

Laboratory Safety and Human Tissue Banking

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Laboratory Safety

Hazards in the laboratory: Chemical, Infectious, Physical, Radiation

Chemical:

1. Toxic or corrosive chemicals
2. Flammable/Explosive/Unstable chemicals
3. Carcinogenic/Mutagenic/Teratogenic chemicals
4. Toxic Compressed Gases

Identification

1. Manufacturer's label
2. Transfer container: should be clearly labeled
3. Laboratory procedures
4. Material Safety Data Sheets (MSDS)

Protection

1. Wear proper personal protection equipment (PPE): gloves, lab coat, and safety glasses
2. Properly labeled container, proper storage
3. Use safety equipment: biohazard hood
4. Disposal: follow university guidelines, check MSDS or Merck index, label as "Excess" not "Waste"
5. Use hand washing after glove removal; personal emergency rinse station for face/body exposures.
6. Spills: contain with bench coat or absorbent pads. Small spill or chemical: use mitt. Large flowing spills can be stopped or slowed with vermiculite or perlite. If spill is large and innocuous (buffer, distilled water) call house keeping. If hazardous, call 5-4444 and evacuate; for additional information, call the Hopkins Biosafety Officer, x5-5918.

Infectious:

Identification: Human blood and other tissue, Cell culture, Viral, Bacterial, Mold and fungus

Protection

Three rules based on Universal Precautions:

1. Consider all patients (and their tissue) as potentially infectious
2. Assume all blood and body fluids and tissue are contaminated with HIV and HBV.
3. Assume all used needles and other sharps are contaminated.

In the lab:

1. Wear proper personal protection equipment (PPE): gloves, lab coat, safety glasses, mask
2. Use safety equipment: biohazard hood
3. Use proper containment, storage, disposal
4. Decontamination: Anything considered infectious must be sterilized (autoclaved) prior to disposal in the biohazard box or mixed with bleach prior to sink disposal. All materials in contact with infectious item must be sterilized; areas in contact with infectious item must be cleaned with a fresh 10% bleach solution.
5. Use hand washing after glove removal; personal emergency rinse station for face/body exposures.

Physical:

Sharps: Needles, glass pipettes, slides, pins, blades. Do not re-cap needles or scalpel blades, do dispose of in sharps container.

Compressed gasses: Monitor all gas gauges when in use, do not exceed tubing or equipment pressure limits, shut down regulators when not in use, secure tanks properly.

Fire: stop, drop, and roll, use personal shower at sink, if fire on bench or hood use extinguisher if qualified or pull fire alarm, call 5-4444, close lab door and evacuate.

Cryogenics: use protective gloves, use lab coat, face shield (for liquid nitrogen)

Vacuum: do not use standard glassware, try to isolate vacuum recovery vessels away from personnel.

GENERAL LABORATORY RULES

1. No eating or drinking in the lab; no food or drink storage anywhere in the lab
2. Wear lab coat, gloves, and eye protection when experimenting
 - certain procedures require specialized protection
 - do not wear protective clothing outside of the work area
 - remove contaminated gloves immediately if possible
 - gloves become porous with use and exposure, so change them often
 - do not wear gloves to touch common-area surfaces (refrigerators, drawers, door knobs, equipment, phones, radios, faucets, manuals, reagent containers, etc.)
 - wash hands after glove use
 - you must wear a radiation-monitoring badge if working with radioactive isotopes
 - no sandals, open-toed shoes, shorts, or short skirts
3. Dispose of laboratory waste in the approved container
 - Biohazard box (red bag lined): general lab waste (gloves, wipes, tips, pipettes, broken glass sealed in smallest box possible, etc.)
 - Sharps container: needles, blades, glass pipettes
 - Radioactive material: approved container for that lab/isotope
4. Wash hands before leaving the laboratory

Important Info.

Fire: pull fire alarm, dial 5-4444 (security), report location, evacuate by nearest exit.

Injury: Call 911 and/or go to Emergency Department

Infectious needle or sharps stick: 5-STIX (5-7849) immediately

Material Safety Data Sheets (MSDS): laboratory file, Office of Safety and Environmental Health (5-5918) or Internet: www.ilpi.com/msds/index.chtml or Sigma/Aldrich or other suppliers

Safety Procedure Questions: Principle Investigator or Hopkins Office of Safety and Environmental Health: x5-5918, www.hopkinsmedicine.org/hse/

Poisoning: Call 911; for additional information, call Maryland Poison Information Center: 1-800-492-2414

In summary, LEARN and UNDERSTAND what reagents and procedures you will be using. KNOW the hazards; KNOW what to do and how to do it if a safety problem should arise. KNOW and use the prudent practices, protective facilities, and protective equipment provided to minimize the risks.

Human lung tissue procurement and tissue banking

Once received, the lung is transferred to 4°C RPMI solution containing penicillin, streptomycin, and amphotericin and dissected with sterile instruments.

Central airways (trachea to tertiary airways) are sent to a neighboring lab for epithelial cell isolation for primary ALI cultures (Vonakis, Sidhaye).

From each lung, airways (tertiary bronchus to alveoli), parenchyma, and lymph nodes are:

1. Fixed in 10% formalin for 24 hours. Some of this tissue is paraffin embedded, the remaining is stored at 4°C in sterile PBS containing 0.02% sodium azide. The latter can but used for frozen sectioning.
2. Another batch is put in RNALater solution in a sterile container for 72 hours, drained, stored in -80°C.
3. Another is put in a sterile container, placed on dry ice for 1-2hours, stored in -80°C.