC.

(d) A permit is valid only for:

(1) The time period and/or term indicated on the permit, and

(2) Only for so long as the permit conditions continue to be met.

(e) A permit can be denied, revoked or suspended if:

(1) The biosafety measures of the permit holder are not commensurate with the hazard posed by the infectious biological agent, infectious substance, or vector, and the level of risk given its intended use; or,

(2) The permit holder fails to comply with all conditions, restrictions, and precautions specified in the permit.

(f) A permit issued under this part is not required for an item if:

(1) It is a biological agent listed in 42 CFR Part 73 as a select agent and its importation has been authorized in accordance with 42 CFR 73.16 or 9 CFR 121.16.

(2) With the exception of bat or nonhuman primate specimens, it is a diagnostic specimen not known by the importer to contain, or suspected by the importer of containing, an infectious biological agent and is accompanied by an importer certification statement confirming that the material is not known to contain or suspected of containing an infectious biological agent, or has been rendered noninfectious.

(3) With the exception of live bats or bat or nonhuman primate products, it is an animal or animal product being imported for educational, exhibition, or scientific purposes and is accompanied by documentation confirming that the animal or animal product is not known to contain (or suspected of containing) an infectious biological agent or has been rendered noninfectious.

(4) It consists only of nucleic acids that cannot produce infectious forms of any infectious biological agent and the specimen is accompanied by an importer certification statement confirming that the material is not known to contain or suspected of containing an infectious biological agent.

(5) It is a product that is cleared, approved, licensed, or otherwise authorized under any of the following laws:

(i) The Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 *et seq.*), or

(ii) Section 351 of the Public Health Service Act pertaining to biological products (42 U.S.C. 262), or

(iii) The Virus-Serum-Toxin Act (21 U.S.C. 151-159).

(6) It is an animal or animal product listed in 42 CFR Part 71 and its importation has been authorized in accordance with 42 CFR 71.52, 71.53, or 71.56.

(g) To apply for a permit**, an individual** must:

(1) Submit a signed, completed CDC Form 0.753 (Application for Permit to Import Biological Agents or Vectors of Human Disease into the United States) to the HHS/CDC Import Permit Program.

(2) Have in place biosafety measures that are commensurate with the hazard posed by the infectious biological agent, infectious substance, and/or vector to be imported, and the level of risk given its intended use.

(h) **Issuance of a permit may be contingent upon an inspection of the importer's facility by the CDC to evaluate whether the importer's biosafety measures (e.g., physical structure and features of the facility, and operational and procedural safeguards) are commensurate with the hazard posed by the infectious biological agent, infectious substance, and/or vector, and the level of risk given its intended use. Checklists for pre-inspection are found at: http://www.cdc.gov/od/eaipp/inspection/index.htm**

(i) Denial, suspension, or revocation of a permit under this section may be appealed to the CDC Director. The appeal must be in writing, state the factual basis for the appeal, and be submitted to the CDC Director within 30 calendar days of the denial, suspension, or revocation of the permit. HHS/CDC will issue a written response to the appeal, which shall constitute final agency action.

[78 FR 7678, Feb. 4, 2013]

C. Other Permits

- United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) permits are required for infectious agents of livestock and biological materials containing animal material. Tissue culture materials and suspensions of cell culture grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origins are controlled by the USDA due to the potential risk of introduction of exotic animal diseases into the U.S. Further information may be obtained by calling the USDA/APHIS at (301) 734-7834 (see www.aphis.usda.gov/vs).

- U.S. Fish and Wildlife Service permits are required for certain live animals, including bats. Please call 1-800-344-WILD for further information (www.fws.gov/).

- Individuals wishing to import select agents and toxins must be registered with CDC's Select Agent Program in accordance with 42 CFR Part 73 (Possession, Use, and Transfer of Select Agents and Toxins; Interim Final Rule) for the select agent(s) and toxin(s) listed on the import permit application. Also, In accordance with 42 CFR Part 73.16(a), an APHIS/CDC Form 2 must be completed and submitted to the CDC Select Agent Program and granted approval prior to the shipment of the select agents or toxins under the import permit. Additional information can be found at www.cdc.gov/od/sap.

D. Exports of Infectious Materials

The export of a wide variety of etiologic agents of human, plant, and animal diseases may require a license from the Department of Commerce. Information may be obtained by calling the Department of Commerce Bureau of Export Administration at 202-482-4811 or through the internet at: www.bis.doc.gov/Licensing/.

E. International Shipments / WHO Guidelines :

http://apps.who.int/iris/bitstream/handle/10665/254788/WHO-WHE-CPI-2017.8-eng.pdf?sequence=1&isAllowed=y

The international regulations for the transport of infectious substances by any mode of transport are
based upon the Recommendations made by the Committee of Experts on the Transport of Dangerous Goods (UNCETDG), a committee of the United Nations Economic and Social Council.

The Recommendations are presented in the form of Model Regulations. The United Nations Model
Regulations are reflected in international law through international modal agreements (links to further
information are provided in Annex 1; in the PDF Text):

Air The Technical Instructions for the Safe Transport of Dangerous Goods by Air published by the International Civil Aviation Organization (ICAO) are the legally binding international
regulations. The International Air Transport Association (IATA) publishes Dangerous Goods
Regulations (DGR) that incorporate the ICAO provisions and may add further restrictions (where necessary such restrictions are included in these guidelines). The ICAO rules apply on all international flights. For national flights, i.e. flights within one country, national civil

aviation authorities apply national legislation. This is normally based on the ICAO provisions,
but may incorporate variations. State and operator variations are published in the ICAO Technical Instructions and in the IATA Dangerous Goods Regulations.

Rail Regulations concerning the International Carriage of Dangerous Goods by Rail (RID) apply to
countries in Europe, the Middle East and North Africa. RID also applies to domestic transport
in the European Union through Council Directive 2008/68/EC.

Road The European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR) applies to 46 countries. In addition, modified versions of the convention are being used by countries in South America and South-East Asia. ADR also applies to domestic transport in the European Union through Council Directive 2008/68/EC.

Sea The International Maritime Dangerous Goods Code published by the International Maritime
Organization (IMO) is of mandatory application for all contracting parties to the International
Convention for the Safety of Life at Sea (SOLAS).

F. Packaging Guidelines

Infectious materials imported into this country must be packaged to withstand breakage and leakage of contents, and labeled, as specified in the following federal regulations:

- DOT 49 CFR PART 173 - Transportation of Etiologic Agents
- https://www.law.cornell.edu/cfr/text/49/part-173

For international shipments, the International Air Transport Association (IATA) Dangerous Goods Regulations should be consulted.

Additional helpful information regarding shipping and packaging guidelines at the following sites:

1. Guidance on regulations for the Transport of Infectious Substances 2013-2014 (World Health Organization):http://apps.who.int/iris/bitstream/10665/78075/1/WHO_HSE_GCR_2012.12_eng.pdf

2. The IATA Dangerous Goods Regulations (International Air Transport Association):https://www.iata.org/publications/store/Pages/infectious-substances-shipping-guidelines.aspx
3. Title 49 Code of Federal Regulations, Parts 100 - 185. Hazardous materials regulations (Department of Transportation): https://www.phmsa.dot.gov/sites/phmsa.dot.gov/files/docs/Transporting_Infectious_Substances_brochure.pdf and https://hazmatonline.phmsa.dot.gov/services/publication_documents/howtouse0507.pdf
4. Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. (CDC/NIH): http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixC.pdf

G. Prior approval of all Icahn School of Medicine at Mount Sinai Shipments to off-site locations.

All shipments of Category A, Category B Infectious / Biological Substances, Diagnostic, Clinical specimens and cultures / cell lines ***must be approved*** through the Environmental Health and Safety Office ***before shipping the material off site***.

