



राष्ट्रीय प्रौद्योगिकी संस्थान कालिकट

NATIONAL INSTITUTE OF TECHNOLOGY CALICUT

School of Biotechnology

biOSafety



Guidelines



Prepared by,
Dr. Ilanila I.P.
Assistant Professor
SoBT

Risk Assessment

- Framework:



Risk Assessment of GE Organisms

Risk Categories

Based on the

- Likelihood of the risk occurring
- Consequence of the risk if it occurs

Risk assessment matrix:

Likelihood	Consequence			
	Marginal	Minor	Intermediate	Major
Highly likely	Low	Moderate	High	High
Likely	Negligible	Low	High	High
Unlikely	Negligible	Low	Moderate	High
Highly unlikely	Negligible	Negligible	Low	Moderate

Risk Assessment

As per DBT guidelines, risk groups (RG) are classified into 4 groups

Risk Group	Description
RG 1	A microorganism that is unlikely to cause human/ animal/plant disease.
RG 2	A microorganism that can cause disease in human /animal/ plant but the laboratory exposures may or may not cause serious infection to individual and risk(s) of spread of infection is limited.
RG 3	A microorganism that usually causes serious/lethal human/ animal/ plant disease but does not ordinarily spread from one infected individual to another.
RG 4	A microorganism that usually causes serious/lethal human/ animal/ plant disease and that can be readily transmitted from one individual to another, directly or indirectly.

Biosafety levels

Table 2. Different biosafety level facilities

Organism	Facility designation	Safety Levels
Microorganisms	Biosafety Level (BSL)	BSL-1 to BSL-4
Animal	Animal Biosafety Level (ABSL)	ABSL-1 to ABSL-4
Plant	Plant Biosafety Level (PBSL)	PBSL-1 to PBSL-4
Arthropods/Insects	Insect Biosafety Level (IBSL)	IBSL-1 to IBSL-4
Aquatic organisms	Aquatic Organism Biosafety Level (AqBSL)	AqBSL-1 to AqBSL-3

