Medical Laboratory Technology - I

INTRODUCTION

The definition of health includes a state of complete and perfect physical, mental, social and spiritual well-being and not just the absence of disease or infirmity and good health is a fundamental right of every living human being on earth. However, modern world, though, has to an extent eliminated infectious diseases. But the focus has now shifted to lifestyle diseases. Pollution of every nature too has taken its toll. About half a century back, the predominant diseases used to be infective ones but now you may find individuals in mid-twenties waiting for their turn for open heart surgeries. Also, modern medicine has increased the longevity of life accompanied by attendant geriatric diseases like Alzheimer's disease and malignancies. The polluted and toxic world has not spared the fetuses in utero and neonates. A new face of disease has emerged, diseases like HIV-AIDS and severe acute respiratory syndrome (SARS), are new entrants in the long list of infective diseases. We may have eradicated smallpox but tuberculosis and malaria have raised their heads with a vengeance. So, do what you might. Some forms of disease, mild or severe will strike every human being living. On getting sick, the patient first comes in contact with a clinician—medical or surgical. The clinician gives a patient hearing (if the patient is conscious) to his problems and symptoms and also takes note of various signs, which he sees or elicits. Sometimes, he may immediately arrive at a diagnosis and may under emergency circumstances institute treatment at first instances. In most cases, however, he will have a differential diagnosis in mind and to arrive at a specific diagnosis he usually orders for a battery of tests.

Various means of diagnosis are available.

1. *Most important*: Clinical laboratory tests which include any tissue or fluid obtained from the body.
2. *Imaging sciences*: X-rays, ultrasound, color Doppler, computerized axial tomography (CAT) scan, magnetic resonance imaging (MRI) scan and the latest positron emission tomography (PET) scan.
3. *Electrical signal processing techniques:* ECG, EMG, EEG and nerve transmission techniques, etc.
4. *Direct visualization techniques*: With the availability of fiberoptic-based technologies, the clinician is now capable of passing small tubes (called scopes) through natural passage ways of the human body (without actually surgically opening up the part),

e.g. gastroscopy, cystoscopy, etc. These techniques, eventually culminate in taking small tissue samples (biopsies) which are sent to histopathology laboratories. So, whenever, any sample from a human body is taken (either voided naturally or obtained by the clinician or the laboratorian), it is referred to the clinical laboratory for investigation. On receipt of a report from the laboratorian, the clinician, then, makes up his mind and starts a unidirectional or specific treatment against the disease thus diagnosed. It would not be wrong to designate medical laboratory personnel as the backbone of the clinicians. But, for these technologists, the clinicians would forever grope in the dark. Gone are the days when diabetes mellitus was presented with the classical triad of symptoms—increased thirst, hunger and urination; likewise, typhoid seldom presents with a step-ladder pattern fever. Blood testing is absolutely mandatory, to know that they exist, their severity and eventually, after treatment; to know that they are under control or cured. Investigations are diagnostic as well as prognostic tools.

Clinical laboratory investigations nowadays are being utilized as future predictors. On getting warning signals, one can take necessary corrective measures (lifestyle and/ or dietary) and can prevent diseases from striking or at least deferring or postponing their arrival. When a clinician is lost, you shall show him the way in the best possible way, you lead him to a diagnosis and let him do his job thereafter. He may come back to you later to determine that his efforts have been fruitful.

While *physiology* is the study of essentially normal structures and functions of a body, *pathology* deals with the study of a diseased organ or system of the body, its ab- normal functions, their mode of origin, their progress to recovery or otherwise. All these studies come under the ambit of a clinical pathology laboratory. A clinical labora- tory has further sub-branches such as: hematology, bio- chemistry, seroimmunology, microbiology, cytogenetics, histopathology, cytopathology, blood banking and last but not least—clinical microscopy. A clinical laboratory can be manned by a qualified doctor specializing in clinical pathology, biochemistry, immunology, blood banking, histopathology, cytopathology, hematology, microbiology or cytogenetics. The pathologist is usually assisted by laboratory technicians or technologists (they are also qualified for the job) and lastly the cleaning and documentation staff. Only by collective efforts of the individuals mentioned above, a proper report can be generated. Be grateful to the clinician for having faith in you and give back nothing except an accurate and correct timely report. A delayed report may at times be too late. The patient may have lost his life by then. A timely correct report is the essence of running a good laboratory.

