Biosafety Level 2 and rDNA Training
Office of Biological Safety
Biosafety Level 2 and rDNA Training

- Difference between Risk Group and Biosafety Level
- NIH and UC policy on recombinant DNA
- Work conducted at Biosafety Level 2
- Biosafety cabinets
- Occupational first aid
- What to do in the event of a biohazard spill
- UC Code of Conduct for researchers

Please refer to The University of Chicago Biosafety Manual for supplementary information about the concepts presented in this training module.
http://biologicalsafety.uchicago.edu/page/university-chicago-biosafety-manual
Biosafety Level 2 and rDNA Training

What is the difference between risk group and biosafety level?
Risk Groups: Classification given to infectious microorganisms/pathogens based on hazard. Characteristics taken into account when performing a risk assessment include:

- Capability to infect and cause disease in a susceptible human or animal host
- Infectious dose
- Virulence as measured by the severity of the disease
- Route of transmission of the natural disease and transmissibility
- Availability of preventive measures and effective treatments for the disease
- Stability in the environment
- Host range and endemic nature

Risk groups correlate with but do not equate biosafety levels.

Biosafety Level: Description of the microbiological practices, safety equipment, and facility safeguards for the corresponding level of risk associated with handling a particular agent. Determination of the appropriate biosafety level for working with an organism will take into account:

- The agent’s risk group
- Nature of work being conducted and procedural protocols
- Origin of agent – indigenous or exotic
- Experience of staff
Risk Groups vs Biosafety Level

- RG1: Not associated with disease in healthy adults (non-pathogenic *E. coli*; *S. cerevisiae*)
- RG2: Cause diseases not usually serious and are often treatable (*S. aureus*; *Legionella*; *Toxoplasma gondii*)
- RG3: Serious diseases that may be treatable (*Y. pestis*; *B. anthracis*; *Rickettsia rickettsii*; HIV)
- RG4: Serious diseases with no treatment/cure (Hemorrhagic fever viruses, e.g., Ebola; no bacteria)
Biosafety Level 1 (BSL-1) relies on standard microbiological practices to manage RG1 organisms. There are no special containment or facility design requirements other than a sink for hand washing.

**Risk Groups vs Biosafety Level**

- BSL-1: Usually corresponds to RG1
  - Good microbiological technique
  - No additional safety equipment required for biological work (may still need chemical/radiation protection)
  - Ability to destroy recombinant organisms (even if they are RG1)
All of the standard microbiological practices conducted at BSL-1 are implemented at BSL-2 as well. BSL-2 builds upon BSL-1 containment by adding engineering features and safety equipment, protective clothing, biohazard signage, and specialized training for staff.
At BSL-3 the use of primary and secondary barriers is emphasized, requiring special engineering and design features.

**Risk Groups vs Biosafety Level**

- BSL-3: Same as BSL-2, PLUS...
  - Specialized clothing (respiratory protection, Tyvek, etc.)
  - Directional air flow is required. Rooms **must** have negative pressure relative to outside the room
  - BSC for ALL activities
BSL-4 work is performed in an isolated facility with complex, specialized ventilation requirements and waste management systems.
Risk Groups vs Biosafety Level

- Biosafety levels are set by the Institutional Biosafety Committee (IBC)
- NIH demands any institution receiving NIH funding and working with rDNA must have an IBC that:
  - Consists of experts in the fields of research at the institution,
  - Has at least two people who live in the area who are not associated with the institution (Community Members), and
  - Meets regularly.
Biosafety Level 2 and rDNA Training

What do I need to do if I work with recombinant DNA at UC?
Recombinant and Synthetic Nucleic Acids (NIH Definition)

- Molecules that a) are constructed by joining nucleic acid molecules and b) can replicate in a living cell, i.e., recombinant nucleic acids;
- Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids; or
- Molecules that result from the replication of those described in either point above.
Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
“The NIH Guidelines”

- Resulted as a concern of safety/ethics of rDNA research in the 1970s
- NIH’s Office of Biotechnology Activities (OBA) published the first issue of the Guidelines in 1976
- Updated periodically: visit http://oba.od.nih.gov/rdna/nih_guidelines_oba.html for the most up-to-date information
The purpose of the NIH Guidelines is to specify practices for constructing and handling:
- recombinant nucleic acid molecules,
- synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and
- cells, organisms, and viruses containing such molecules.
The Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules were established by the NIH to ensure safe, secure, and ethical work with rDNA.

These “NIH Guidelines” apply to all research at any institution that receives NIH funding. Therefore, all recombinant or synthetic nucleic acid work at the University of Chicago is subject to the NIH Guidelines, even if the lab does not have NIH funding.