Names of the microorganisms corresponding to each risk groups are listed in the

Annexure 1 of the DBT-Guidelines

BSL and RG essentials

RISK GROUP	BIOSAFETY 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

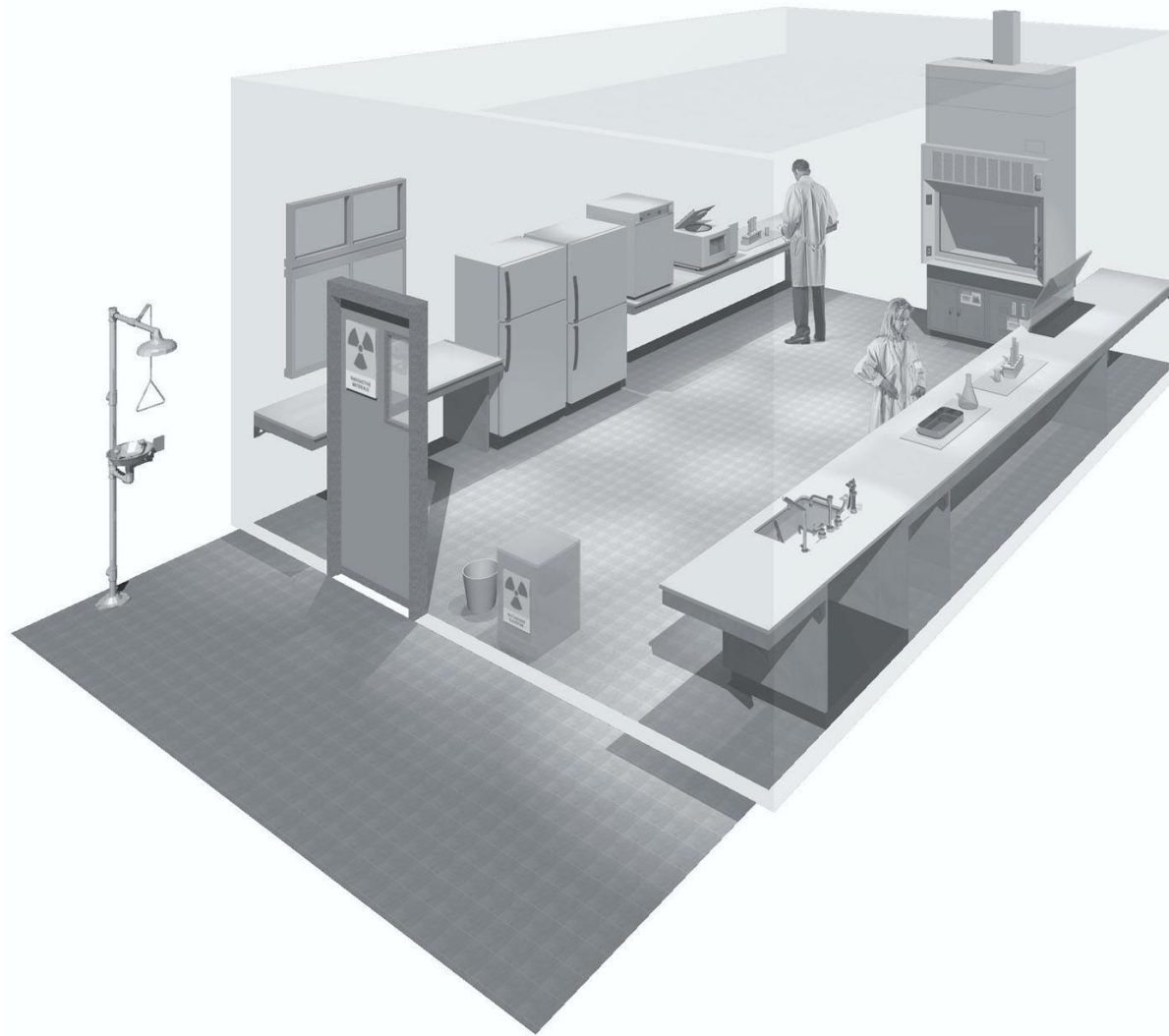


BSL requirements

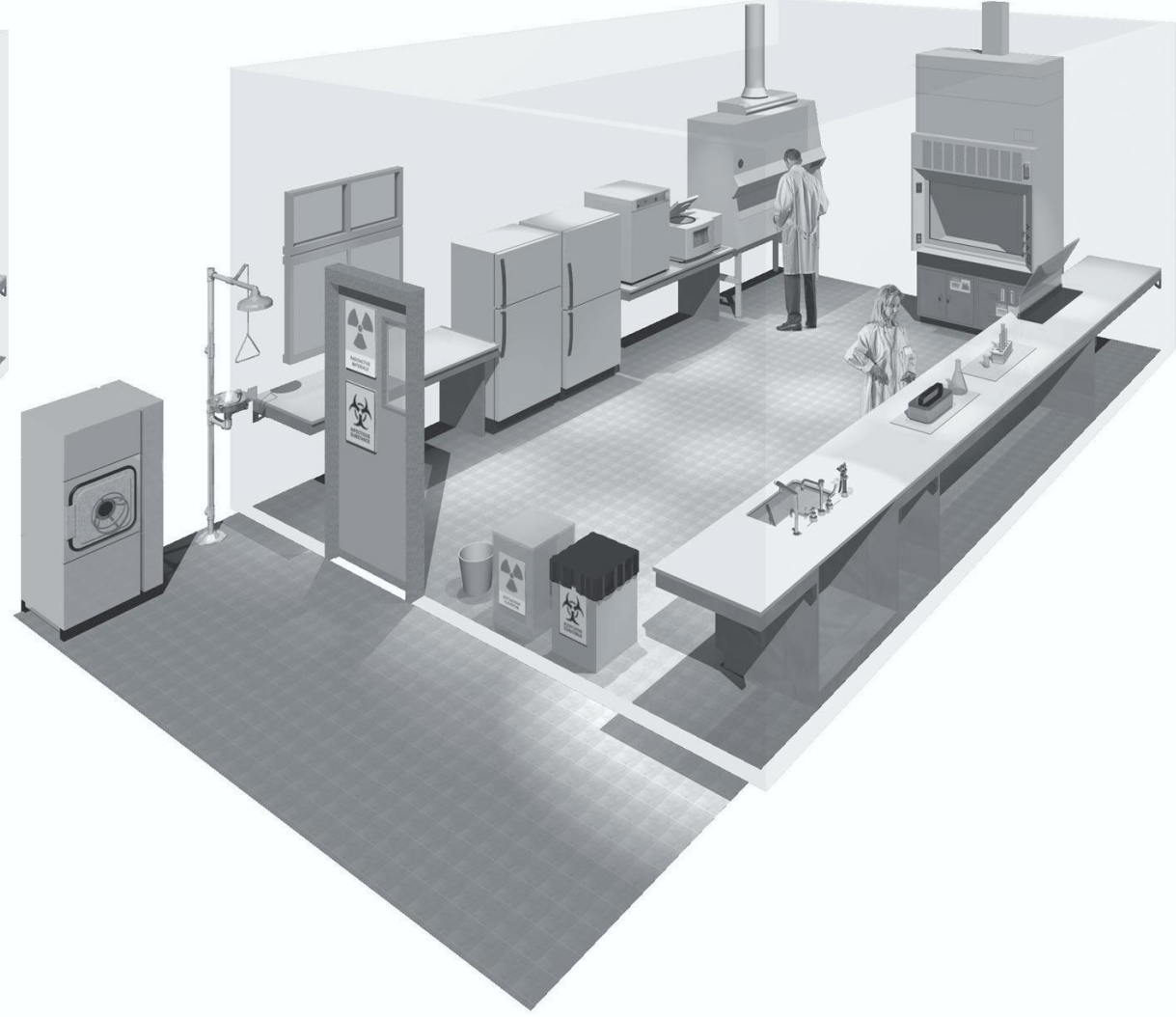
	BIOSAFETY LEVEL			
	1	2	3	4
Isolation ^a of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
— inward airflow	No	Desirable	Yes	Yes
— controlled ventilating system	No	Desirable	Yes	Yes
— HEPA-filtered air exhaust	No	No	Yes/No ^b	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	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
Personnel safety monitoring capability ^d	No	No	Desirable	Yes