The cycle of health-disease with all intermediaries is given in Figure 1.1. Just as there are primary, secondary and tertiary health centers, there are also the primary, secondary and tertiary laboratories too. In India, there are no specific guidelines as to what or how much they can do and overlapping can occur. A superior laboratory may perform all functions of an inferior laboratory too.

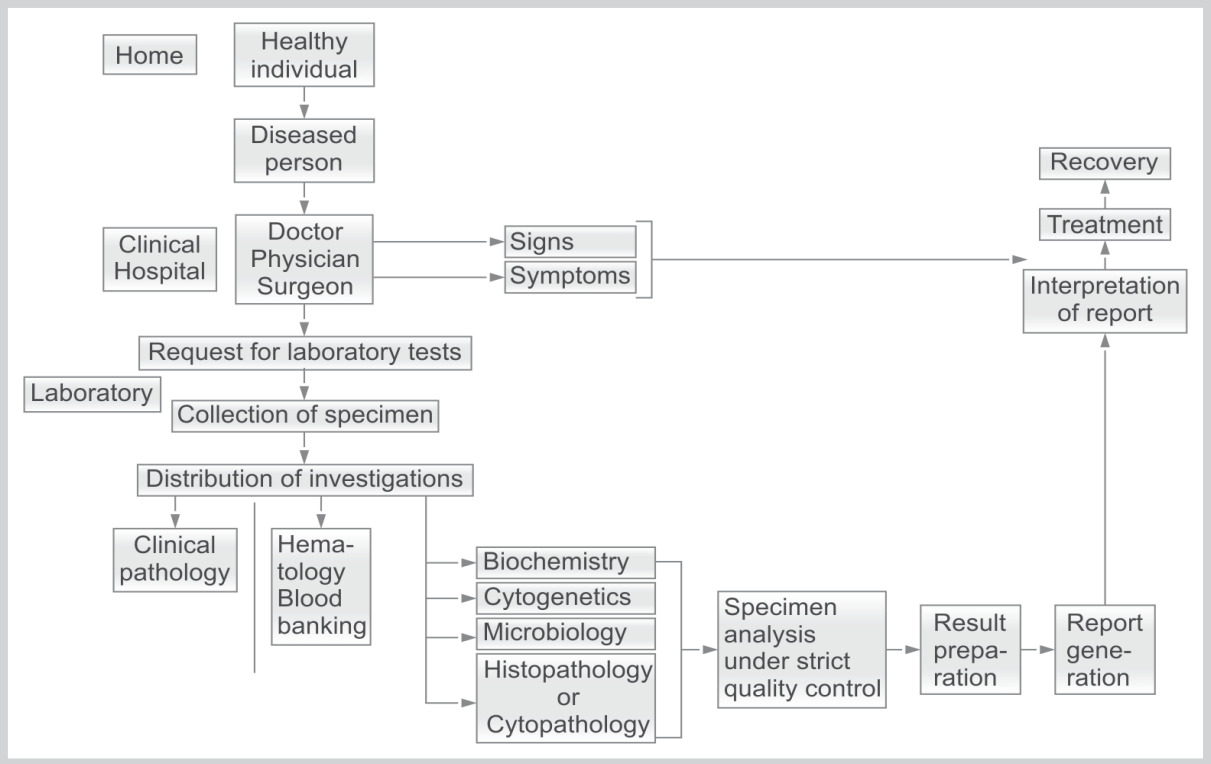


FIG. 1.1: Health-disease-health cycle

Primary Laboratory

In rural setups, for instance, a primary laboratory may provide only the basic investigations. These investigations are simple to perform and do not involve expensive machinery usage. Such laboratories are also attached to physician chambers nowadays, so that clinicians may obtain basic inputs right in their own premises. These primary laboratories may provide the following simple investigations:

- ✓ Hemograms (hemoglobin estimation, total and differential counts, erythrocyte sedimentation rate and packed cell volume with basic peripheral smear study including the reporting of hemoparasites)
- ✓ Routine and microscopic studies of urine and stool. Routine examination also entails chemical examin- ation either by laborious and time-consuming old chemical methods or by new generation dipstick tests. These may include tests for glucose, bilirubin, ketones, hemoglobin, leukocytes, pH, nitrites, protein, urobilinogen and specific gravity in case of urine. For stool samples, reducing substances, pH and occult blood may be performed. Basic spot/latex/device tests (e.g. pregnancy test) may be conducted.

Secondary Laboratory

These are laboratories that assist a clinician to confirm a clinical suspicion or establish a diagnosis. Therapy and prognosis monitoring can also be provided from these laboratories. Such laboratories are staffed by qualified personnel who are trained and experienced to perform the tests. They also have a perfect knowledge of the equipment and machines they use. They should be aware of quality control essentials and be well versed with interpretational aspects of the reports generated by their laboratories. In addition to what has been mentioned under primary laboratories, secondary laboratories also perform:

- ✓ Routine immunohematological tests.
- ✓ Routine examination of all body fluids, e.g. semen, cerebrospinal fluid (CSF), sputum, etc.
- ✓ Routine bacteriologic studies including stains, cultures and antibiograms. Routine mycological investigations would include—primary cultures, isolation and identi- fication techniques along with microscopic evaluation.
- ✓ Routine immunoserological tests. These can include tests like Widal, STS, ELISA or strip or device tests HIV I and II, hepatitis B and hepatitis C, etc.
- ✓ Routine biochemistry investigation and organ profile tests, e.g. lipid, cardiac, liver and renal profiles.
- ✓ Under hematology, these laboratories may also provide RBC indices, platelet, reticulocyte count and absolute eosinophil counts. They can also classify anemias and should be able to indicate hematologic malignancies. When headed by a pathologist, they should be in a position to report bone marrow smears/ preparation too.

Tertiary laboratory

These kinds of laboratories should be able to perform all kinds of sophisticated and delicate/precise investigations. The tertiary laboratories can branch out in very special fields and not cater to all aspects of specialized tests. Besides doing all investigations that are conducted in secondary laboratories, they also carry out the following:

- ✓ Specialized hematological (e.g. leukemia type), coagu- lation profiles and immunohematological investigations. They are equipped with 18 parameter cell counters with differentials and flow cytometry
- ✓ Complete biochemical assays, commonly referred to as SMA-12, SMA 27, etc. Also included are elemental assays, e.g. zinc, magnesium, iron, total iron binding capacity (TIBC), lithium, etc. special enzymes like HBDH, lipase and isoenzymes, etc.
- ✓ Complete immunology based assays for hormones, cancer markers, hepatitis markers, rheumatic/auto- immunity etiology-based profiles, TORCH profiles, rare infectious diseases (e.g. brucellosis leptospirosis, cysticercosis, echinococcosis, etc.)
- ✓ All microbiological processes, e.g. cultures—aerobic, anaerobic, fungal, tubercular, etc. with antibiograms.

The techniques for these investigations may vary. They may be ELISA, chemiluminescence, turbidimetry, PCR, etc. These laboratories are totally automated and have sizable workload. Furthermore, they also undertake all histopathology (simple H and E, special staining techniques, immunohistochemistry methods) and cytopathology processing and reportings. They may also undertake cytogenetic investigations, e.g. chromosomal analysis. The dissemination of reports from these laboratories is in keeping with recent trends in telecommunications, e.g. fax, e-mail, etc. In the United States of America, these laboratories though classified differently (with a few differences) are covered under the Clinical Laboratory Improvement Act (CLIA) of 1988.

LABORATORY SET-UP

Unless the laboratory is hygienic and provides necessary physical and operative comfort, it would be wrong to expect perfect results. To get perfect results, one has to provide a perfect set-up for people to work in.

