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# BSL3 Practices

**Sandia National Laboratories**  
***Laboratory Biosecurity and Biosafety***  
***for BSL3 Laboratories***  
**India**  
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**[www.biosecurity.sandia.gov](http://www.biosecurity.sandia.gov)**

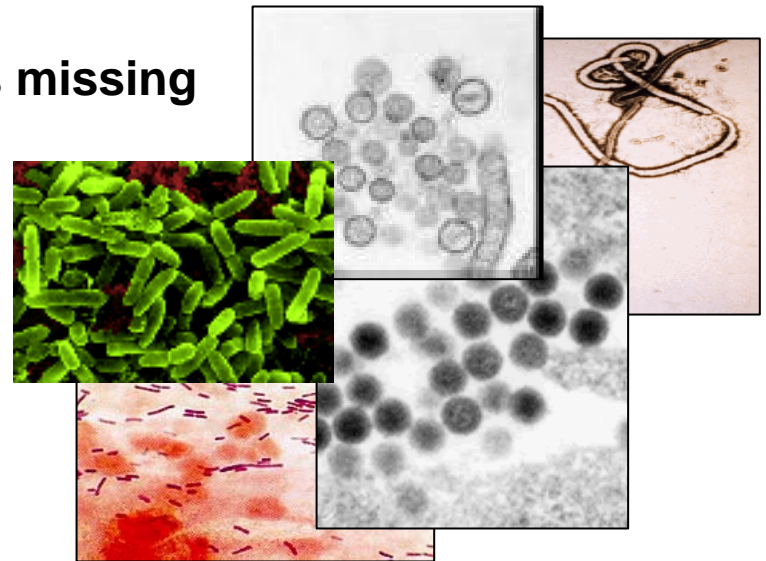
# BSL 3 Practices

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- **Administrative**
  - Written SOPs – discussed in Program Management briefing
  - Material control & accountability
- **Access controls**
  - Training requirements
  - Personnel screening / reliability
  - Visitor controls / protocols
- **Standard & special lab practices**
- **Personnel protective equipment (PPE)**

# Administrative Procedures: Material control & accountability

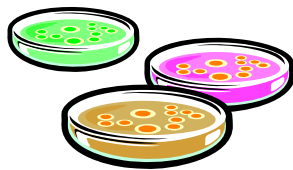
- Ensure the complete and timely knowledge of:
  - What materials exist
  - Where the materials are
  - Who is accountable for them
- NOT: to detect whether something is missing
- What materials in what forms
- Agent
  - What agents are high risk?
  - Viable? Whole organism or DNA?
- Quantity
  - Any amount can be significant
  - A threshold amount for toxins
- Form
  - Repository stocks, working samples, in host, contamination
- Detail—what level is adequate for MC&A?
  - Material as *items*
  - Each vial as a separate inventory record?
- Capture—when does MC&A start & stop?
  - Naturally occurring; clinical samples; disposition



# Administrative Procedures: Material control & accountability

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- **All material should have an associated “accountable person”**
  - The person best in a position to answer questions about the associated material
  - Not someone to blame!
  - Ensure that no material is “orphaned”
  
- **Procedures should ensure accountability**
  - Experimental work: laboratory procedures
  - Inventory: know what you have
  - Reporting: document routine MC&A practices
  - Audit/ assessment: is this working?
    - Ensures effective *implementation* of MC&A
  - Training: personnel understand requirements



# Access Controls: Establishing Restricted Areas

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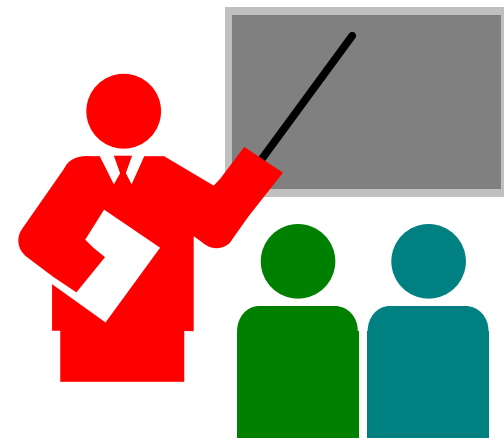
- **Access control ensures that only authorized individuals are allowed into certain areas**
  - Increasingly strict controls as you move toward higher risk assets
- **Limited Areas**
  - Unique item
  - Controlled possession
  - Electronic or physical key
- **Exclusion Areas**
  - Unique item
  - Unique knowledge
  - Controlled possession
  - Electronic key card and keypad or biometric deviceor
  - Controlled key and second individual to verify identity



# Access Controls: Training Requirements

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- **Increased need for staff training as risk increases**
- **All lab and support personnel receive training on hazards, precautions, exposure procedures, escort responsibilities, biosecurity procedures**
- **All lab personnel must demonstrate proficiency in lab-specific procedures**
- **Training should be repeated every year and when procedures change**
- **All training is documented**



# Access Controls: Personnel Screening

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- **Goal: Know that the individual is properly trained and can be trusted to work with the biological materials in the lab**
  