BSL – 1 lab

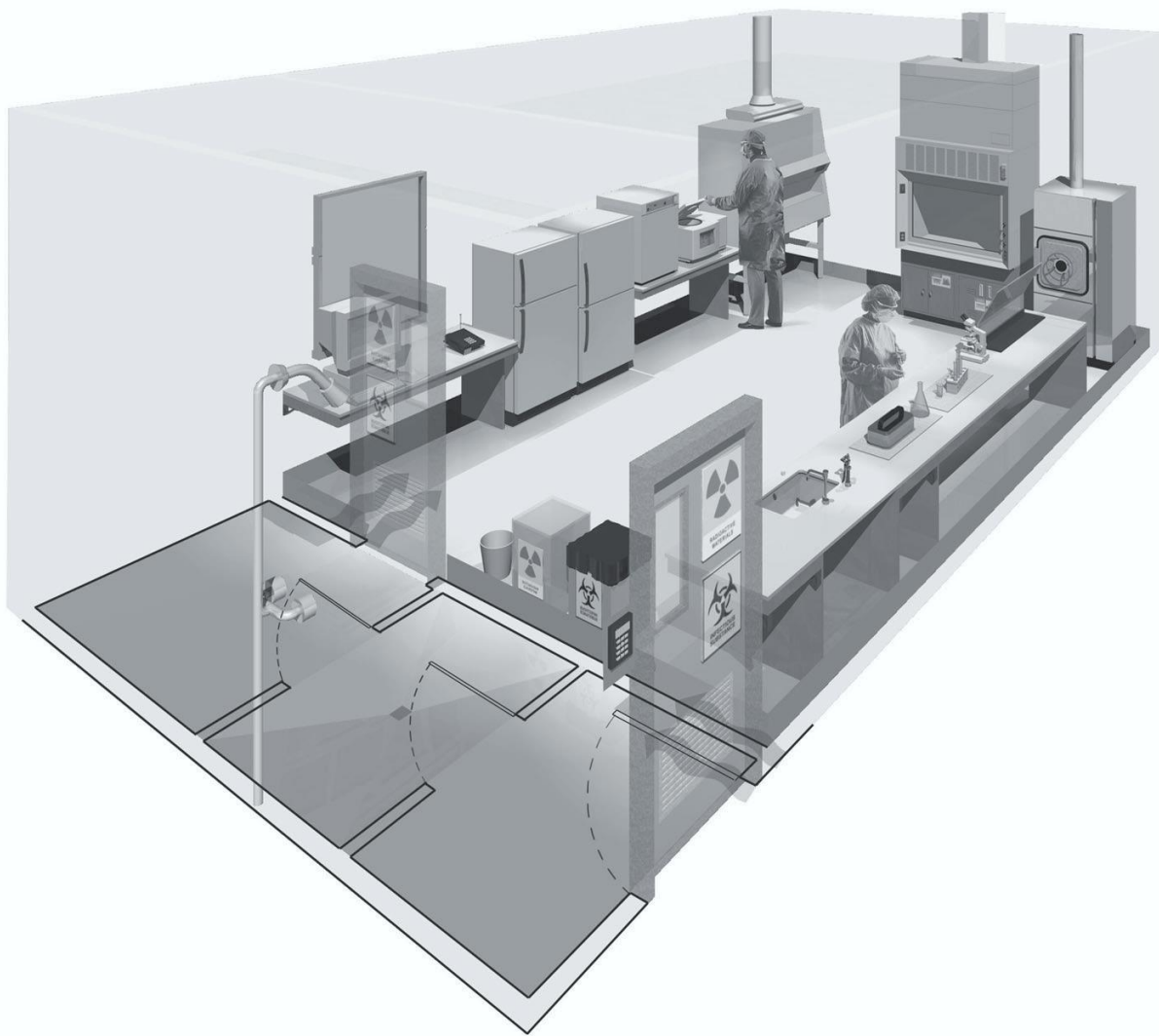


BSL – 2 lab



All images are taken from WHO handbook

BSL – 3 lab



BSL – 4 lab



All images are taken from WHO handbook



Lab Safety



Prepared by,
Dr. Ilanila I.P.
Assistant Professor
SoBT

All labs should have the details
pasted on the entrance



BIOHAZARD

PHOTOGRAPH

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.



Dressing for Lab



FACE SHIELD



GLOVES



BOOTS



LAB COAT



GOGGLES



PPE

High Hazard PPE

“PERSONAL PROTECTIVE EQUIPMENT (PPE) WORN IN HIGH HAZARD AREAS”

EYE PROTECTION

must always be worn in designated laboratory areas.
Safety glasses that meet ANSI Z87.1 specifications are required for work with chemical, biological, radioactive materials and physical hazards.

GOGGLES AND CHEMICAL RESISTANT FACE SHIELD

should be used when...
-working with large volumes of corrosive liquids, organic solvents, hazardous chemicals, water or air reactive chemicals or flammable compounds splash is a hazard
-working with an apparatus with contents under pressure or vacuum
-performing tasks such as sanding or grinding
-working with power tools and other cutting devices

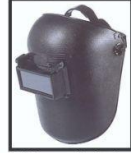
Eye protection and Face Shield is used for protecting eyes and face from flying particles, airborne dust, poisonous and corrosive materials

SHADED GOGGLES / GLASSES



should be worn when...
-performing activities that expose eyes to intense UV light or lasers and lenses must be appropriately shaded with optical density based on beam parameters

WELDING HELMET



should be worn with safety glasses for work with hot materials and open flames such as welding, soldering and brazing

HALF FACE AIR PURIFYING RESPIRATOR

must be worn for work with chemical vapors or particulates
-must be properly fit tested every year - facial hair may impede proper seal
-medical evaluation and training is required
-air purifying respirator with appropriate filter cartridge protects against various particulates, vapors, dust, mists, fumes
-respirators should be worn as a last resort when other engineering controls (fume hood) are not available

CHEMICAL RESISTANT SHOE COVER

For work with large amounts of chemicals or during spill cleanup



FLAME RESISTANT LAB COAT (such as Nomex® fiber)

should be worn when working...
-with welding equipment
-in environments where quick fires are a threat
-with small quantities of pyrophoric or other highly flammable materials

RUBBER APRON

should be worn...
-for work with large volumes of corrosive liquids, solvents or flammable compounds
-when working with apparatus under pressure
-when working with water or air reactive chemicals

GLOVES

must be selected specifically for the type of hazard to protect hands from chemicals, radiation, biological hazards, abrasions, cutting, heat and cold.
-skin contact is a potential source of exposure to toxic materials
-chemical resistant gloves may be made of rubber, neoprene, polyvinyl chloride, nitrile, butyl, etc.
-select glove material based on industry best practice with regards to chemical resistance to substance(s) being handled
-wear gauntlet length gloves to protect forearms
-go to www.safety.nmsu.edu for guidance on glove selection

What to Do and Not to Do with Gloves

replace gloves periodically
contaminated gloves should be rinsed and carefully removed
do not wear gloves out of lab
do not use gloves when touching common surfaces such as telephones, computers, door knobs
do not wear gloves around moving machinery

LONG PANTS

required to cover the legs for work in laboratory
if fire is a threat, fire resistant clothing is required

CLOSED-TOE SHOES



must always be worn
Should have slip resistant soles for work in wet or slippery areas

EMPLOYEES MUST BE TRAINED ON HOW TO SELECT, PROPERLY WEAR, CARE FOR, CLEAN, AND MAINTAIN PPE.
INFORM SUPERVISOR OF NEED TO REPAIR OR REPLACE PPE.
CONTAMINATED PPE MAY BE A HAZARDOUS WASTE, AND SHOULD NEVER BE TAKEN HOME.

