
Components of Laboratory Biosafety – Part II

Laboratory Biosafety and Biosecurity Workshop

**Cairo, Egypt
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www.biosecurity.sandia.gov

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Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company,
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Exposure Routes

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

Standard and Special Lab Practices: Role in Preventing Exposure

- 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)



Personal Protective Equipment

Personal Protective Equipment

- **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

- **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

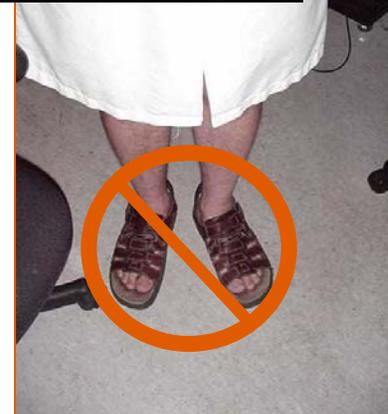


Personal Protective Equipment: Respiratory Protection Program

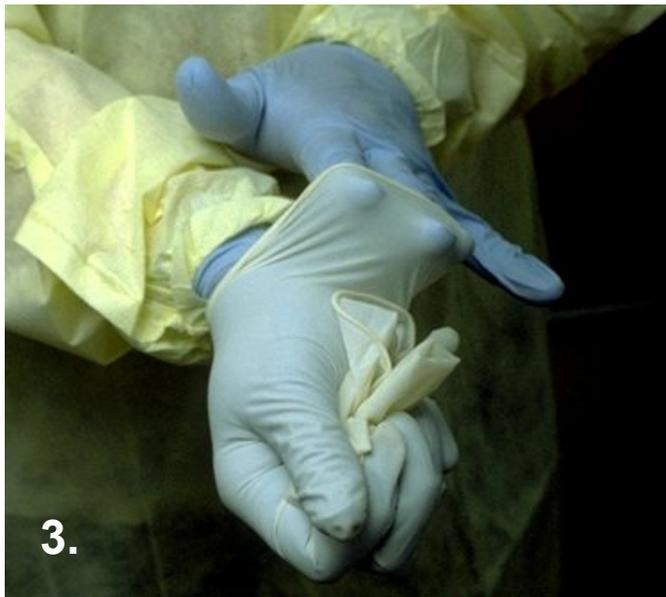
- **Medical evaluation**
 - Determine individual's fitness to use a respirator
 - Physician or other healthcare provider
 - Medical evaluation questionnaire
 - Physical exam at physician's discretion
- **Fit test**
 - Accepted/approved qualitative or quantitative protocol
 - When
 - Prior to initial 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



Decontamination

Definitions

- **Germicide: Any antimicrobial chemical agent used for disinfection, antisepsis or sterilization – regardless of whether its action is microbicidal or microbistatic; any microbicidal disinfectant, antiseptic or sterilant**
- **Contamination: Introduction of microorganisms into tissues or sterile materials**
- **Decontamination: Disinfection or sterilization of infected articles to make them suitable for use**
- **Disinfection: Selective elimination of certain undesirable microorganisms in order to prevent their transmission**
- **Antisepsis: Destruction of vegetative forms of microorganisms but not their permanent forms**
- **Sterilization: Complete killing of all microorganisms**

Effectiveness of Germicides

- **Concentration of the Germicide**
- **Concentration of the Agent**
- **Type of Agent**
- **Time of Contact**
- **Environmental Conditions Present**
- **Expiration Issues**

Surface Disinfectants

- Alcohols (Ethyl alcohol)
- Halogens (Sodium and Calcium hypochlorite)
- Quaternary Ammonium Compounds
- Phenolics (Lysol)
- Aldehydes (Formalin)
- Hydrogen peroxide



Autoclaves



Principles of Autoclave Sterilization

- Direct exposure of each item to steam at the required temperature and pressure for a specific time
- 121° C - 123° C (250° F - 254° F)
- 15 pounds per square inch (psi) at sea level; 1.05 kg/cm²
- Time
 - Minimal time required depends on the weight and specific nature of the objects to be sterilized

- Steam must contact all areas of a load
- Loosely gathered biohazard bags
- Add 500 ml of water to bags prior to packaging and transport to allow for steam saturation

Why Autoclave

- Properly used autoclaves may be used to sterilize instruments, other media, and biohazard waste
- It is recommended that gaseous emissions from the autoclave be actively exhausted through a canopy vent located above the autoclave



What can be Autoclaved?