The following form **MUST** be completed and sent to EnvH&S ***before*** packaging the materials as shown in the figures above.

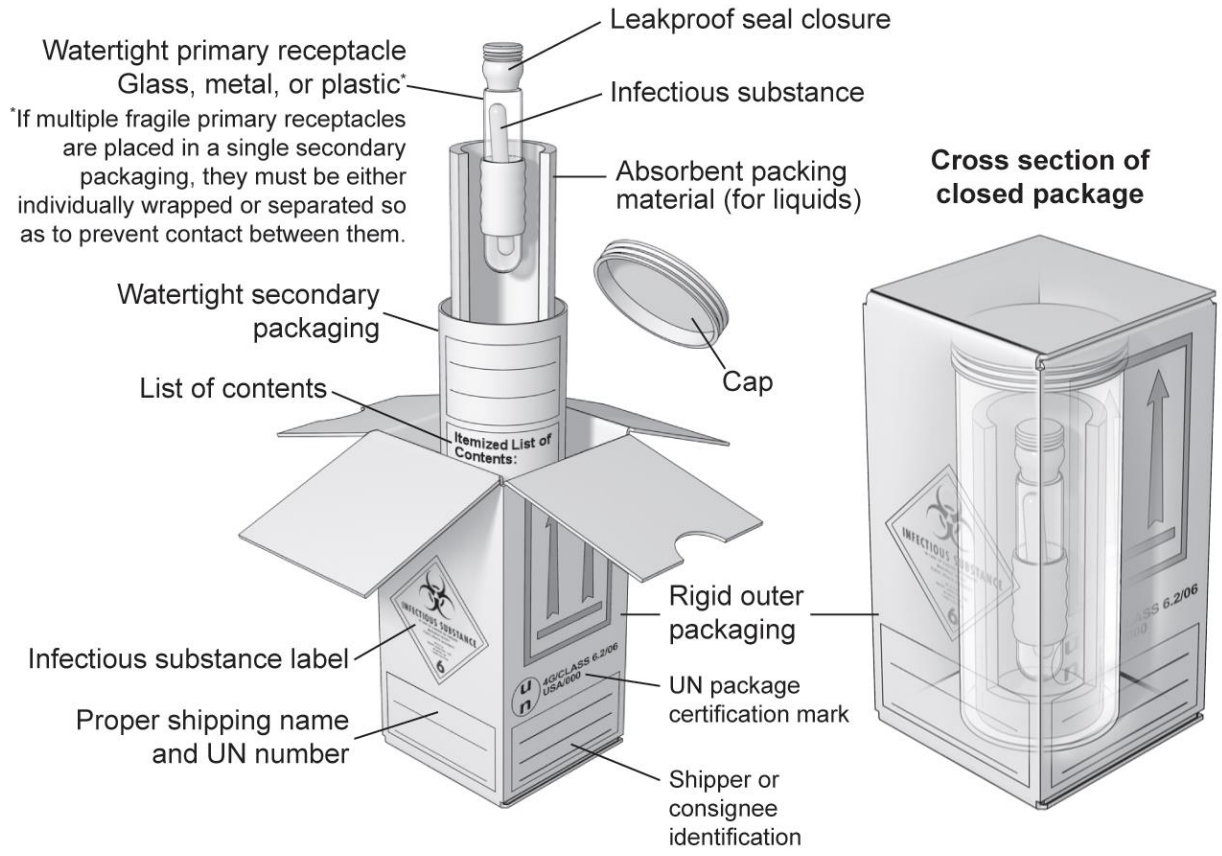
Additionally, anyone offering the above-stated materials for interstate or overseas transport must have awareness level training ***at a minimum*** in the IATA Dangerous Goods shipping procedures and / or US Department of Transportation Hazardous Goods shipping. This training is available through the EnvH&S Office.

Please contact the Biological Safety Officer at 241- 5169 regarding any biological agent shipping questions. **https://icahn.mssm.edu/research/institutional-biosafety**

To Submit the *Approval for Chemical / Biological / Radiological Materials Shipment Form* to EnvHS, Send to: **#Askehs@mountsinai.org**

Current package and Waybill designation is:

Category A Materials, UN 2814 (Infectious Substances Affecting Humans) and UN 2900 (Infectious Substances Affecting Animals [Only])



CDC Homepage

OHASIS OHS Internet

Figure 1

Current package and Waybill designation is:

