

Safety in virology Lab

- Diseases caused by viruses have great public health significance
- A diagnostic virology lab. should be located in a separate building or room to:
 - restrict entry of visitors
 - prevent contamination
 - facilitate maintaining biosafety standards
- In lab-acquired infections, main routes of infection reported are:

1. Oral

- Eating, drinking, & smoking in the lab.
- Mouth pipetting,
- Transfer of microorganisms to mouth by contaminated fingers or articles



2.Through the skin

- **Injuries by needles**
- **sharp instruments, or glass**
- **Animal bites and scratches**
- **Cuts and scratches**

3. Through the conjunctiva

- **Splashes of infectious material into the eye**
- **Transfer of pathogens to eyes by contaminated fingers**

4.Through the lungs

Inhalation of airborne microorganisms

Biosafety requirements

- **Viral pathogens divided into 4 risk groups based on the risk posed to :**

- **individuals**
- **communities**

(Box 1)

Box 1: Classification of infective micro organisms by risk group

Risk Group 1 (no or low individual and community risk)

- A microorganism that is unlikely to cause human or animal disease.
- Example - Adeno-associated virus (AAV) types 1-4

Risk Group 2 (moderate individual risk, low community risk)

- A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment.
- Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
- Examples include herpes viruses, foot-and-mouth disease viruses, adenoviruses and unconventional slow viruses.

Risk Group 3 (high individual risk, low community risk)

- A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
- Examples include human immunodeficiency virus (HIV), hepatitis B virus (HBV), hantaviruses, Japanese encephalitis virus (JEV), rabies, rift valley fever and yellow fever virus

Risk Group 4 (high individual and community risk)

- A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.
- Examples include Lassa fever, filoviruses, smallpox, Crimean-Congo haemorrhagic fever, avian influenza viruses, Nipah virus, Russian spring-summer encephalitis and Kyasanur forest disease viruses.

- **Biosafety infrastructure must be designed on the basis of risk assessment for handling specific pathogens**
- **An organism that is assigned to Risk Group 2 (RG2) require:**
 - **biosafety Level 2 (BSL2) facilities**
 - **equipment**
 - **practices**
 - **procedures for safe conduct of work**
- **Desired BSL are established on the basis of professional judgement based on risk assessment**

(Tab.1)

Table 1: Relation of RG to BSL, practices & equipment

Risk group	Biosafety level	Laboratory type	Laboratory practices	Safety equipment
1.	Basic—in Biosafety Level 1	Basic teaching, research	Good microbiological techniques (GMT)	None; open bench work
2.	Basic—in Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus biological safety cabinet (BSC) for potential aerosols
3.	Containment—in Biosafety Level 3	Special diagnostic services, research	Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4.	Maximum containment—in Biosafety Level 4	Dangerous pathogen units	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

(WHO Manual on Biosafety, 2004)

- **Every country should have at least one National**

Lab. that is equipped with BSL3 facility

Re: most of the viral pathogens that have emerged in the recent past have been agents that require BSL 3 facilities for handling specimens & cultures

But, if resources are limited, a BSL 2 lab. With negative pressure is essential to handle most emerging pathogens