- Pathogenic plant matter
- Culture and stocks of infectious agents (bacteria, mold, viruses)
- Contaminated solids (paper towel, cloth, plastic pipette tips, glassware)
- Discarded live and attenuated vaccines
- Recombinant DNA, plant and animal specimens
- Animal tissues
- Animal cage waste



What should NOT be Autoclaved?

- Items containing solvents, volatiles or corrosive chemicals
- Radioactive material (s)
- Sealed and pressurized containers



Autoclave Safety Procedures

- Follow manufacturers' guidelines
- Do not open when chamber is pressurized
- Avoid standing directly in front of autoclave door when opening
- Divide and Conquer
 - Divide small volumes into small
 - Autoclaving dense materials is not recommended
- Do not place sealed containers into autoclave
- **Careful - liquids are hot**
- Use shallow metal pans for best results and heat transfer
- Place autoclave on preventive maintenance schedule to ensure it is working according to specifications of the manufacturer
 - Annual inspection by manufacturer-certified technician



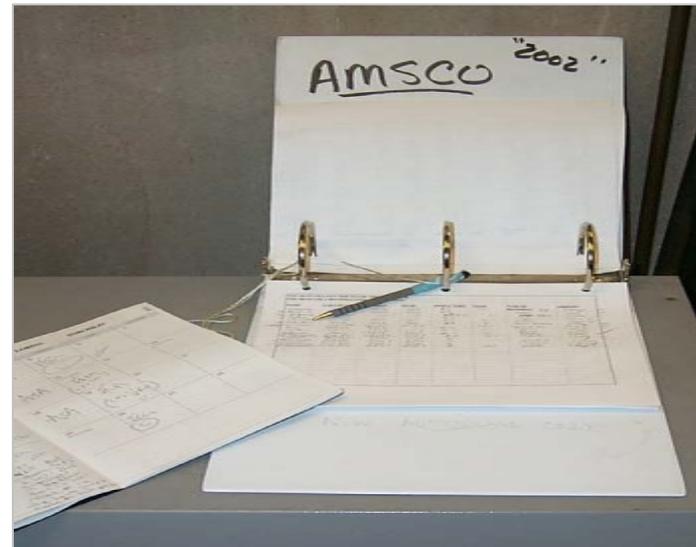
Biological Indicators for Autoclaves

- When the bioburden is unknown, the most appropriate method to validate sterilization is the overkill method.
- This method involves demonstrating that 10^6 spores (*Geobacillus stearothermophilus*) will be killed in a half cycle.
- Thus a full cycle would result in a 12-log reduction of spores and produce a **s**terility **a**ssurance **l**evel (**SAL**) of 10^{-6} or a one-in-a-million chance of a non-sterile sample.
- Monitoring of biohazard waste using biological indicators should be performed weekly



Record Keeping for Autoclaves

- Record all information in a Log book
 - Name
 - Laboratory Location
 - Phone Number
 - Date
 - Dwell Time
 - Temperature
 - Type and amount of material
 - Date of service visits



Cleaning Autoclaves

- **Clean trap at least once a week**
- **Clean surrounding area after every use**
- **Remove broken glass**
 - **Using forceps or other tools – not your hands**
- **Clean up overflowing liquids immediately**



Biological Waste

Types of Biological Waste



- **Solid, non-sharp waste**
 - Plastic labware (flasks, tubes, plates, bottles, vials)
 - Lab waste (stocks, specimens, cultures, swabs)
 - Tissue or carcass waste (pathological)
 - Gloves, apparel, wipes
 - Pipettes (could also be sharps!)

- **Liquids**
 - Aspirates, culture fluids, rinses, washes, etc.
 - Sera, body fluids
 - Spill clean-up waste

- **Sharps**
 - Anything with a point or edge capable of piercing or cutting human skin
 - Glass labware (sometimes also broken plastic)
 - Needles, scalpels, blood tubes, Pasteur Pipettes
 - Syringes (*with* and sometimes *without* needles)

Solid Biowaste Collection



Intermediate vs. Final Treatment

- **Intermediate Treatment**
 - Usually performed for worker protection
 - Autoclaving most common method
 - Standard microbiology lab practice
 - Performed before transport to final treatment
- **Final Treatment**
 - On-site treatment by facility staff
 - Off-site treatment by disposal contractor



Biosafety Levels, Practices, and Equipment

Table 2. Relation of risk groups to biosafety levels, practices and equipment

RISK GROUP	BIO SAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

From: WHO LBM 3rd edition