UN 3373 Biological Substances, Category B

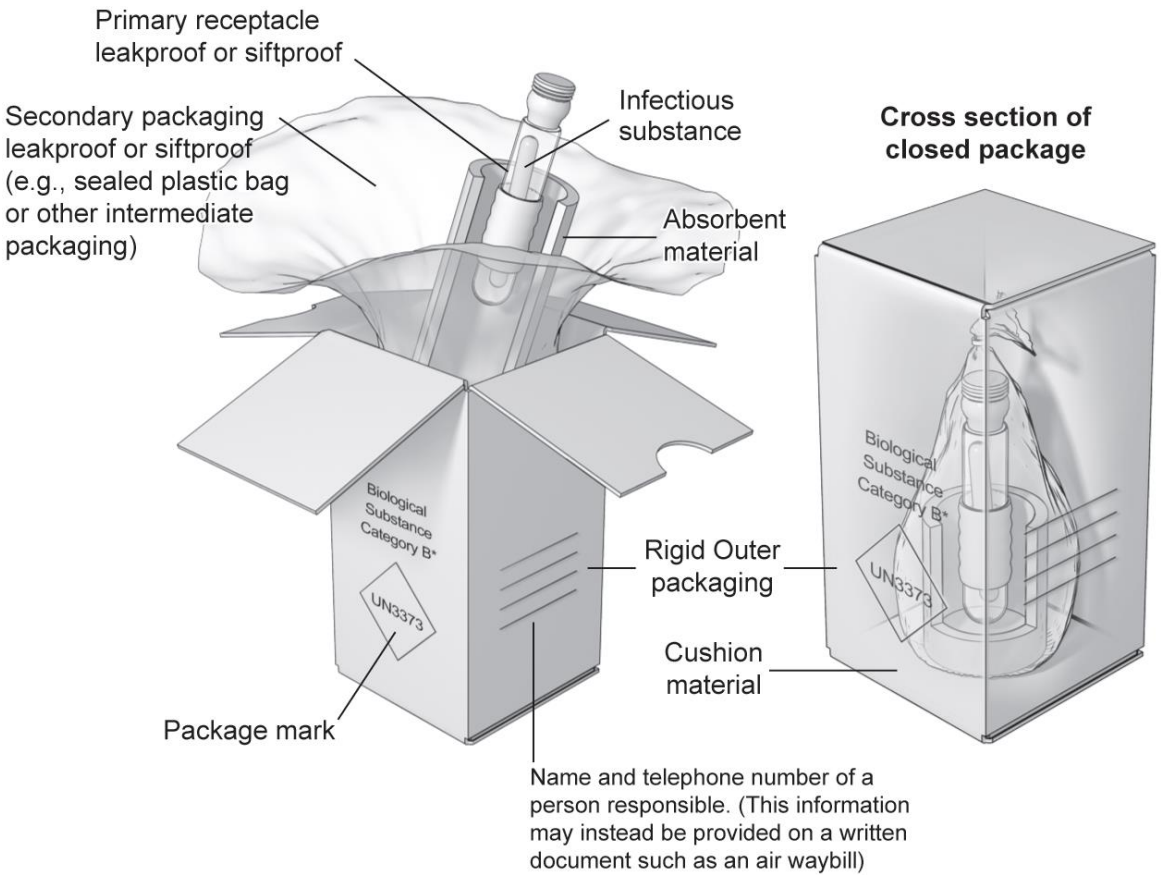


Figure 2

Links:

ISM-MS Guidance on Shipping and Importing:

http://intranet1.mountsinai.org/compliance/envhs/hazardous_materials_shipping.asp

CDC Import / Export:

http://www.cdc.gov/od/eaipp/

USDA / APHIS Permits for Interstate Transport

http://www.aphis.usda.gov/permits/

Chapter 11: Recombinant DNA, RNA, Synthetic Molecules, and Gene Therapy / Transfer Projects

A. Introduction: *The NIH Guidelines April 2016 (also "Guidelines")*

Icahn School of Medicine is a recipient of grant funding from the National Institutes of Health. As a recipient contractor, *ALL* research whether funded directly by the NIH, or by other granting sources, falls under the *NIH Guidelines,* which is the short version for: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). All Principal Investigators involved with rDNA and related genetic work should become familiar with *the Guidelines* and its requirements.

NIH has a FAQ base at: https://osp.od.nih.gov/biotechnology/nih-guidelines-faqs/

The Guidelines sets out the roles and responsibilities of the NIH, RAC, OBA, ISMMS and the Principal Investigators conducting research with recombinant DNA, Gene Therapy / Transfer, or products derived from genetic recombination in Sections I - IV.

B. Overview of *NIH Guidelines* Sections and Appendices

Sections I – IV are provided below. ***Section II*** discusses conducting a risk assessment based on content in ***Sections I and II***. ***Section III*** specifically gives *the range of experiments* covered by *the Guidelines* and conditions for filing projects with OBA, RAC (Major Actions, Gene Therapy); OBA and ISMMS IBC before initiating work; and reporting to the ISMMS IBC before or simultaneously with the initiation of the project; and finally those projects EXEMPT from reporting to either OBA or the ISMMS. Several Flow charts are included at the end of this chapter to use in determining where your experiments reside in the Section III requirements.

Section IV outlines the responsibilities of the Principal Investigator to ISM-MS, to the ISM-MS Biosafety Committee and to the NIH – OSP, as an awardee / contractor. It is strongly recommended that a PI read and become thoroughly familiar with Sections III and IV, especially if NIH-OSP is planning on conducting a site visit.

Refer to:

Appendix B

https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html#_Toc446948379

Subsequent sections of *the Guidelines* cover Biosafety as related to Recombinant DNA and related topics. All italicized content is directly from *the Guidelines*. Although there is much overlap with the CDC's Biosafety levels, in some respects the Biosafety Levels in Appendix G are more strict in the *Guidelines*, in that the NIH is concerned with the inadvertent release of a genetically modified agent to the natural environment. The two appendices given below are used in determining the risk posed by working with a GMMO, and the required protection such work requires.

APPENDIX G. PHYSICAL CONTAINMENT contains the requirements for BSLs 1-4.

The objective of physical containment is to confine organisms containing recombinant or synthetic nucleic acid molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant or synthetic nucleic acid molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

APPENDIX Q. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULE RESEARCH INVOLVING ANIMALS

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms

tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede Appendix G (Physical Containment) when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see Appendix Q-III-D, Footnotes and References for Appendix Q). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry. Appendix Q contains the requirements for BSLs 1-4 relating to animal research (ABSL's in the BMBL). The CDC levels were given earlier in this manual.

Other Appendices of the Guidelines are:

APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

***This appendix reflects the current state of knowledge** and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the Agent Summary Statements of the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories (see Sections V-C, V-D, V-E, and V-F, Footnotes and References of Sections I through IV. Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.*

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the NIH Guidelines.

A summary table is given below. A very rough rule of thumb is that BSL-1 practices go with RG-1, BSL-2 with RG-2 agents and so on up the scale. An exception is HIV, which requires only BSL-2, yet is a RG3 - agent. Another consideration is that the RGs are based on the risks and hazards presented by the agent, whereas the BSLs address the performance-based safety practices needed to protect researchers and the environment from exposure.

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that 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)

APPENDIX C.EXEMPTIONS UNDER SECTION III-F-8

Section III-F-8 states that exempt from these NIH Guidelines are "those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), NIH Director--Specific Responsibilities), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, Exemptions under Sections III-F-8, for other classes of experiments which are exempt from the NIH Guidelines." The following classes of experiments are exempt under Section III-F-8:

Recombinant or synthetic nucleic acid molecules ***containing less than one-half of any eukaryotic viral genome*** *(all viruses from a single family being considered identical -- see Appendix C-IX-E, Footnotes and References of Appendix C), that are propagated and maintained in cells in tissue culture* ***are exempt*** *from these NIH Guidelines with the exceptions listed in Appendix C-I-A.*

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, Footnotes and References of Sections I through IV) or cells known to be infected with these agents, (iii) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates), and (iv) whole plants regenerated from plant cells and tissue cultures are covered

by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

APPENDIX D. MAJOR ACTIONS TAKEN UNDER THE *NIH GUIDELINES*

As noted in the subsections of Section IV-C-1-b-(1), the Director, NIH, may take certain actions with regard to the NIH Guidelines after the issues have been considered by the RAC. Some of the actions taken to date include the following: ***Appendix D-1(ca.1988) - Appendix D-118 (4 /6 / 2010)***

This is a compendium of preceding determinations made by RAC and OBA regarding vectors and gene therapy transfers. This Appendix can inform PIs and research Coordinators of past filings with OBA and RAC, and the need to file their projects as Ma*jor Actions.*

APPENDIX E. CERTIFIED HOST-VECTOR SYSTEMS (See Appendix I, Biological Containment)

While many experiments using Escherichia coliK-12, Saccharomyces cerevisiae, and Bacillus subtilis are currently exempt from the NIH Guidelines under Section III-F, Exempt Experiments, some derivatives of these host-vector systems were previously classified as Host-Vector 1 Systems or Host-Vector 2 Systems. A listing of those systems is presented in Appendix E. Biological containment is a unique concept in the Guidelines, since it relies on the host's biological properties to restrict or prevent the release of a GMMO to the environment.