Laboratory Building and Space

Ample working space is absolutely essential. For smaller laboratories up to 25 square meters (Fig. 1.2), the working platforms can be arranged along the walls while the central area is kept free for movement.

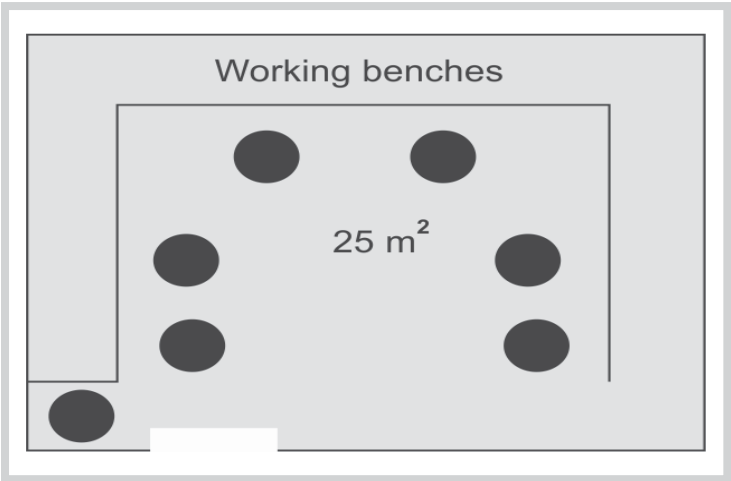


FIG. 1.2: A typical small laboratory

For larger areas, partitions can be made which would create separate spaces for different sections (Fig. 1.3). The chief pathologist must have casual access to all sub- units of the laboratory. If possible, he should be able to directly see into the cabins either through glass windows or through closed circuit cameras. In the cabins again, the central region should be kept free and benches be placed against the walls and away from the doors.

- ✓ Hygiene is of utmost importance. The whole facility should be absolutely clean, uncrowded and devoid of any hindrances to movement of men and materials. Never, should a chance arise where two people would clash or contaminated material would be spilt all over
- ✓ Scratch proof matt finish vitrified floor (slip resistant) should be provided. The walls should preferably have white ceramic tiles. Such provisions are resistant to chemicals and disinfectants
- ✓ All benches should be preferably 2½ feet high and those to be used while standing should be at least 3 feet high. The bench surfaces should be solvent and acid proof.

Every laboratory and/or its section must have at least one sink and one hand wash basin. The hand wash basin should not be used for any other purpose; the sink can be utilized for laboratory purposes like washing off stains from slides or washing glassware or discharging non- contaminated laboratory refuse.

Sero-immunology ELISA's, PCRs, drugs, Cancer markers	Biochemistry	Microbiology
Pathologist's chamber	Collection of specimens and report delivery	Hematology + Clinical pathology
Histopathology Cytopathology		Toilet

FIG.1.3: A typical large/complete laboratory plan

Physical Aspects of a Laboratory

- ✓ The ambient temperature should be within the comfort zone of a human body. It should between 21 and 27°C. If the laboratory is in a cold zone, it must have heating provision, and conversely, if it is in a hot zone, it must have cooling or air conditioning. The environment control appliances like air conditioners or heaters must not directly discharge air at the working bench zone.
- ✓ A good exhaust system is a must for all laboratories. This removes dirty air (aerosols), which may at times be foul smelling. The sample collection zone too, must have excellent exhaust provision
- ✓ Adequate ventilation is also essential but without strong currents of air
- ✓ Lighting should be more than adequate and places where very delicate or fine processes are being conducted should have additional lighting provision. As far as possible, do not use excessive heat producing bulbs and lamps. The new CFLs are ideal
- ✓ Windows that are exposed to bright sunlight can be internally fitted with reflective films or blinds

- ✓ There should be sufficient running water for the laboratory and all must have sufficient number of sinks and hand wash basins
- ✓ As most machines consume a lot of electricity, sufficient power load (a little in excess) must be available to the laboratory

Provisions and Precautions

Every working room or cabin should have adequately spaced provision of water, electricity, gas, sinks lighting and exhausts. All aspects, whether plumbing, electrical systems or gas connection must pass through regular inspections and a log book should be maintained of such preventive exercises. Preventive maintenance should be carried out by knowledgeable and qualified persons.