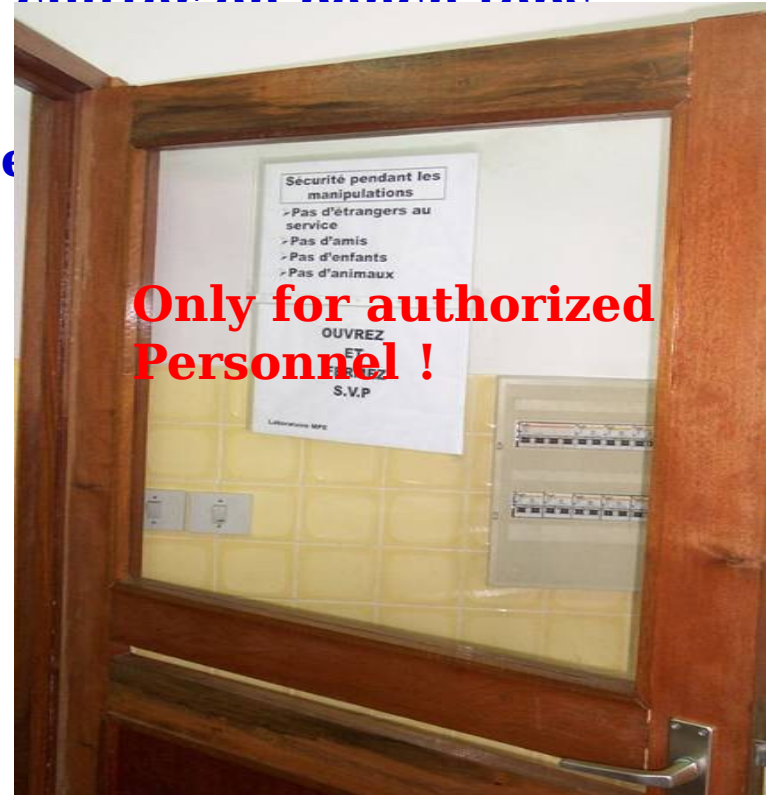
Tab.2 Summary of biosafety level requirements

	Biosafety level			
	1	2	3	4
Isolation ^a of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
• Inward airflow	No	Desirable	Yes	Yes
• controlled ventilating system	No	Desirable	Yes	Yes
• HEPA-filtered air exhaust	No	No	Yes/No ^b	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	Yes	Yes
Airlock with shower	No	No	Optional	Yes
Anteroom	No	No	Yes	-
Anteroom with shower	No	No	Yes/No ^c	No
Effluent treatment	No	No	Yes/No ^c	Yes
Autoclave:				
• on site	No	Desirable	Yes	Yes
• in laboratory room	No	No	Desirable	Yes
• double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personal safety monitoring capability ^d	No	No	Desirable	Yes
Viruses that can be handled		herpes viruses (HSV, VZV, EBV, CMV and HHV8), measles, mumps, HTLV, rubella, hepatitis A-E viruses, dengue virus, enteroviruses (polio, Coxsackie, ECHO and entero)	Arboviruses (JE, West Nile, Chikungunya), Rabies, Nipah, Chandipura, HIV and Avian influenza.	Marburg, Ebola, Lassa, Kyasanur Forest disease, Small pox virus

Apart from the BS requirements, the following need to be considered in virology lab

- Adequate space must be provided for the safe conduct of laboratory work & for cleaning & maintenance**
- Ideally, the entire lab. should be air conditioned to maintain a dust free environment & an ambient Temp. of 22-25°C**
- Walls, ceilings & floors should be smooth, easy to clean, resistant to liquids & resistant to chemicals & disinfectants normally used in the lab.**
- Floors should be slip-resistant**
- Bench-tops should be impermeable to water & resistant to disinfectants, acids, alkalis, organic solvents & moderate heat**

- **Illumination should be adequate for all activities**
- **Open spaces between and under benches, cabinets & equipment should be accessible to cleaning**
- **Storage space must be adequate to hold supplies for immediate use and thus prevent clutter on bench tops**
- **All doors should have vision panels preferably be self-closing**



**Only for authorized
Personnel !**

- Facilities for storing outer garments, personal items as well as having tea & lunch & restrooms should be provided outside the lab. working area
- Hand-washing basins with running tap water should be provided in each lab. room, preferably near the exit door
- There should be reliable & adequate:
 - ✓ electricity supply
 - ✓ emergency lighting for safe exits

- **Safety systems should co**

- fire



- electrical emergency

- emergency shower

- eyewash facilities

- Waste disposal



- **Avoiding overcrowdin**

- **Preventing unauthori
entry into the lab. area**



BIOHAZARD

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

Biosafety Level: _____

Responsible Investigator: _____

In case of emergency call: _____

Daytime phone: _____ Home phone: _____

Authorization for entrance must be obtained from
the Responsible Investigator named above.