APPENDIX F. CONTAINMENT CONDITIONS FOR CLONING OF GENES CODING FOR THE BIOSYNTHESIS OF MOLECULES TOXIC FOR VERTEBRATES

Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} of < 100 nanograms per kilograms body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriaeneurotoxin) are covered under Section III-B-1

APPENDIX H. PATHOGENIC AGENT SHIPMENTS

In addition to DOT / IATA Class 6.2 Regulations, The Guidelines regulate shipments of GMOs and GMMOs in domestic and International Shipments. *Host organisms and viruses will be shipped as etiologic agents if they contain recombinant or synthetic nucleic acid molecules when: (i) the recombinant or synthetic nucleic acid molecule includes the complete genome of a host organism or virus regulated as a human or animal pathogen or a plant pest; or (ii) the recombinant or synthetic nucleic acid molecule codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome*

and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage; or (iii) the recombinant or synthetic nucleic acid molecule comes from a host organism or virus regulated as a human or animal pathogen or as a plant pest and has not been adequately characterized to demonstrate that it does not code for a factor involved in eliciting human, animal, or plant disease.

APPENDIX I. BIOLOGICAL CONTAINMENT (See Appendix E, *Certified Host-Vector Systems*)

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant or synthetic nucleic acid molecule and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment shall be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other non-laboratory hosts. The following levels of biological containment (host-vector systems) for prokaryotes are established. Appendices I-I-A through I-II-B describe levels of biological containment (host-vector systems) for prokaryotes. Specific criteria will depend on the organisms to be used.

APPENDIX J. BIOTECHNOLOGY RESEARCH SUBCOMMITTEE

The National Science and Technology Council's Committee on Fundamental Science determined that a subcommittee should be continued to identify and coordinate Federal research efforts, identify research needs, stimulating international cooperation, and assess national and international policy issues concerning biotechnology sciences. The primary emphasis will be on scientific issues to increase the overall effectiveness and productivity of the Federal investment in biotechnology sciences, especially regarding issues which cut across agency boundaries. This subcommittee is called the Biotechnology Research Subcommittee.

APPENDIX K. PHYSICAL CONTAINMENT FOR LARGE SCALE USES OF ORGANISMS CONTAINING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES

Appendix K specifies physical containment guidelines for large-scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant or synthetic nucleic acid molecules. It shall apply to large-scale research or production activities as specified in Section III-D-6, Experiments Involving More than 10 Liters of Culture.

APPENDIX L. GENE THERAPY POLICY CONFERENCES (GTPCS)

In order to enhance the depth and value of public discussion relevant to scientific, safety, social, and ethical implications of gene therapy research, the NIH Director will convene GTPCs at regular intervals.

As appropriate, the NIH Director may convene a GTPC in conjunction with a RAC meeting. GTPCs will be administered by NIH/OSP. https://osp.od.nih.gov/biosafety-biosecurity-and-emerging-biotechnology/

APPENDIX M. POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF PROTOCOLS FOR THE TRANSFER OF recombinant or synthetic NUCLEIC ACID MOLECULES INTO ONE OR MORE HUMAN RESEARCH PARTICIPANTS (POINTS TO CONSIDER)

Appendix M applies to research conducted at or sponsored by an institution that receives any support for recombinant or synthetic nucleic acid molecule research from NIH. Researchers not covered by the NIH Guidelines are encouraged to use Appendix M (see Section I-C, General Applicability).

Research proposals involving the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from such nucleic acid molecules, into human subjects (human gene transfer) will be considered through a review process involving both NIH/OBA and RAC. Investigators shall submit their relevant information on the proposed human gene transfer experiments to NIH/OBA. Submission of human gene transfer protocols to NIH will be in the format described in Appendix M-I-A, Submission Requirements for Protocol Submission. ***Submission to NIH shall be for registration purposes and will ensure continued public access to relevant human gene transfer information conducted in compliance with the NIH Guidelines.*** *Investigational New Drug (IND) applications should be submitted to FDA in the format described in 21 CFR, Chapter I, Subchapter D, Part 312, Subpart B, Section 23, IND Content and Format.*

Institutional Biosafety Committee ***approval must be obtained from each institution at which recombinant or synthetic nucleic acid molecule material will be administered to human subjects*** *(as opposed to each institution involved in the production of vectors for human application and each institution at which there is ex vivo transduction of recombinant or synthetic nucleic acid molecule material into target cells for human application).*

This is a very important Appendix, since the requirements for applying for gene therapy projects are contained here. There are very important IRB requirements as well as IBC, OBA and RAC requirements, which the Principal Investigator or Research Coordinator *MUST* be aware of. Failure to file an Appendix M can result in the suspension of a research grant by the NIH until the infraction is resolved to the satisfaction of the NIH.

ISMMS researchers must be aware that even though they are funded by an international Pharmaceutical company to conduct a clinical trial, either the parent Pharmaceutical company *OR THE ISMMS PI MUS*T have a valid Appendix M filed with and approval from NIH OBA before empaneling subjects in the trial. The key point is ***that all research conducted at ISMMS*** **comes under NIH Guidelines regulation**, and

international companies may not be aware of this requirement to submit an Appendix M. The obligation then falls on the ISMMS researcher to submit the appendix.

APPENDIX P. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULE RESEARCH INVOLVING PLANTS

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant or synthetic nucleic acid molecule-containing plants, plant-associated microorganisms, and small animals. The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule-derived organisms associated with plants.

Currently, there is no plant –related research conducted at the ISMMS.

C. INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)

The Institutional Biosafety Committee is the committee that reviews and approves all experiments that come under the NIH Guidelines, except those that fall under Section III-E which are designated "Exempt" from reporting to the IBC. The IBC is still interested in Non-exempt research projects and requests that the Biosafety Officer keep records on all exempt DNA activities. The Charter for the Committee that delineates the committee's responsibilities under NIH-OBA and was approved is provided below. MSSM has now become ISMMS.

1. The Mount Sinai School of Medicine (MSSM) will maintain an Institutional Biosafety Committee consistent with the National Institutes of Health (NIH) Guidelines published in Published in Federal Register, July 5, 1994 (**59 FR 34496**) and its most recently published amendment.[Currently, November 2013]

2. Membership of the committee will consist of no fewer than 5 individuals with experience and expertise in recombinant DNA (rDNA) technology and other biosafety issues. At least two members shall not be affiliated with the MSSM and should represent the interests of the surrounding community with respect to public health and protection of the environment. At least one member shall have expertise in animal containment principles and one member shall be a Biological Safety Officer

3. The responsibilities of the IBC include, but are not limited to the following:

   a. Review rDNA, pathogen, oncogene, toxins and toxic chemical use in research conducted at MSSM. These reviews shall include:

(1) independent assessment of containment levels

(2) assessment of the facility's procedures, practices, training and expertise of the personnel involved in research

involving rDNA, pathogens, oncogenes, toxins and toxic chemicals.