NMSU ENVIRONMENTAL HEALTH & SAFETY 646-3327

GO TO NMSU SAFETY WEB AT safety.nmsu.edu

Minimum Lab PPE

PERSONAL PROTECTIVE EQUIPMENT (PPE) FOR THE LABORATORY WORKER

EYE PROTECTION

must always be worn in designated laboratory areas.
Safety glasses that meet ANSI Z87.1-1989 standards are required as minimum PPE for work in a laboratory

GLOVES

must be selected specifically for the type of hazard to protect hands from chemicals, radiation, biological hazards, abrasions, cutting, heat and cold.
-skin contact is a potential source of exposure to toxic materials
-chemical resistant gloves may be made of rubber, neoprene, polyvinyl chloride, nitrile, butyl, etc.
-select glove material based on industry best practice with regards to chemical resistance to substance(s) being handled
-wear gauntlet length gloves to protect forearms
-go to www.safety.nmsu.edu for guidance on glove selection

INSULATED CRYOGEN



Use to protect against ultra-cold temperatures when working with liquid nitrogen and other cryogenic hazards

LEATHER



Use to guard against injuries from sparks or scraping against rough surfaces

ALUMINIZED



Use gloves to insulate hands from intense heat such as molten materials

METAL MESH



Use gloves when using cutting tools or other sharp instruments

What to Do and Not to Do with Gloves

replace gloves periodically
contaminated gloves should be rinsed and carefully removed
do not wear gloves out of lab
do not use gloves when touching common surfaces such as telephones, computers, door knobs
do not wear gloves around moving machinery

HAIR CARE

long hair should be tied back to prevent contamination
must use hair net to prevent entanglement in rotating equipment

LAB COAT

LAB COAT should be...
-selected to assure proper fit
-worn buttoned/snapped and sleeves rolled down
-made of a material which is suitable for your work, some synthetic fabrics are extremely vulnerable to particular chemicals
-fire resistant (synthetic fabrics can burn and melt and must not be worn in areas where open flames or other sources of fire might present the potential of igniting the coat)

LAB COAT should be removed when...
-there is reasonable chance that it is contaminated by chemical, radioactive material or biological material

LAB COAT should be worn...
-to prevent splatter and spills from contacting skin and/or contaminating your street clothes
-to prevent non-obvious contamination and impede sanitation of regular clothes and skin
-when operations involve harmful liquids or toxic solid materials, even in tiny quantities.

Care and Maintenance:

Protective equipment that has been used in a laboratory and is potentially contaminated with hazardous materials should NOT be taken home.
When lab coats become contaminated with hazardous materials they should be cleaned on site at the university or sent out for cleaning by professionals who have been informed of the potential hazards or disposed of as hazardous waste.

LONG PANTS

are required as minimum PPE for work in a laboratory

CLOSED-TOE SHOES

are required as minimum PPE for work in a laboratory

EMPLOYEES MUST BE TRAINED ON HOW TO SELECT, PROPERLY WEAR, CARE FOR, CLEAN, AND MAINTAIN PPE.
INFORM SUPERVISOR OF NEED TO REPAIR OR REPLACE PPE.
CONTAMINATED PPE MAY BE A HAZARDOUS WASTE, AND SHOULD NEVER BE TAKEN HOME.

NMSU ENVIRONMENTAL HEALTH & SAFETY 646-3327

GO TO NMSU SAFETY WEB AT safety.nmsu.edu

Safe Lab Practices

Never store food or drink, or personal items such as coats and bags in the laboratory

Activities such as eating, drinking, smoking, and applying cosmetics are only to be performed outside the laboratory.

Never put materials, such as pens, pencils or gum, in the mouth while inside the laboratory, regardless of whether gloves are worn or not.

Wash hands thoroughly, preferably with warm running water and soap, after handling biological material and/or animals, before leaving the laboratory or when hands are known or believed to be contaminated.