Standard Safety

Practices
DO NOT!



Standard Safety Practices

DO!



Safety signs



- Toxic. Damage to health



- Harmful or irritant. Lesions



- Corrosive. Lesion (eyes)



- Flammable. Easily ignite



- Combustive. No O₂ needed



- Explosive.



- Hazardous for environment

Do you see anything wrong?

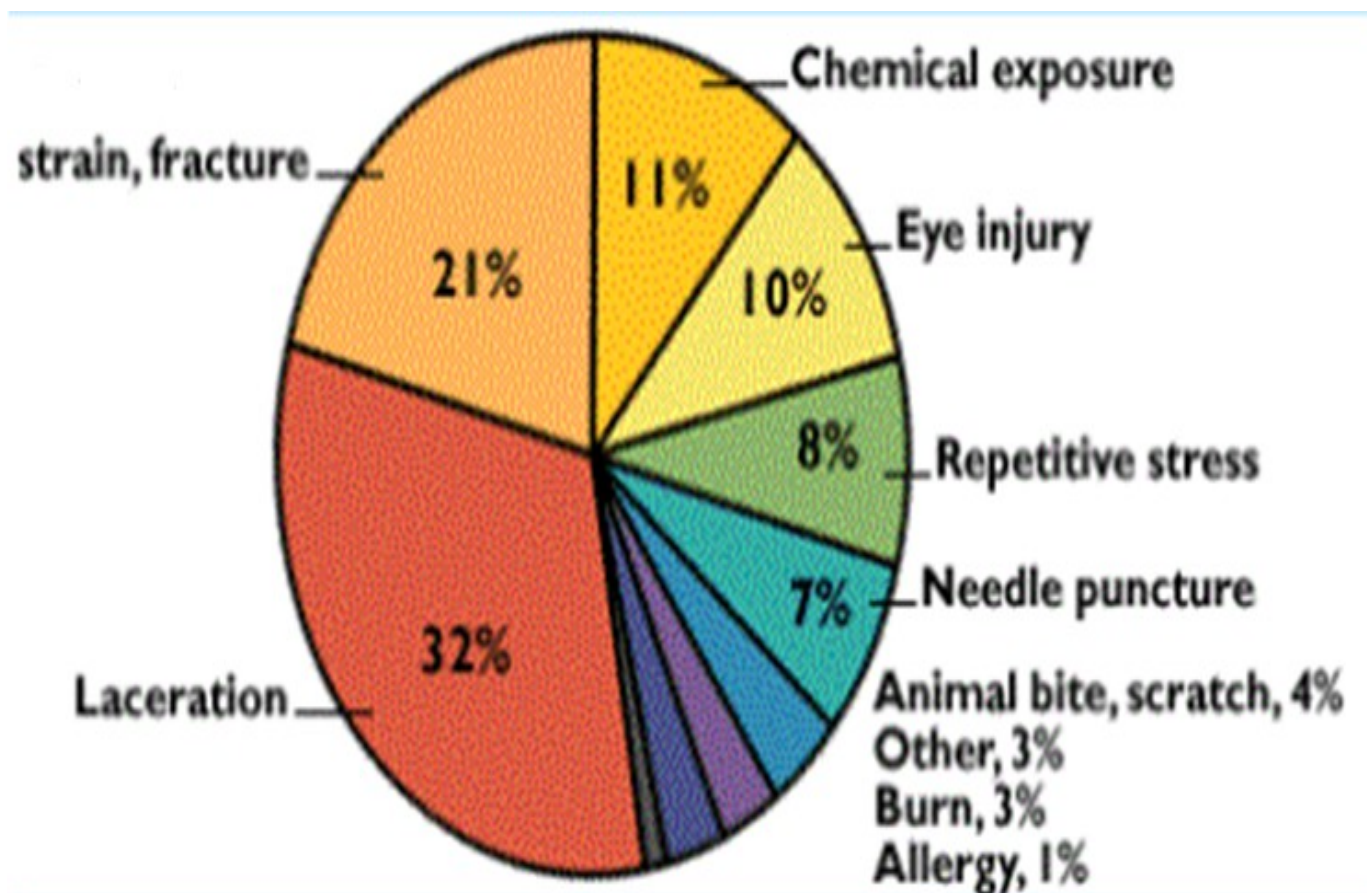


The risks for lab.workers

Most frequently reported infections in USA (1979-1999)