(3) verification and assignment of the classification of the rDNA research in accordance with the NIH Guidelines.

b. Notify the Principal Investigator of the results of the IBC review and approval.

c. Set appropriate containment levels for experiments as specified in the most recent edition of the *NIH Guidelines.*

d. Provide for the adjustment of containment levels for certain experiments as specified in the *NIH Guidelines* and CDC/NIH *BMBL* (latest edition).

e. Conduct periodic reviews of rDNA, pathogen, oncogene, toxin and toxic chemical research conducted at the MSSM for compliance with the *NIH Guidelines* and CDC/NIH *BMBL.*

f. Adopt emergency plans covering spills and personnel contamination from containment laboratories.

g. Report any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and the NIH within 30 days.

h. Provide an open forum for the discussion of biosafety concerns and assist in the resolution of any biosafety issues brought before the committee.

i. Review of all Standard Operating Procedures (SOP's) for significant hazards.

j. Provide training for members of the committee.

4. ***Meetings of the IBC will be held at a minimum of quarterly per calendar year. Additional meetings may be called at the discretion of the Chairperson. A quorum of Five IBC Members***

including at least One Non-institutional member will be required to review activities and approve protocols.

5. Subcommittees may be established by the Chairperson in order to review and resolve a variety of biosafety issues. Examples of these subcommittees are the Recombinant DNA Technical Review Subcommittee and the Pathogen Technical Review Subcommittee. Meetings of the subcommittees can be arranged at the discretion of the members.

The Chair of the committee will update the committee regularly on relevant issues.

6. Investigators must notify the IBC Chair and the Grants and Contracts Office in writing of any adverse event within 24 hours of the event and in an annual report.

7. Any research related accident must be reported ***in writing*** to the Biosafety Officer.

8. This directive will be included in the information packet given to Principal Investigators.

9. The Committee will develop training materials, including on-line exercises, for investigators. More extensive training will be provided for those who must access the BSL-3+ facility.
10. References:

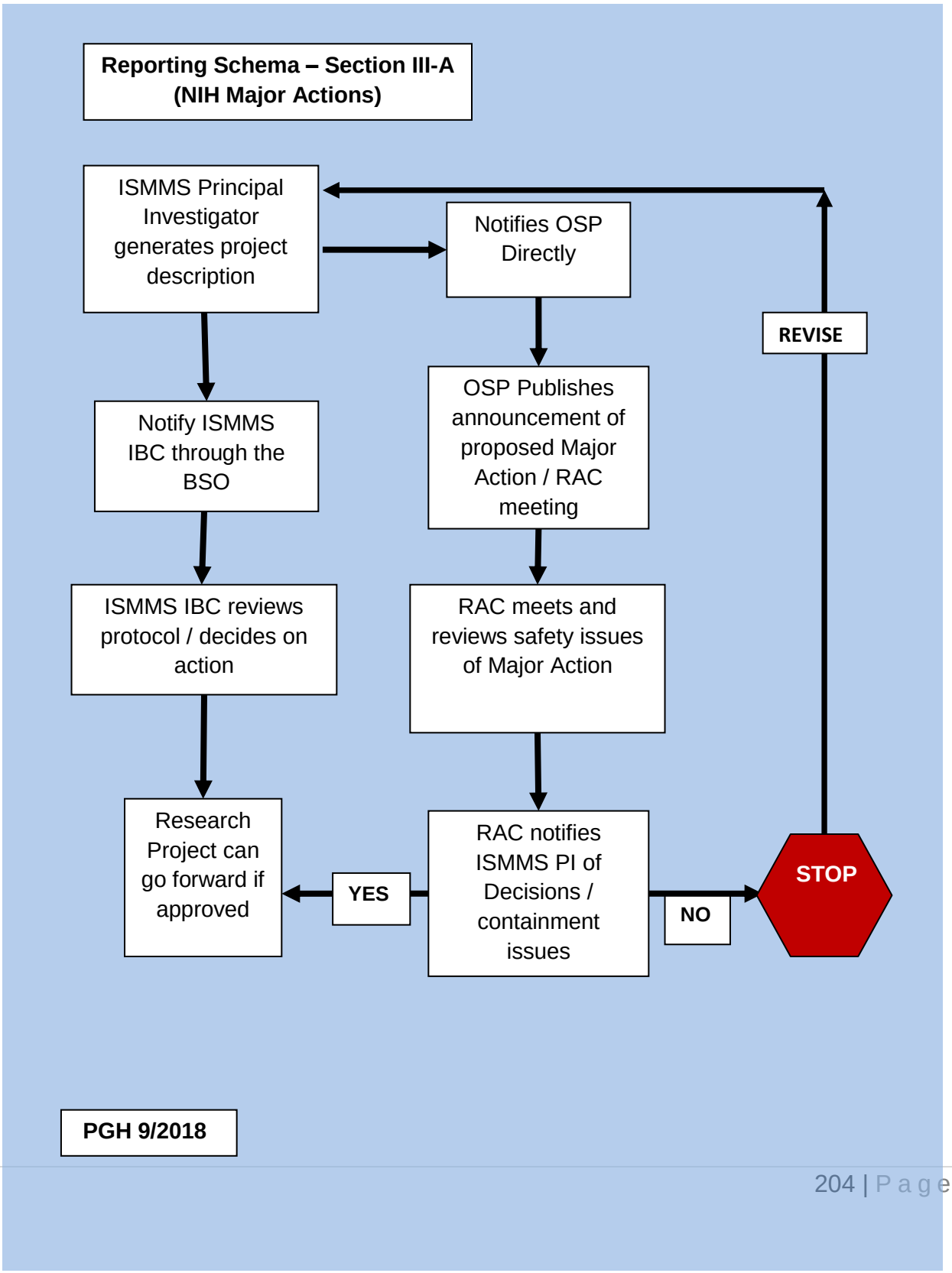
1. NIH Guidelines for Research Involving Recombinant DNA. (current edition).