Fire Prevention

- ✓ Install appropriate fire extinguishing system and timely testing of such a system be conducted at regular intervals
- ✓ Color code and place firefighting equipment at an easily visible and reachable location. Check the working capability of all such systems at regular intervals
- ✓ Provide adequate ventilation in zones where flammable chemicals are used. Before these substances reach combustible or explosive concentration, they should be removed by mechanical exhausts.
- ✓ Post "No-smoking" signs in zones where smoking can be hazardous.
- ✓ Lastly, mark clearly the emergency exit points. Keep the emergency exit route free from obstructions.

Electrical Installations

- ✓ Hire a proper, qualified electrical engineer and explain to him the purpose of the premises being taken. As far as possible, all points where sparks can be generated should be kept out of room/cabins where explosive chemicals are likely to be used
- ✓ Use earthing everywhere and install fire-resistant cables in the laboratory
- ✓ Employ only certified products
- ✓ Use one electrical socket for a single device or machine.
  Overloading is usually the cause of accidents.

Liquefied and Compressed Gases

- ✓ Color code and identify each gas container. Check their valves regularly
- ✓ Keep all such cylinders away from sources of heat and electrical sparks
- ✓ When not in use, replace protection/safety caps back on the cylinder mouths.

Chemicals and Radioactive Substances

- ✓ Label all bottles with proper names of contents and affix warning signs and symbols as applicable to them
- ✓ Clearly display the warning charts (both chemical and radioactive) next to such containers. All staff members working in such areas should be well trained to handle accidents of any kind that can happen
- ✓ A stringent record of stocks should be maintained of all persons and radioactive

substances being used in the laboratory. A bottle lost or stolen is invitation to problem.

Stores

- ✓ Every bottle/container should be labeled. Affix the hazard intensity on the bottle or the container
- ✓ Ensure in every possible way that the containers cannot under any circumstances fall or spill. This can be done by placing the most dangerous chemical at the bottom or at the floor level
- ✓ Proper ventilation should be ensured in storage zones that house flammable chemicals. Keep fires extinguishing equipment handy? Post "No smoking" signs that are clearly visible. Make sure that the place remains free from pests.

Staff Safety and Facilities

The most important asset of any institution is the man- power that works for it. It holds true for laboratories too. Absence of staff due to morbidity or mortality can stifle your working capacity, capability and reputation. Provide adequate facilities to your team. (Designate a room or space meant exclusively for retiring or resting and consuming foodstuffs).

- ✓ Hot and cold running water with soap and disinfectants should always be provided. Clean hand towels should be replaced daily
- ✓ A clean toilet for use by staff members is mandatory as are the changing rooms. If possible, separate units for male and female members should be provided
- ✓ Biomedical wastes and non-biomedical wastes should be discarded properly and safely. Chemical treatment of liquid wastes and incineration of solid wastes should not be overlooked. Wastes handled properly ensures good health of your working team
- ✓ Designate a room or space meant exclusively for retiring or resting and consuming foodstuffs. Under no circumstances, laboratorians should eat or drink on their workbenches. Provide safe drinking water to all
- ✓ Each room/cabin must have a first-aid box kept at an identified place that is easily accessible. Every person working in the laboratory must be aware of all hazards that exist and must also know about the remedial measures that should be taken if something happens. What can be managed in house should be managed, when required, assistance of other specialists must be taken. Contact numbers of such institutions/specialists must be displayed prominently
- ✓ All members of your team must be immunized as relevant to the laboratory work. Make sure no single person works alone in a room or cabin. Two compatible persons should work together always.

Basic Laboratory Safety

- ✓ Use only certified safe equipment in the laboratory
- ✓ Decontaminate all equipment regularly and before their servicing or maintenance, use appropriate disinfectants correctly
- ✓ As far as possible, use disposable plastic ware to avoid contamination (chemical, biological, etc.) and breakages with ensuing dangers.
- ✓ Regularly test and service biological safety cabinets and fume cupboards.