Agent	No. of Cases
<i>M.tuberculosis</i>	223
Q fever	176
Hantavirus	169
Hepatitis B virus	84
<i>Brucella</i> sp.	81
<i>Salmonella</i> sp.	66
<i>Shigella</i> sp.	56
Hepatitis C virus	28
<i>Cryptosporidium</i> sp.	27
Total	1074

Research Employee Injuries (1993-1997)



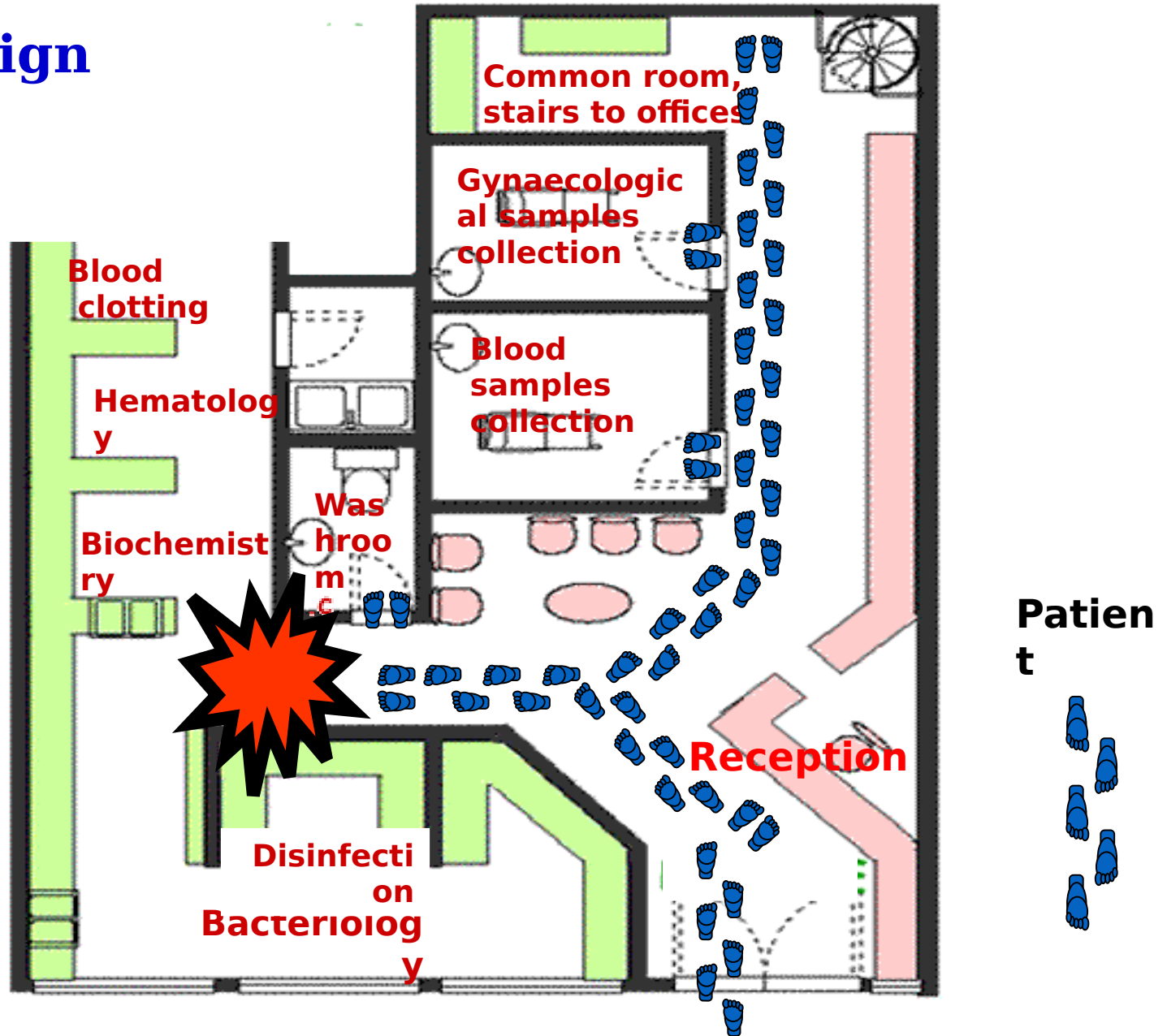
Howard Hughes Medical Institute, Office of Lab. Safety

