Biosafety Level 1 and rDNA Training

Office of Biological Safety

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Biosafety Level 1 and rDNA Training

- Difference between Risk Group and Biosafety Level
- NIH and UC policy on recombinant DNA
- Work conducted at Biosafety Level 1
- UC Code of Conduct for researchers

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Biosafety Level 1 and rDNA Training

What is the difference between risk group and biosafety level?

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Risk Groups vs Biosafety Level

- Risk Groups: Assigned to infectious organisms by global agencies (NIH, CDC, WHO, etc.)
- In US, only assigned to human pathogens (NIH)
- Biosafety Level (BSL): How the organisms are managed/contained (increasing levels of protection)

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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)

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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)

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Risk Groups vs Biosafety Level

- BSL-2: Same as BSL-1, PLUS…
  - Biohazard signs
  - Protective clothing (lab coat, gloves, eye protection, etc.)
  - Biosafety cabinet (BSC) for aerosols is recommended but not always required
  - Negative airflow into room is recommended, but not always required

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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

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Risk Groups vs Biosafety Level

- BSL-4: Same as BSL-3, PLUS…
  - Airlock entry; Shower exit
  - Special waste disposal
  - Complete isolation of agent from worker (Positive pressure suits)

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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.

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Biosafety Level 1 and rDNA Training

What do I need to do if I work with recombinant DNA at UC?

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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.

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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

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Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
"The NIH Guidelines"

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.

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Mandate for the Institutional Biosafety Committee (IBC)

- Required by the NIH Guidelines
- Applicable to all recombinant or synthetic nucleic acid research sponsored by institutions receiving NIH funds.
- Ensures oversight at the local/institutional level

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Important Sections of the NIH Guidelines

- Experiments covered by the Guidelines (Section III)
- Responsibilities (Section IV)
  - Biosafety Officer (BSO), IBC, Institution, and Investigator
- Pathogen Risk Groups (Appendix B – human pathogens)
- Biosafety Levels (Appendix G)
- Animal Containment (Appendix Q)
- Plant Containment (Appendix P)

http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

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Experiments Covered by the NIH Guidelines (Section III)

- III-A. Major actions (includes adding clinically relevant drug-resistant markers to pathogens)
  - Requires approval from the IBC, rDNA Advisory Committee (RAC), and NIH Director
  - Approval required before project can start
- III-B. Cloning of toxins
  - Requires approval from IBC and NIH-OBA
  - Approval required before project can start

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Experiments Covered by the NIH Guidelines (Section III)

- III-C. Administration of rDNA to humans (Human Gene Transfer)
  - Requires IBC approval, IRB approval, and OBA approval
  - Approval required before project can start
- III-D. rDNA with pathogens, animals, large cultures, etc.
  - Requires IBC approval
  - Approval required before project can start

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Experiments Covered by the NIH Guidelines (Section III)

- III-E. Less hazardous rDNA work (transgenic rodents and plants)
  - Approval by IBC
  - Approval is NOT required to begin work, but IBC notification is required
- III-F. Non-hazardous rDNA work
  - Exempt from the Guidelines

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What Are IBC Requirements by UC?

- UC IBC requirements are more stringent than what NIH requires
- You need an IBC protocol to begin work at UC if you:
  - Manipulate rDNA
  - Work with Risk Group 2 organisms or higher (regardless of presence of rDNA)
  - Work with transactive (e.g., TAT) or infectious proteins (e.g., prions)
  - Work with one of the biological toxins listed on the next slide

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Toxins Regulated by the UC IBC

- Abrin
- Aerolysin
- Botulinum toxin
- b-bungarotoxin
- *C. difficile* enterotoxin A
- *C. perfringens* lecithinase
- *C. perfringes* perfringolysin O
- *C. perfringes* delta toxin
- *C. perfringes* epsilon toxin
- Conotoxin
- Diacetoxycirpenol
- Diphtheria toxin
- Listeriolysin
- Modeccin
- Pertussis toxin
- Pneumolysin
- *Pseudomonas* toxin A
- Ricin

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Toxins Regulated by the UC IBC

- Saxitoxin
- Shiga toxin
- *Shigella dysenteriae* neurotoxin
- *Staphylococcus* enterotoxins A-F
- Streptolysin O
- Streptolysin S
- T-2 toxin
- Taipoxin
- Tetanus toxin
- Tetrodotoxin
- Volkensin
- *Yersinia pestis* murine toxin

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Biosafety Level 1 and rDNA Training

What is required for work at Biosafety Level 1 (BSL-1)?

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

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When Is BSL-1 Work Required?

- When working with most Risk Group 1 agents

- BSL-1 work is NOT sufficient if you are using:
  - Human-derived cells (even cell lines)
  - Any clinical material from humans or non-human primates.
  - Biological toxins

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What Is Required at BSL-1?

- Lab supervisor enforces policies that control 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

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What Is Required at BSL-1?

- 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

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What Is Required at BSL-1?

- 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

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What Is Required at BSL-1?

- Decontaminate work surfaces after work and after any spill of potentially infectious material
- Destroy any organisms carrying rDNA before disposal
  - Recombinant organisms cannot enter the food chain (NIH regulation)
    - *E. coli* carrying plasmids
    - Transgenic animals (includes *Drosophila* or other insects)
- If needed, effective pest management program should be in place

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Decontamination When Using Recombinant Organisms

- 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

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Decontamination When Using Recombinant Organisms

- 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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What Is Required at BSL-1?

- **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

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SAFETY EQUIPMENT: Primary Barriers

Personal Protective Equipment (PPE)

- Special containment (such as biosafety cabinets) are usually not needed
- Lab coat: Wear in lab/Remove when leaving lab
- Gloves should be worn when needed
  - Latex or nitrile (if allergic to latex)
  - Remove before leaving lab
  - Wash hands after removing
  - Don't reuse
  - If you leave and need to protect your sample
    - Remove one glove
    - Use bare hand to open doors, touch elevator buttons, etc.
- Eye protection: If you might generate splashes of hazardous material

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LAB FACILITIES: Secondary Barriers

- Lab should have doors for access control
- 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

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Code of Conduct for Researchers at UC

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

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Code of Conduct for Researchers at UC

(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

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Call or Visit!

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- Joe Kanabrocki: 4-7496
- Allen Helm: 4-6756

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