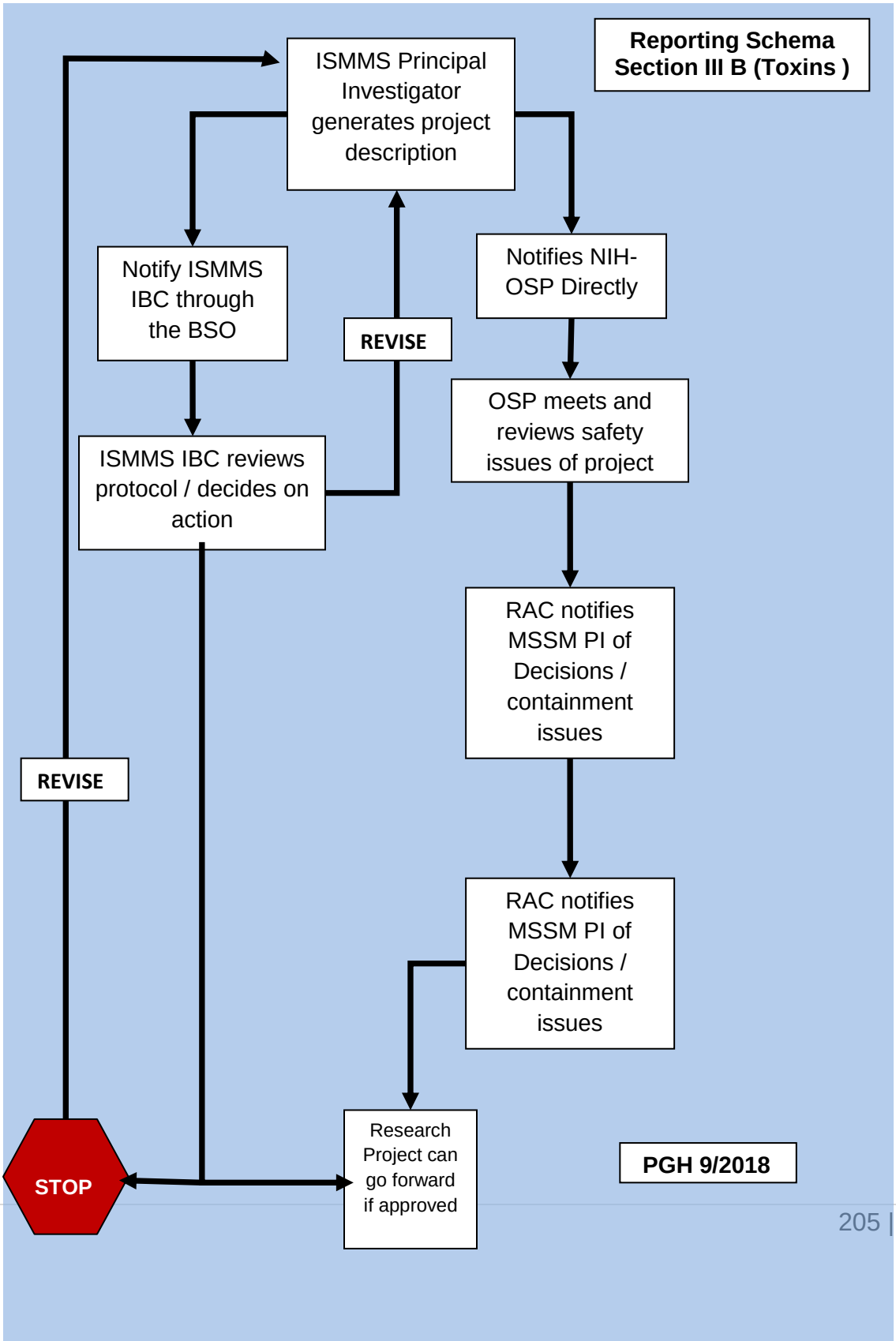
2. CDC-NIH Biosafety in Microbiological and Biomedical Laboratories (current edition).

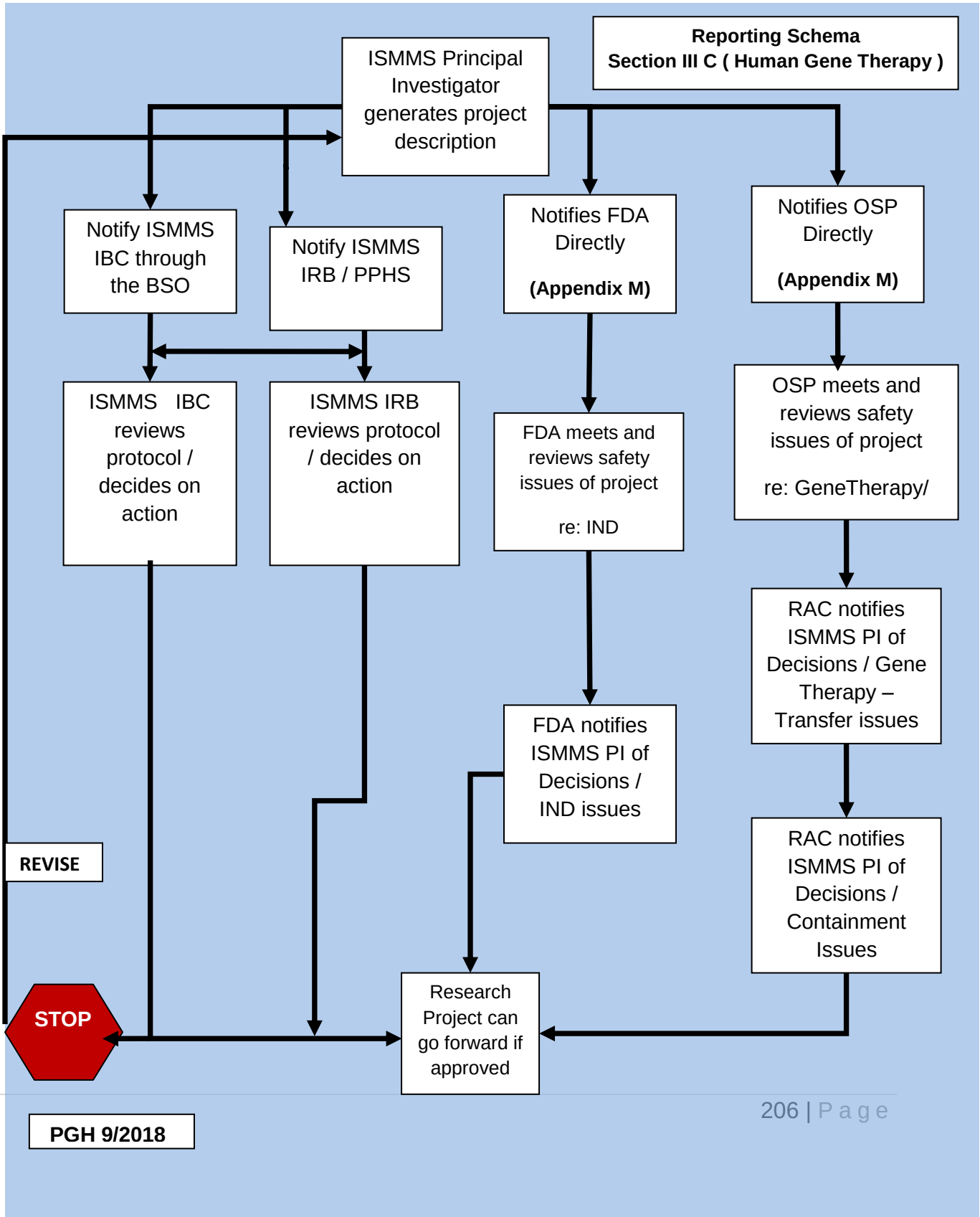
Proposed : Sept 8, 2004
Revised: Jan.10, 2005 Pgh:
Revised: 9/25/2018 Pgh:

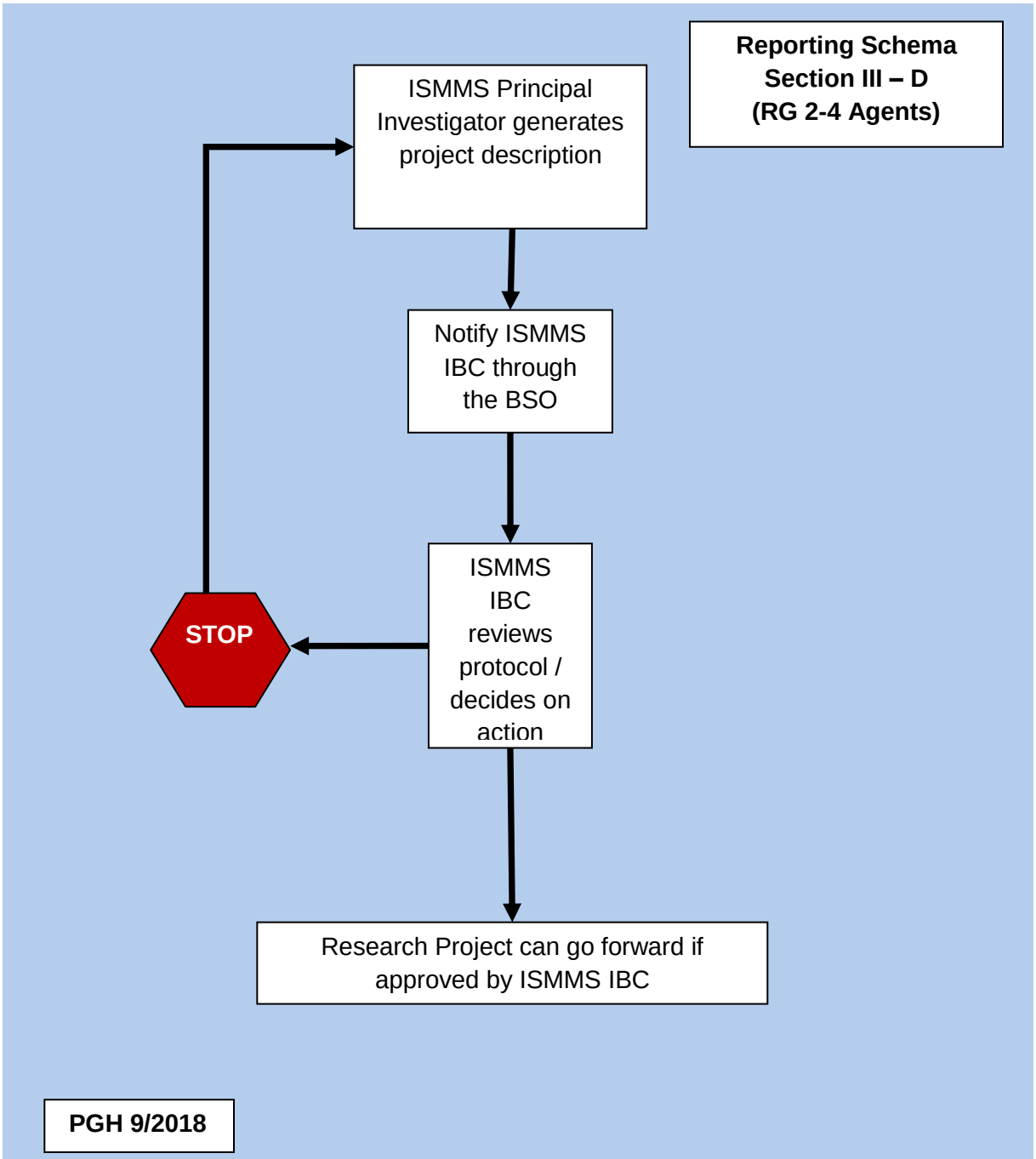
D. Flow Charts of Reporting Schemas for Section III / *NIH Guidelines*

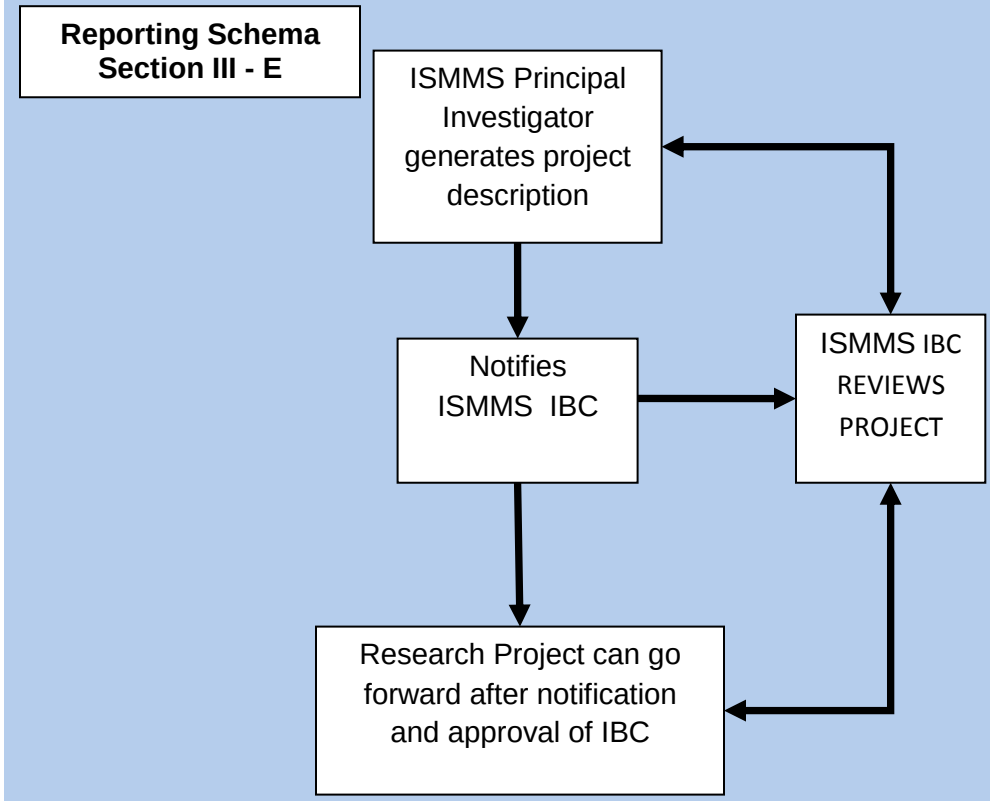
Following pages contain outline schemas in flow chart format showing the interaction of NIH OSP, ISM-MS, ISM-MS Biosafety Committee and PI together under specific activities.


Reporting Schema – Section III-A
(NIH Major Actions)
ISMMS Principal Investigator generates project description
Notifies OSP Directly
REVISE
Notify ISMMS IBC through the BSO
OSP Publishes announcement of proposed Major Action / RAC meeting
ISMMS IBC reviews protocol / decides on action
RAC meets and reviews safety issues of Major Action
Research Project can go forward if approved
YES
RAC notifies ISMMS PI of Decisions / containment issues
NO
STOP
PGH 9/2018