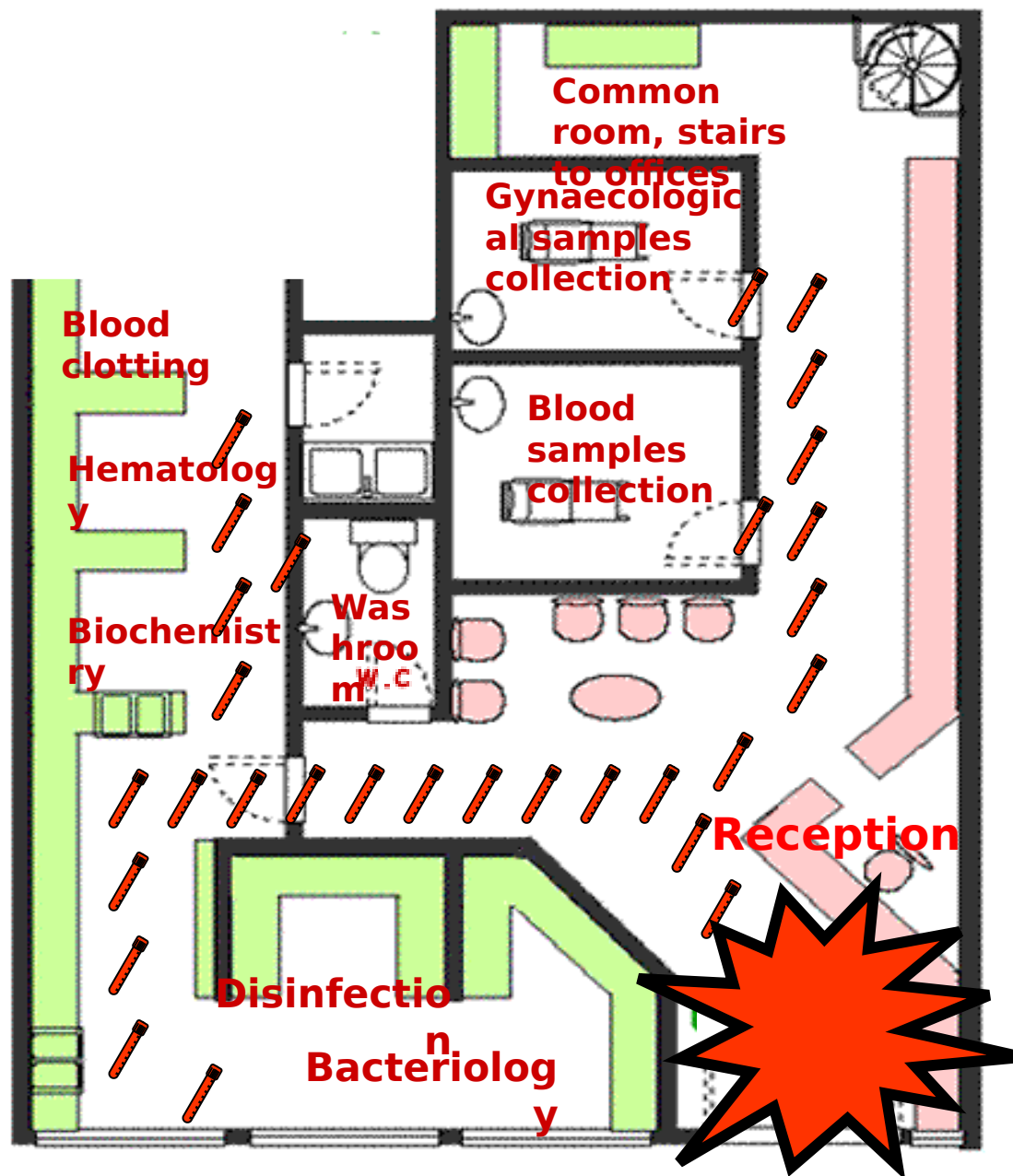
- **Specimen reception/collection rooms**
 - **Should be separated from offices**
 - **Treated as a potentially dangerous area**
 - **Floors & surfaces must be leak-proof & amenable to decontamination procedures**
 - **A hand basin must be available**
 - **Access should be restricted to authorized persons only!**