- **Before granting access:**
  - **Verify credentials**
  - **Verify completion of all pre-requisite training**
  - **Check references**
  - **Mentor and observe their lab skills**
  - **Ensure extensive experience at BSL 2 first**
  - **Medical surveillance**
  - **Assure supervision by competent scientist**
  - **Criminal history**
  - **In-depth background investigation**



# Access Controls: Visitors

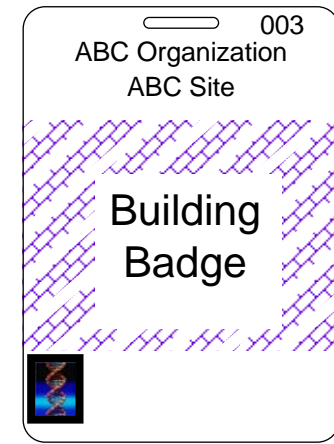
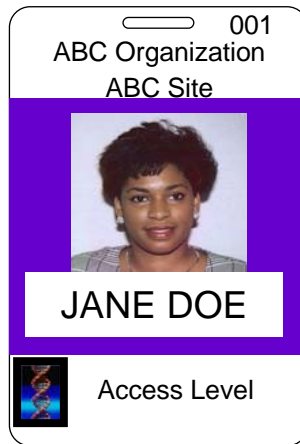
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- **Types**
  - **Personal Visitors**
    - Family members
  - **Casual Visitors**
    - Tours, seminars
    - Equipment repair technicians
  - **Working Visitors**
    - Visiting researchers
    - Facility maintenance personnel
- **Controls**
  - All visitors should have a host at the facility
  - Visitors should be escorted in restricted areas



# Access Controls: Badges

- **Badges should be issued to those individuals authorized to be in restricted areas**



- **Badge return**
  - Upon employee termination
  - Daily or at the conclusion of a limited term for visitors
- **Report lost or stolen badges**
- **Consider safety implications**

# Exposure Routes

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- **Airborne (A)**
- **Skin contact (S)**
- **Mucous membrane contact (M)**
- **Ingestion (I)**
- **Percutaneous injection (P)**

# **Standard and Special Lab Practices: Role in Preventing Exposure**

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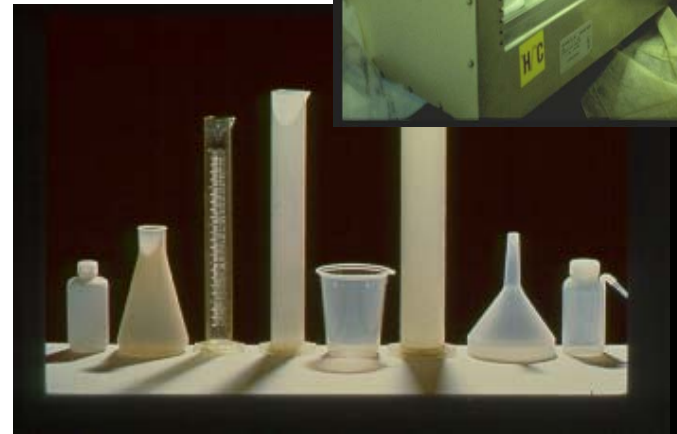
- **No mouth pipetting: A, I, S**
- **Careful manipulation of fluids: A, S, M**
- **Care with sharps: P, S, A, M**
- **Use of PPE: S, M, A, I**
- **Frequent hand washing: S, M, I**
- **Decontamination of work surfaces: S, M, I**
- **No eating, drinking, etc in labs: S, I**

**Airborne (A)**  
**Skin contact (S)**  
**Mucous membrane contact (M)**  
**Ingestion (I)**  
**Percutaneous (P)**



# Standard and Special Laboratory Practices

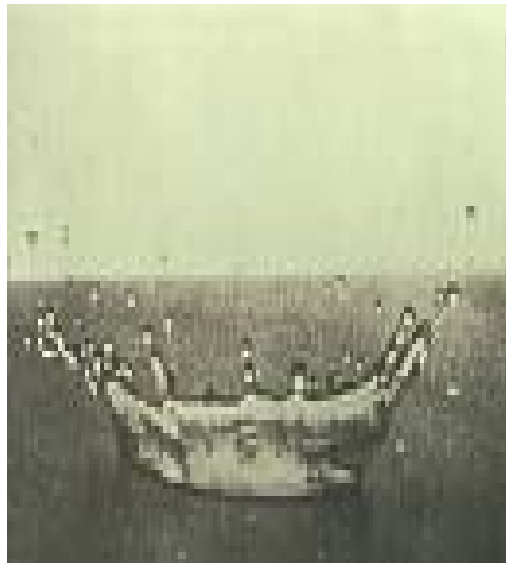
- All procedures with live agent must be performed inside the biosafety cabinet
- No work with open vessels on open bench
- Use of paper covering on work surface assists clean-up
- Use of appropriate disinfectant in cabinet
- Substitute plastic for glass
- Careful pipetting techniques
- Wash hands often
- No mouth pipeting
- No eating or drinking in lab
- Minimize aerosol generation
- Decontaminate work surfaces
- Safe sharps handling
- Wear applicable PPE