Protect written documents from contamination using barriers (such as plastic coverings), particularly those that may need to be removed from the laboratory.



Safe Lab Practices 2

Ensure open flames or heat sources are never placed near flammable supplies and are never left unattended.

Ensure that cuts or broken skin are covered before entering the laboratory.

Before entering the laboratory, ensure that there are adequate supplies of laboratory equipment and consumables, including reagents, PPE and disinfectants, and that these items are suitable for the activities envisaged.

Ensure that supplies are stored safely and according to storage instructions to reduce accidents and incidents such as spills, trips and falls.

Ensure proper labelling of all biological agents and chemical and radioactive material.



Safe Lab Practices 3

Ensure that the work is performed with care and without hurrying.

Avoid working when fatigued.

Keep the work area tidy, clean and free of non-essential objects and materials.

Prohibit the use of earphones

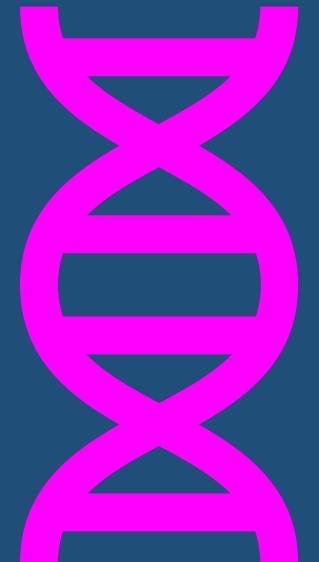
Cover or remove any jewelry that could tear gloves

Cleaning and decontamination of jewelry or spectacles should be considered.

Refrain from using portable electronic devices when not specifically required

Keep electronic devices where they cannot easily become contaminated

Ensure that the devices are either protected by a physical barrier.



Avoiding ingestion of biological agents and contact with skin and eyes

- ✿ Wear disposable gloves at all times when handling specimens known or reasonably expected to contain biological agents.
- ✿ Disposable gloves must not be reused.
- ✿ Avoid contact of gloved hands with the face.
- ✿ Remove gloves aseptically after use and wash hands as instructed during the training.
- ✿ Shield or otherwise protect the mouth, eyes and face during any operation where splashes may occur, such as during the mixing of disinfectant solutions.
- ✿ Secure hair to prevent contamination.
- ✿ Cover any broken skin with a suitable dressing.
- ✿ Prohibit pipetting by mouth.

Avoiding injection of biological agents

- ☛ Wherever possible, replace any glassware with plastic-ware.
- ☛ If required, use scissors with blunt or rounded ends rather than pointed ends.
- ☛ If you are using glassware, check it on a regular basis and discard it if anything is broken, cracked or chipped.
- ☛ Use ampoule openers for safe handling of ampoules.
- ☛ Minimize the risk associated with the use of syringes or needles by using blunt syringe needles, alternative devices or engineered sharp safety devices, where possible.
- ☛ However, be aware that sharp safety devices also pose a risk when not handled properly.
- ☛ Never use syringes with needles as an alternative to pipetting devices.
- ☛ Never re-cap, clip or remove needles from disposable syringes.
- ☛ Dispose of any sharps materials in puncture-proof or puncture-resistant containers fitted with sealed covers.
- ☛ Disposal containers must be puncture-proof/-resistant, must not be filled to capacity, must be never reused and must not be discarded in landfills.

Preventing dispersal of biological agents

- ☺ Discard specimens and cultures for disposal in leak-proof containers with tops appropriately secured before disposal in dedicated waste containers.
- ☺ Place waste containers, preferably unbreakable (such as plastic, metal), at every workstation.
- ☺ Regularly empty waste containers and securely dispose of waste.
- ☺ Ensure all waste is properly labelled.
- ☺ Consider opening tubes with disinfectant-soaked pad/gauze.
- ☺ Decontaminate work surfaces with a suitable disinfectant at the end of the work procedures and if any material is spilled.
- ☺ When disinfectants are used, ensure the disinfectant is active against the agents being handled and is left in contact with waste materials for the appropriate time, according to the disinfectant being used.

In the Event of a Lab Accident...

- All incidents must be reported to the appropriate personnel, usually a laboratory supervisor (in NITC, the PI in-charge for the specific lab), in a timely manner.
- A written record of accidents and incidents must be maintained, in line with national regulations where applicable.
- Any incident that occurs must be reported and investigated in a timely manner.
- Results from incident investigations must be used to update laboratory procedures and emergency response.