Sample collection room



Lab. Design





Spécimen

Collection, transport & receipt of infectious materials

- **Specimens are transported by various means b/n different health facilities & lab. every day**
- **During shipment, containers or packages enclosing them may be handled by many people**
- **Containers may be involved in accidents & be damaged or broken**
- **Containers may also be lost or stolen & opened by unauthorized persons. This could present a hazard to any person in contact with the specimen.**
- **Therefore, the collection, packaging, transport and unpacking should be strictly regulated**
- **Postal & transport authorities of different countries have different regulations governing the transport of infectious materials**

Collection of Specimens

Specimen containers must be :

- Sufficiently robust to withstand stresses
- Leak-proof & screw-capped
- Reusable containers (e.g. glass) must remain leak-proof

- **Labelling specimens & containers**
 - Labels and stickers used should be self-adhesive
 - **"Danger of Infection" & "High Risk"** labels for specimen containing HBV or HIV
 - Specimens labelled "Danger of Infection" should be placed in self-sealing plastic bags
 - Pins & staples should not be used as they may prick the hands of any persons handling the bag
 - Request forms should be separate from the specimen

Transport of specimens

1. Transport within or b/n hospitals

- **Use secure transport containers with secure lids**
- **Containers must be capable of withstanding autoclaving**
- **Withstand prolonged exposure to disinfectants**
- **Containers should be inspected daily for leakages**
- **Be decontaminated & washed frequently**

2. By inland post : the Post Office Guide (1986)

- Hazard Group 4 organisms must not be posted**
- Hazard Group 3 material must have a "Danger of Infection" label on the container**
- Container used must be robust & wrapped in enough absorbent packing material**
- Place the container in a self-sealing plastic bag (optional)**
- The specimen must be sent by first class mail**

3. By air

- **Before any material is sent to another country by air mail, the sender & receiver must ascertain that it is acceptable to the authorities of that country**
- **The material should be placed in a watertight "primary container" which is then packed in a watertight "secondary container" & the whole packed in an "outer container"**
- **Documentation required are:**
 - 1. An International Air Traffic Association's Shippers Certificate for Restricted Articles**
 - 2. Labels giving contents, description & quantity of infectious material**
 - 3. Green customs label**
 - 4. Official address label**

Management of laboratory waste

- **Ideally, lab. waste should be separated into color-coded containers**
- **Recommended colors:**
 - **Yellow** for incineration
 - **Light blue or transparent with blue label...**
for autoclaving
 - **Black....** For normal household waste
 - **White or clear plastic.....**for soiled linen

Waste disposal

- **All waste should be treated prior to disposal**
 - **No potential to cause harm**
- **All staff & environment must be protected from harm**
- **Proper separation of waste**



- **3 practical methods of treating contaminated lab. waste:**
 - **Sterilization by autoclaving**
 - **Chemical disinfection**
 - **Incineration**
- **Sterilization & disinfection are lab. processes**
- **Incineration involves the transport of material off-site**
- **Sterilization : killing of all forms microorganisms;**
- **Disinfection: kills most microorganisms but not all**