One of the mandates of the NIH Guidelines is that each institution has an Institutional Biosafety Committee (IBC) to oversee rDNA research.
Experiments covered by the NIH Guidelines (Section III): This section describes six categories of experiments involving recombinant or synthetic nucleic acid. These six categories are presented in descending order in terms of the degree of oversight required.
Experiments that fall into these categories require approval before commencement of work.

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

**Example:** Rickettsial infections are treated in the clinic with tetracycline antibiotics. A tetracycline resistance gene cannot be cloned into *Rickettsia* species without first having approval from the IBC, RAC, and NIH Director.

**III-B:** Experiments involving the cloning of toxin molecules with LD$_{50}$ of less than 100 nanograms per kilogram body weight.

**Example:** The LD$_{50}$ for botulinum toxin is 1.2 ng/kg. Cloning and expression of the genes that encode this toxin requires first obtaining approval from the IBC and NIH-OBA.
Experiments that fall into these categories require approval before commencement of work.

**III-C:** Experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules into one or more human research participants.

Human gene transfer is the deliberate transfer into human research participants of either:
1. Recombinant nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, or
2. Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:
   A. Contain more than 100 nucleotides; or
   B. Possess biological properties that enable integration into the genome; or
   C. Have the potential to replicate in a cell; or
   D. Can be translated or transcribed.

**Example:** Direct administration of rDNA to humans via gene gun or administration of microorganisms harboring rDNA to humans would require IBC, IRB, and OBA approval prior to the start of the experiment.

**III-D:**
- Experiments using RG2, RG3, RG4, or restricted agents as host-vector systems.
- Experiments in which DNA from RG2, RG3, RG4, or restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems.
- Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of a helper function in tissue culture (e.g., lentivirus, adenovirus).
- Experiments involving whole animals in which the animal’s genome has been altered by stable introduction of rDNA, or DNA derived there from, into the germ-line (transgenic animals) and experiments involving viable rDNA-modified microorganisms tested on whole animals. This excludes transgenic rodents managed at BSL-1.
- Experiments to genetically engineer plants by rDNA methods, to use such plants for other experimental purposes, to propagate such plants, or to use plants together with microorganisms or insects containing rDNA.
- Experiments involving more than 10 liters of culture.
-Experiments with influenza virus generated by recombinant or synthetic methods.
Experiments that fall into category III-E require IBC notice simultaneous with initiation of work.

**III-E:**
- Experiments involving the formation of rDNA molecules containing no more than 2/3 of the genome of any eukaryotic virus.
- Experiments involving the rDNA-modified whole plants, and/or experiments involving rDNA-modified organisms associated with whole plants, except those that fall under III-A, III-B, III-D, III-F.
- Experiments involving the generation of rodents in which the animal’s genome has been altered by stable introduction of rDNA, or DNA derived therefrom, into the germ-line (transgenic rodents) requiring BSL-1 containment.

**III-F: Experiments Exempt from NIH Guidelines**
- Those involving synthetic nucleic acids that (1) can neither replicate nor generate nucleic acids that can replicate in any living cell, (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight.
- Those that are not in organisms, cells, or viruses, and that have not been modified or manipulated to render them capable of penetrating cellular membranes.
- Experiments that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.
- Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- Those that consist entirely of DNA from a prokaryotic host when propagated only in that host.
- Those that consist entirely of DNA from an eukaryotic host when propagated only in that host (or a closely related strain of the same species).
- Consist entirely of DNA segments from different species that exchange DNA by known physiological processes.
- Those that do not present a significant risk to health or the environment, as determined by the NIH Director, with advice of the RAC.
III-F Experiments that are exempt from the NIH guidelines are **NOT** exempt from oversight by the UC IBC if they involve rDNA or RG2 or higher organisms.

For information specific to The University of Chicago, please visit the UC IBC website at [http://researchadmin.uchicago.edu/ibc/](http://researchadmin.uchicago.edu/ibc/)
If you are working with these toxins, you need to submit an IBC protocol (regardless of whether you are using rDNA or infectious agents).

More information on IBC-regulated toxins can be found at http://researchadmin.uchicago.edu/docs/ibc/UC_ibc_Toxin.pdf
If you are working with these toxins, you need to submit an IBC protocol (regardless of whether you are using rDNA or infectious agents).

More information on IBC-regulated toxins can be found at http://researchadmin.uchicago.edu/docs/ibc/UC_ibc_TOxin.pdf
Biosafety Level 2 and rDNA Training

What is required for work at Biosafety Level 2 (BSL-2)?