Instruction for Emergencies

- Even when carrying out low-risk work and following all core requirements for biosafety, incidents can still occur. To reduce the likelihood of exposure to/release of a biological agent or to reduce the consequences of such incidents, a contingency plan must be developed that provides specific SOPs to be followed in possible emergency scenarios that apply to the work and local environment. Personnel must be trained on these procedures and have periodic refresher training in order to maintain competency.

Biological spill response

If a spill occurs where there is a high initial risk (due to a large formation of aerosols, a large volume/high concentration of liquid spilt, and/or high pathogenicity of the biological agent involved) the following protocol should be followed

- Personnel must immediately vacate the affected area.
- Exposed persons should be referred immediately for medical evaluation.
- The room containing the spill should not be entered for a length of time that allows aerosols to be carried away and heavier particles to settle. If the laboratory does not have a central air exhaust system, entrance should be delayed for longer.
- **SIGNS MUST BE POSTED INDICATING ENTRY IS FORBIDDEN.**
- The laboratory supervisor and the biosafety officer must be informed as soon as possible after the event has occurred.
- After the necessary amount of time has elapsed, decontamination must proceed; depending on the size of the spill, this may require help or supervision, for example, by the biosafety officer.
- Suitable protective clothing and respiratory protection may be needed for the spill clean-up.

Waste Disposal

- As per WHO,

CATEGORY OF LABORATORY WASTE MATERIAL	TREATMENT
Uncontaminated (non-infectious) material	Can be reused or recycled or disposed of as general municipal waste
Contaminated sharps (hypodermic needles, scalpels, knives and broken glass)	Must be collected in puncture-proof containers fitted with covers and treated as infectious
Contaminated material for reuse or recycling	Must be first decontaminated (chemically or physically) and then washed; thereafter it can be treated as uncontaminated (non-infectious) material
Contaminated material for disposal	Must be decontaminated onsite OR stored safely before transportation to another site for decontamination and disposal
Contaminated material for incineration	Must be incinerated onsite OR stored safely before transportation to another site for incineration
Liquid waste (including potentially contaminated liquids) for disposal in the sanitary sewer system	Should be decontaminated before disposal in the sanitary sewer

At the End of Your Lab Time...

Make sure

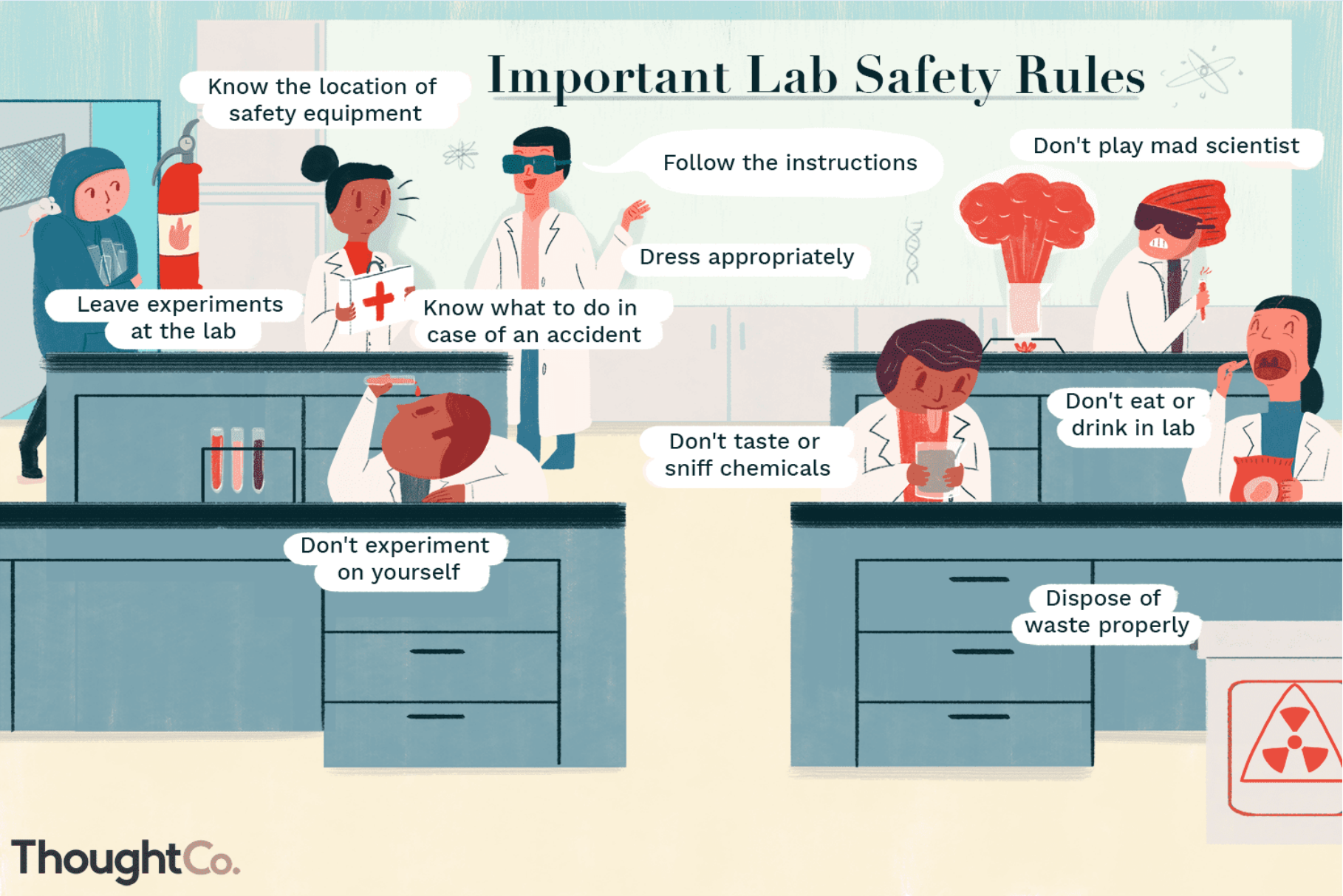
- You have entered in the respective log books
- All chemicals are replaced at respective places.
- All chemicals are labelled.
- You remove the gloves without contaminating the hands
- You wash your hands and face (if needed) with soap thoroughly.