Summary of waste management

Type of Waste	Treatment	Disposal Method
Band Aids	none	Regular Trash
Gloves (unsoiled)	none	
Paper / Towels / Mats (unsoiled)	none	Regular Trash
Personal Protective Equipment (uncontaminated)	none	
Pipette Tips (unused)	none	Regular Trash
Urine Transfer Pipettes / Specimen Containers / Test Strips	none	
Agar Plates (used)	Autoclave	Regular Trash
Agar Plates (unused)	none	
Blood Culture Bottles (used)	Autoclave or Bleach	Regular Trash
Capillary Tubes (used)	Autoclave or Bleach	
Culturette Swabs (used)	Autoclave or Bleach	Regular Trash
Glass Tubes containing blood / body fluids	Autoclave or Bleach	
Gloves (visibly soiled w/ blood)	Autoclave	Regular Trash
Loops (inoculating)	Autoclave or Bleach	
Lyophilized Specimens	Autoclave or Bleach	Regular Trash
Media (spent solid)	Autoclave	
Micro-titre Plates (contaminated / used)	Autoclave or Bleach	Regular Trash
Paper / Towels / Mats soiled with blood / body fluids	Autoclave	
Personal Protective Equipment (contaminated)	Autoclave	Regular Trash
Specimen Bags grossly soiled	Autoclave	
Specimen Containers with blood / body fluids	Autoclave or Bleach (depending on volume)	Regular Trash
Syringe without needle (not containing blood / body fluids)	None	
Tissue / Specimens (unfixed)	Autoclave or Bleach	Regular Trash
Tissue / Specimens (fixed)	none	
Towel / paper / etc. soiled with blood / body fluids	Autoclave	Regular Trash
Tubes (used / contaminated) culture, vacutainer, microfuge	Autoclave	
Tubes (unused / uncontaminated) culture, vacutainer, microfuge	none	Regular Trash
Test Tubes containing blood / body fluids	Autoclave or Bleach	
Pipette Tips (used)	Autoclave or Bleach	Regular Trash
Pasteur Pipettes Plastic (used)	Autoclave or Bleach	
Pipettes – Plastic (used)	Bleach or Autoclave	Regular Trash
Glass Tubes (empty-unused)	none	
Pipettes – Plastic or Glass (unused)	none	Regular Trash
Slides (fixed – used or unused)	none	
Pipettes – Glass (used)	Bleach or Autoclave	Regular Trash
Pasteur Pipettes Glass (used)	Autoclave or Bleach	
Animal Blood / Body Fluids (potentially infectious)	Autoclave or Bleach	Regular Trash
Blood – human	Bleach	
Body Fluids – human (except urine)	Autoclave or Bleach	Regular Trash
Liquid – blood / body fluids	Bleach	
Media (spent liquid)	Autoclave or Bleach	Regular Trash
Urine Samples	none	
Blades	none	Regular Trash
Lancets	none	
Needles	none	Regular Trash
Razor Blades	none	
Slides (unfixed used or unused)	none	Regular Trash
Syringe containing blood / body fluids with or without needle	None	
Animal Carcass / Tissue	Incinerate	Incineration
Pathological Waste	Autoclave or Bleach	Morgue

Summary

Lab. safety is dependent on:

- **a responsible supervisor /safety officer**
- **a safety manual & SOPs**
- **trained personnel**
- **assessment of risks**
- **Lab. Design**

(patients & patient samples do not have common pathways)

Exercise

- **Identify gaps/challenges that hinder the implementation of the required level of bio safety in the different Lab. rooms in the Regions.**
 - **Molecular Lab**
 - **PCR room**
 - **Mycobacteriology lab**

References

Guidelines on establishment of virology laboratory in developing countries, WHO, 2008

Biosafety in Microbiological and Biomedical Laboratories. CDC, 5th Ed.

Lab. Biosafety manual. WHO, 3rd edition, 2004

Each laboratory should have the WHO Laboratory Biosafety Manual/Document WHO/CDS/CSR/LYO/2004.11, available at:

<http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf>)

END