The following requirements are based on *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition* (BMBL)

The complete text of BMBL can be found online at [http://www.cdc.gov/biosafety/publications/bmbl5/](http://www.cdc.gov/biosafety/publications/bmbl5/)
In general, the greatest risks for laboratory personnel at BSL-2 include accidental percutaneous or mucous membrane exposure or ingestion of infectious materials. However, a risk assessment is always required to identify hazards specific to the organism, facility, and procedures involved.
Washing your hands is the best way to remove biohazardous contamination from your hands and prevent further spread. Hand-sanitizing solutions/gels/foams do **NOT** replace hand washing as a means of decontamination. Hand sanitizer usually relies on ethanol as a germicide and is ineffective against non-enveloped viruses, such as adenovirus, and spore-forming bacteria. Hand washing with soap and water also provides mechanical removal of microorganisms, which is equally as important as the soap used.

What Is Required at BSL-2?

- Lab supervisor controls access to the lab
- Workers must wash hands:
  - After working with anything hazardous
  - ALWAYS before leaving the lab
- Washing hands is one of the simplest and most reliable things you can do in lab safety
What Is Required at BSL-2?

- Lab supervisor should ensure that staff is trained for working safely in the lab
- Personal health status should be considered when conducting any laboratory work
  - Immunocompromised individuals
  - Pregnant women
  - Sensitivities to chemicals and/or allergens
  - LET YOUR PERSONAL PHYSICIAN KNOW WHAT YOU DO!
What Is Required at BSL-2?

- The following is prohibited while in lab:
  - Eating, drinking, or smoking
  - Handling contact lenses
  - Applying cosmetics, lotions, etc. (including Chapstick®)
  - Storing food in lab
  - Mouth pipetting
What Is Required at BSL-2?

- Policies for safe handling of sharps (needles, scalpels, broken glass, etc.) must be established and followed
- Minimize creation of splashes and/or aerosols
- Aerosols vs Droplets
  - Droplets are much larger and often visible
  - Aerosols are invisible
  - Gravity will cause droplets to settle on surfaces
  - Aerosols behave as a gas, will not settle, and are removed by the room HVAC system

Safe handling of sharps:

- Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, forceps or other mechanical means. Plastic ware should be substituted for glassware whenever possible.
Why use a BSC if my bug is not transmitted via aerosol route?

**Answer:** Working in a BSC provides other advantages in addition to containment of aerosols. Utilization of a BSC confines the work space where biohazards are manipulated, reducing their accidental spread beyond the BSC. This confined space can often be easier to decontaminate than large open open benches.
What Is Required at BSL-2?

- Decontaminate work surfaces after work and after any spill of potentially infectious material
- Decontaminate any cultures, stocks, and other potentially infectious material before disposing
  - This includes non-infectious material that is carrying rDNA
- If needed, effective pest management program should be in place
Decontamination

- Decontaminate work surfaces daily
  - Wipe down at beginning and end of day
  - Wipe down any time there is a spill or possible spill
- Waste should be decontaminated prior to disposal
- The proper means of decontamination will be found in your lab’s IBC protocol. Ask your PI for a copy!
  - Bleach, EtOH, etc.
  - Autoclaving
### Decontamination

- What should be used for decontamination?
  - 10% household bleach (made weekly) for spores or non-enveloped viruses
  - 70% EtOH for most vegetative bacteria or enveloped viruses; 70% EtOH is better than 100% EtOH
  - When using bleach, follow with EtOH to eliminate bleach from surface
  - Decontamination of toxins depends on which toxin is being used
  - Consult IBC protocol or Biosafety Office

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**Decontamination:**

The proper way to decontaminate your work surface and potentially infectious material will depend on the microorganism that you work with. The information regarding the proper means of decontamination for your work will be listed in the Agent Profile Form of your lab’s IBC Protocol. Ask your PI or contact the IBC office to obtain a copy of the IBC protocols that describe your work with biohazards.

If you’re going to use EtOH for decontamination, 70% EtOH is better than 100%.

For more information about choosing the correct decontamination method, please refer to the University of Chicago Biosafety Manual at [http://biologicalsafety.uchicago.edu/page/university-chicago-biosafety-manual](http://biologicalsafety.uchicago.edu/page/university-chicago-biosafety-manual) or contact the Office of Biological Safety.
What Is Required at BSL-2?

- **Immunizations, tests, serum baselines, etc.**
  - May be recommended or required
  - Declination forms
  - Alternative work assignment
  - Discuss with PI and your physician

- **Sharps**
  - Minimize use when possible
  - Use appropriate SHARPS container for disposal

- **Disposal of biohazard waste**
  - Marked containers
  - Containers should be leak-proof

What Is Required at BSL-2?