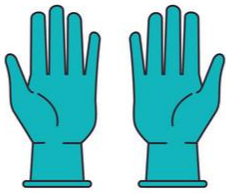
Signs to be put up in the lab



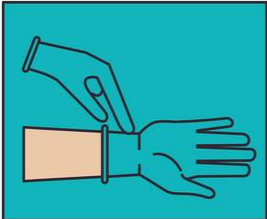
Signs to be put up in the lab



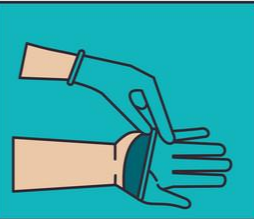
Signs to be put up in the lab



HOW TO REMOVE GLOVE SAFELY



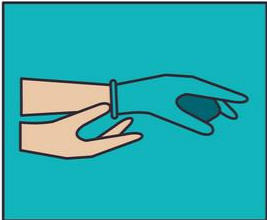
GRASP THE OUTSIDE OF THE GLOVE IN THE WRIST AREA



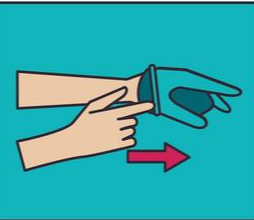
PEEL THE GLOVE AWAY FROM YOUR BODY, TURN IT INSIDE-OUT



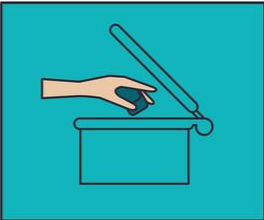
HOLD THE INSIDE-OUT THE GLOVE IN THE OTHER HAND



SLIDE YOUR FINGERS UNDER THE WRIST OF THE OTHER GLOVE



PEEL THE GLOVE AWAY FROM YOUR BODY, TURN IT INSIDE-OUT, LEAVE THE FIRST GLOVE INSIDE THE SECOND



DISPOSE THE GLOVES SAFELY



shutterstock.com · 1509602516



BIOHAZARD

ADMITTANCE TO AUTHORISED PERSONNEL ONLY

Biosafety Level: BSL 2 _____

Principal Investigator: _____

In case of emergency call: _____

Office / Mobile Phone: _____

Authorization for entry must be obtained from
Principal Investigator of the Laboratory

The left side of the slide features four vertical bars of varying widths and colors: a wide orange bar, a medium-width yellow bar, a thin white bar, and a medium-width green bar.

Thank you