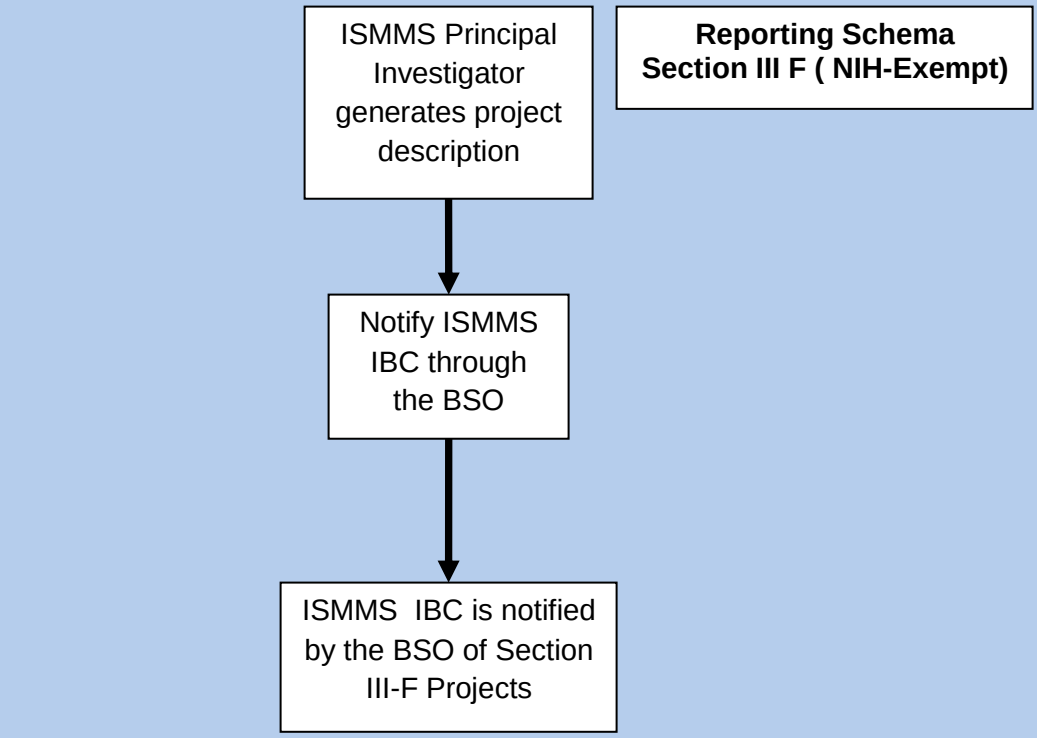

Reporting Schema
Section III B (Toxins)
ISMMS Principal Investigator generates project description
Notify ISMMS IBC through the BSO
Notifies NIH-OSP Directly
REVISE
OSP meets and reviews safety issues of project
ISMMS IBC reviews protocol / decides on action
RAC notifies MSSM PI of Decisions / containment issues
REVISE
RAC notifies MSSM PI of Decisions / containment issues
STOP
Research Project can go forward if approved
PGH 9/2018


Reporting Schema
Section III C (Human Gene Therapy)
ISMMS Principal Investigator generates project description
Notify ISMMS IBC through the BSO
Notify ISMMS IRB / PPHS
Notifies FDA Directly
(Appendix M)
Notifies OSP Directly
(Appendix M)
ISMMS IBC reviews protocol / decides on action
ISMMS IRB reviews protocol / decides on action
FDA meets and reviews safety issues of project
re: IND
OSP meets and reviews safety issues of project
re: GeneTherapy/
RAC notifies ISMMS PI of Decisions / Gene Therapy – Transfer issues
FDA notifies ISMMS PI of Decisions / IND issues
RAC notifies ISMMS PI of Decisions / Containment Issues
REVISE
STOP
Research Project can go forward if approved


Reporting Schema
Section III – D
(RG 2-4 Agents)
ISMMS Principal Investigator generates project description
Notify ISMMS IBC through the BSO
ISMMS IBC reviews protocol / decides on action
STOP
Research Project can go forward if approved by ISMMS IBC
PGH 9/2018


Reporting Schema
Section III - E
ISMMS Principal Investigator generates project description
Notifies ISMMS IBC
ISMMS IBC REVIEWS PROJECT
Research Project can go forward after notification and approval of IBC

PGH 9/2018



Source: https://osp.od.nih.gov/biotechnology/nih-guidelines/

PGH 9/2018

Table 4: Carcinogens

Compound	Use Condition	Principal Investigator Approval Level	Laboratory or Branch Chief Approval Level	Occupational Health and Safety Committee Approval Level
Benzene; Carbon Tetra-chloride;Chloroform;1,2,Dibromo-3-chloropropane;1,1 Dimethyl-ethylenimene; p-Dioxane; Ethylene dibromide; Propy-leneimine	Storage Normal Operation(1) Complex Operation(2)	<10liters(=)* < I liter(=) < 0.1 liter	> 10 liters > I liter 0.1 to 1.0 liter	— — > 1.0 liter
Bromoethyl methanesulfonat;Chlorormethyl methylether;Di-epoxybutane; 1,1-Dimethyl-hydrazine;1,2-Dimethylhydrazine;Ethylenimine;Ethyl methanesulfonate;Hydrazine; Methylhydrazine; Methyl meth-anesulfonate; N-Nitrosodiethy-lamine;N-Nitrosodimethylamine; N-Nitrosodi-n-butylamine; N-Nitrosodi-n-propylamine;N-Nitroso-N-ethylurethane; N-Nitrosopiperidine; Polychlorinated biphenyls; β-Propiolactone;	Storage Normal Operation Complex Operation	<1000 g(=) < 100 g(=) < 10 g	>1000 g > 100 g 10 g to 100 g	— — > 100 g
N-Acetoxy-2-acetylaminofluorene; 2-Acetylaminofluorene; Afla-toxins; o-Aminoazotoluene; 2-Aminofluorene; Benz(a)an-thracene;Benzo(a)pyrene; Chlorambucil; Cycasin; Diazo-methane; Dibenz[a,h]anthracene; 7,12-Dimethylbenz(a)anthracene; 4-Dimethylamino-azobenzene; 3-3'-Dimethyl-benzidine; 1,4-Dinitrosopiperazine; N-Hydroxy-2-acetyl-aminofluorene; 3-Methylcholanthrene; 4,4'-Methylene bis(2-chloroaniline); 1-Mehtyl-3-nitro-1-nitrosoguanidine; N-[4-(5-Nitro-2-furyl)-2-thiazoyl]-formamide; N-Nitroso-N-ethylurea; N-Nitroso-N-methylurea; 4-Nitro-quinoline-1-oxide; Procarbazine; 1,3-Propane sultone; m-Toluenediamine; Uracil mustard; Vinyl Chloride	Storage Normal Operation Complex Operation	< 100 g < 10 g < 1 g	100gto1000g 10g to 100 g 1 g to 10 g	> 1000 g > 100 g > 10 g
Bis(chloromethyl) ether	Storage Normal Operation Complex Operation	— — —	< 1 liter(=) < 0.01 liter(=) —	> 1 liter > 0.01 liter Any Quantity
4-Aminophenyl;Benzidine;3,3'-Dichlorobenzidine;3,3'-Dimeth-oxybenzidine;2-Naphthylamine; 4-Nitrobiphenyl	Storage Normal Operation Complex Operation	— — —	< 100 g(=) < 1 g —	> 100 g > 1 g Any Quantity

Note: Approval levels apply to principal investigators and laboratory/branch chiefs who have successfully completed the NIH course in the recognition and control of chemical hazards in the laboratory.

(5) **Normal Operation**: Any operation involving simple manipulations or reactions where the potential for release of the material is remote (e.g. dilutions; qualitative, controlled transfer of test materials; use of analytical standards).

(6) **Complex Operation**: Any operation involving the handling, manipulation or reaction of materials where the potential for release of the material is significant (e.g. rapid exothermic reactions; imparting of sufficient energy to a test system (heating, mixing, delivery under pressure) so that uncontrolled release of material could occur; transfer of electrostatic powders).

(7) *< 10 liters(=); quantity less than or equals 10 liters

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