- Decontaminate any potentially contaminated equipment
  - Think about your bug and your equipment when deciding on disinfectant
  - Ask Biosafety if you have questions about how to decontaminate
- Spill protocol should be included in Biosafety Manual
  - It is listed on IBC protocol
  - All workers should be familiar with their lab’s spill protocol
- No animals in lab unless they are part of experiment
A “primary barrier” is the first line of defense between a biohazard and the worker. A BSC is considered a primary barrier even when a biohazard is contained within a tube because once the tube is opened, the BSC becomes the first line of defense.

BSCs are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed.
Lab Coats: Lab coats should be provided by your supervisor and should be laundered routinely. Lab coats are never to be worn outside of the lab or taken home for any reason. Laundry facilities are available to you on campus. Contact your PI or department administrator for information regarding how to have your lab coat laundered. Never launder your lab coat at home.
LAB FACILITIES:
Secondary Barriers

- Hand wash sink
- Lab is easily cleaned
  - Reduce clutter
  - Bench tops easy to wipe down
  - Cloth-upholstered chairs not recommended
- Eye wash station in each room
  - Test once a week by flushing for three minutes each week
  - Maintain accessibility
  - No clutter near eyewash
Biological Safety Cabinets
Types of Particulate Protection

- Personnel Protection: Aerosols generated within the cabinet are contained and kept away from the researcher.

- Product Protection: Air within the work space of the cabinet has been filtered so that it is virtually free of airborne particles and organisms, thus protecting your work from outside contamination.

- Environmental Protection: Aerosol generated within the unit is removed from the air before the air is discharged.
# Types of Ventilation Equipment

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<td>Class III BSC</td>
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Fume Hoods

- Personnel protection only
- Exhausts air to the outside
- Does not offer product or environmental protection
- Draws contaminants in the laboratory air directly over the product being worked on
- Used for work with chemical hazards
Clean Benches

- Product protection only
- Provided by creating airflow generated through a HEPA filter
- Discharge air goes directly into workroom
- Used when the product is not hazardous but must be kept contaminant free
- Preparation of non-hazardous mixtures and media
- Never use a clean bench for working with biohazardous material!
Classes and Types of BSCs

- Class I
- Class II
  - Type A1
  - Type A2
  - Type B1
  - Type B2
- Class III
Ultraviolet (UV) Lights

- The CDC, NIH, and ABSA all agree that using UV lamps for decontamination of BSCs is not recommended or required.

- UV light in your BSC is only useful as an extra precaution in keeping the work area decontaminated between uses.

- UV light has very little power to penetrate even through a dust particle.
HEPA Filters

- 99.97% effective on particles 0.3 microns in size

- Higher effectiveness on particles less than and greater than 0.3 microns

- The 0.3 micron is the “most penetrating particle size”
  - i.e., the least efficiently filtered particle
  - Particles larger and smaller than 0.3 μm are filtered more efficiently
HEPA Filters

Capture particles via:

– Impaction
– Interception
– Diffusion
HEPA Filters

- Filters may increase effectiveness as more particles are trapped on filter
- This can lead to increased resistance and wear on motor/blower
- No evidence that particles are released from filters during normal use
Biological Safety Cabinets
Class II – A2 and B1
Class II BSC Airflow

- Personnel Protection
  Intake air rushing into the front grille prevents aerosols in the work area from escaping

- Product Protection
  HEPA-filtered down-flow air in the rear of the front grille prevents outside contaminants from entering the work area

- Environmental Protection
  All air leaving the BSC is HEPA filtered to prevent aerosols from escaping in the exhaust air
Class II Type A2

- Exhaust 30% of total air handled and recirculate 70%
- Minimum inflow air velocity of 100 fpm
- May exhaust HEPA-filtered air back into the room or through a canopy to the outdoors
- Plenums under negative pressure or surrounded by negative pressure ducts and plenums
- A2 cabinets may be used with small quantities of toxic chemicals and small amounts of radionuclides, if they are exhausted through exhaust canopies
Class II Type B1

- Exhaust 70% of total air handled and recirculate 30%
- Air in the back of the cabinet is exhausted to the outdoors through a dedicated exhaust plenum and the air in the front is recirculated
- Minimum inflow air velocity is 100 fpm
- Must be hard ducted to the outside for the cabinet to function
- May be used with small quantities of toxic chemicals and small amounts of radionuclides
Preparation for Working in a BSC