# Standard and Special Laboratory Practices: Minimizing Aerosols

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- Use careful pipetting practices
- Avoid drops onto hard surfaces
- Wipe up spills promptly with appropriate disinfectant
- Use capped tubes when mixing, blending, or vortexing
- Pour liquids carefully
- Avoid bubbles



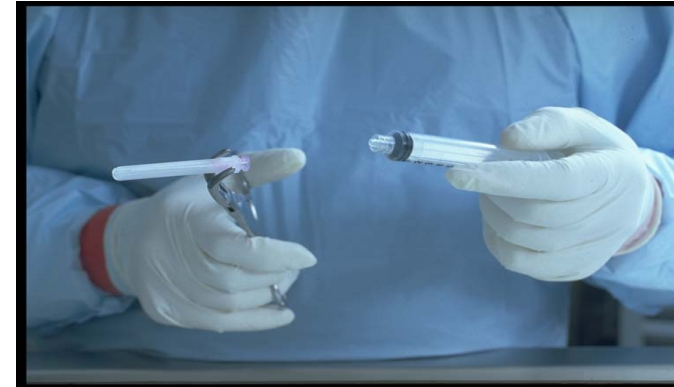
# Standard and Special Laboratory Practices: Careful Pipetting Techniques

- Never blow out last drop in pipette
- Use pipetting aids with filters
- Use horizontal pipette collection tubs
- Never mix by suction + expulsion
- Discharge liquid down side of container, using tip-to-wall contact
- Deliver as close as possible to contents
- Work over plastic-backed absorbent matting



# Standard and Special Laboratory Practices: Safe Use of Sharps

- **Sharps includes scalpels, blades and wires, anything that can cut or puncture**
- **Percutaneous exposure risk**
  - Employ safe work practices
  - Only use sharps if absolutely required as part of a process
- **Aerosol risk**
  - Use biosafety cabinet for removal of air from needle
- **Utilize safe sharp devices**
- **Keep hands away from needles**
- **Use mechanical methods for needle removal**
- **Never bend, recap or manipulate sharps by hand.**
- **Dispose of entire unit into sharps container**
- **Collect reusable sharps in labeled, leak-proof container**





# Personal Protective Equipment

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- **Why:**
  - Provides a barrier against skin, mucous membrane or respiratory exposure to infectious agents
  - To prevent spread of contamination
- **Types:**
  - Gloves
  - Gowns, lab coats, coveralls, scrubs
  - Goggles
  - Safety glasses with side shields
  - Face shield
  - Booties, head covers
    - Closed toe shoes (no sandals)
  - Respirators
- **Limitations:**
  - Does not eliminate the hazard
  - Integrity wanes with use (change gloves frequently)
  - Not all gloves created equal - select best glove for the task





# Personal Protective Equipment

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- **Personal protective equipment is NOT worn outside of the lab or taken home to be laundered!**
- **Reusable protective clothing should be autoclaved on-site**
- **For BSL 3**
  - **Back closing lab coat**
    - **Wrap around gowns with tight cuffs**
  - **Gloves**
    - **Single pair for entry**
    - **Double gloving for work in BSCs, transport, spill clean-up**
  - **Shoe coverings**
  - **Face protection**
    - **Safety glasses or goggles**
  - **Respiratory protection may be required (i.e. M.tb, SARS)**
- **Staff must be trained in aseptic removal procedures-  
gloves last**



# Personal Protective Equipment: Respirators

- **Uses a filter medium to remove contaminant**
  - Reduces aerosol exposure
- **N95 Respirator**
  - Disposable
  - Classified by:
    - Filter efficiency – 95% (N95), 99% (N99), 99.97% (N100)
    - Series – N (not oil resistant), R (oil resistant), P (oil proof)
- **Powered Air Purifying Respirator (PAPR)**
  - Disposable hood
  - Breathing tube
  - Motor/blower unit
  - Cartridges
  - Nickel Cadmium (NiCad) battery pack
  - Used when
    - Persons with facial hair or facial anomalies that interfere with the seal cannot wear an N95 respirator
    - High-risk aerosol generating procedures present
- **Surgical masks are not respirators**
  - Provide droplet protection, not aerosol protection
  - Provide patient protection
  - Keeps hands out of mouth



# Personal Protective Equipment: Respiratory Protection Program

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- **Medical evaluation**
  - Determine individual's fitness to use a respirator
  - Physician or other healthcare provider
    - Medical evaluation questionnaire
    - Physical exam at physician's discretion
- **Fit test**
  - Accepted/approved qualitative or quantitative protocol
  - When
    - Prior to initial use
    - Annually
    - Whenever different respirator is worn
    - Whenever a problem reported
    - Whenever a change (e.g. facial change, weight loss) is reported
- **Training**
  - Criteria for respirator selection
  - Limitations of respirator types
  - Proper method for donning
  - Checking facepiece for seal and proper operation
  - Respirator maintenance



# PPE Examples





# Removing Gloves