Setting up the Cabinet

- Turn on blower and wait at least 2 minutes before loading equipment. This is to purge the BSC of contaminated air.
- Disinfect work area and work surfaces before setting up;
- Check that the sash is in the correct position;
- Make sure that everything needed to complete the work is in the cabinet;
- Segregate clean items from ones that will get contaminated;
- Work from “clean” to “dirty”;
- Do not block grille or airflow slots.
Working in a BSC

The Operator

- When seated, armpits are level with bottom of the window
- Move smoothly and deliberately in and out of the unit
- Work as far into the work area as comfortably possible - at least 6 inches
- Schedule uninterrupted work times
- Only one person at the cabinet at a time
- Do not put your head inside an operating cabinet
- Respond appropriately to all warnings and alarms
What to Do in the Event You Are Exposed to a Biohazardous Agent
## Occupational Exposure

### First Aid

**Skin Contamination**
- Disinfect by submersing or exposing the area with 70% alcohol for 10 minutes.
- Wash with soap and water

**Needle Sticks, Contaminated Cuts, and Animal Bites/Scratches**
- If bleeding, squeeze out blood, spray with 70% alcohol
- Disinfect with iodine solution (povidone-iodine) or peroxide for 20 minutes
- Wipe surrounding tissue with iodine solution or peroxide
- Wash with soap and water

**Eye Contamination**
- Flush eyes in eye wash for 15 minutes.

**Aerosol Exposure**
- Remove PPE and leave lab
Occupational Exposure
First Aid

After leaving lab:
• *Immediately* contact the University of Chicago Occupational Medicine (UCOM) Bloodborne Pathogen/Needle Stick hotline at 773-753-1880, enter pager number 9990, followed by #
• Notify supervisor/PI
• Notify Biosafety Office
Occupational Exposure
First Aid

Life threatening situations:
• Call 911 if on or off campus
• If health emergency and on campus, call 147 from a campus phone
  – CART Team (resuscitation)
  – Faster than 911
Spill of Less Than 1 Liter of Infectious Material (Outside of a BSC)

Part I

- Contain spill by covering with paper towels.
- Warn others in the immediate area of the potential infectious spill and leave the area under normal SOP for 30 minutes. Post signage of the spill prohibiting access.
  - Allows droplets to settle
  - Aerosols get removed by building HVAC system
  - Post signage of the spill prohibiting access
- Notify your supervisor and if necessary, the Biosafety Office.
- Remove contaminated clothing, wash all contaminated body parts, and flush exposed mucous membranes.
- Re-enter the area and cover the spill with paper towels and saturate with 10% bleach; Leave room and allow 30 minutes of contact time.
Spill of Less Than 1 Liter of Infectious Material
(Outside of a BSC)

Part II

- Re-enter the area and pick up paper towels, working from the outside of
  the spill inward, and place them in a leak-proof container lined with a
  plastic bag.
- If broken glass or sharps are present, handle with tongs, forceps, brush
  and dustpan, or other mechanical means. Place broken glass in sharps
  container. Do not use your hands.
- Treat all waste as biohazardous. Autoclave material from the clean-up.
- Exit the area under normal SOP and wash all potentially exposed body
  parts.
Spill of Greater Than 1 Liter of Infectious Material (Outside of a BSC)

• Leave the room and alert others to do the same

• Call UC Police at 123 from a campus phone

• They will provide you with instructions and assistance
Biosafety, Post-9/11

Biosafety: Keeps bad bugs away from good people

Biosecurity: Keeps bad people away from bad bugs

Biosurety: Keeps only good people near bad bugs
- Hiring and maintaining a trustworthy, responsible, and ethical work force.
UC Biosurety Program
Code of Conduct

In the realm of research involving the study of pathogens and toxins, additional responsibilities include:

• Awareness of and adherence to all safety protocols
• Knowledge and awareness of spill and exposure protocols
• Knowledge of and adherence to reporting requirements related to spills, exposures, or potential releases
• Knowledge and awareness of all emergency response protocols (e.g., fire, tornado, inclement weather)
• Completion of all training requirements
• Completion of all proficiency training requirements
UC Biosurety Program
Code of Conduct

(continued)

- Completion of all Occupational Health requirements, including documentation of required physicals, medical clearances, and/or vaccinations
- Immediate reporting to the Principal Investigator/Employee Assistance of any situation that compromises an individual's ability to perform as required in a BSL-2 or ABSL-2 laboratory, including physical or psychological issues
- Immediate reporting to the Principal Investigator and the UC, where appropriate, of behavior or activities that are inconsistent with safety and security plans
- Awareness of and adherence to security protocols
Office of Biological Safety

Call or Visit!

• Abbott 120
• Joe Kanabrocki: 4-7496
• Allen Helm: 4-6756