Appropriate safety measures taken by you will go a long way in enhancing productivity.

As a rule, the place for receiving or withdrawing the specimens should be separate from the working compartment. To avoid specimen mixing (hazardous), each sample should be carefully labeled. The label should clearly mention the alloted specimen number, the date and time of receipt of specimen, the investigations to be done and most important the name of the patient. Both, the clinical and the paraclinical workers are equally at risk of acquiring transmissible diseases through the patient or through the test samples. The risk of these can be lessened by taking appropriate vaccinations. In addition, one should attend to one's general hygiene and prevent fomite transmission of any infectious disease. Disinfect the working benches and as far as possible autoclave (or chemically disinfect) various glassware used in the laboratory. Use a rubber teat for sucking/filling the pipettes. To avoid strain on the eyes, keep both eyes open while doing microscopic work. Before leaving the laboratory, one should thoroughly wash one's hands with soap and water, and then rinse them well in a disinfectant lotion.

CODE OF CONDUCT FOR MEDICAL LABORATORY PERSONNEL

1. Place the well-being and service of the sick above your own interests.
2. Be loyal to your medical laboratory profession by maintaining high standards of work and strive to improve your professional knowledge.
3. Work scientifically and with complete honesty.
4. Do not misuse your professional skills or knowledge for personal gain.
5. Never take anything from your place of work that does not belong to you.
6. Do not disclose to a patient or any unauthorized person the result of your investigations.
7. Treat with utmost confidentiality and personal information that you may learn about a patient.
8. Respect and work in harmony with the other members of your hospital staff or health center team.
9. Be at all times courteous, patient, and considerate to the sick and their relations.
10. Promote health care and the prevention and control of disease.
11. Follow safety procedures and know how to apply first aid.
12. Do not drink alcohol during laboratory working hours or when on emergency stand-by.
13. Use equipment and laboratory-ware correctly and with care.
14. Do not waste reagents or other laboratory supplies.
15. Fulfill reliably and completely the terms and conditions of your employment.

Always remember that you can be a patient tomorrow. Treat others as you would want them to treat you.

ACCIDENTS

Safety Measures in the Laboratory

You must remain alert and cautious while working in the laboratory. You must know that careless handling of reagents, glassware or specimens to be tested in the laboratory can cause serious injury and is dangerous to life.

Hazards in the Clinical Laboratory

Clinical laboratory workers may encounter three types of hazards:

1. Physical,
2. Chemical, and
3. Biological hazards.

Physical Hazards

Physical hazards are present in ordinary equipment or surroundings. Electrical equipment, open flames, laboratory instruments and glassware can all be hazardous if improperly used.

Electricity

- ✓ All electrical equipment must be properly grounded following the manufacturer's instructions
- ✓ Even minor repairs, such as replacement of the micro- scope bulbs, require that instrument be disconnected from the power supply before the work is begun
- ✓ All electrical cords and plugs be kept in good shape and order with no frayed cords or exposed wires
- ✓ Avoid overloaded circuits
- ✓ Extension cords present several safety hazards and should not be used except in emergency.

Fire

Fire is a potential danger in the workplace:

- ✓ Though rare, they can occur when open flames are used in the vicinity of flammable liquids
- ✓ Make sure that loose clothing and long hair do not catch fire
- ✓ Instead of open flames, use hot plates, microwave ovens, electric incinerators and slide warmers
- ✓ Store flammable chemicals in a flameproof cabinet, away from heat sources and well-ventilated area. A flameproof cabinet can protect flammable chemical from flames until firefighters arrive and also allow workers more time to escape
- ✓ All laboratory workers must know about the escape route and procedure to follow if that exit is blocked
- ✓ All workers must know the location of fire extinguishers and how to use them
- ✓ Inspect all fire extinguishers periodically and log the date of inspection.

Usual Causes of Fire in the Laboratory

- ✓ Naked flames (do not work with loose clothing and long hair near naked flames). Naked flames can also ignite flammable liquids and gases
- ✓ Electrical overloading. Use one socket for one equipment only. Do not operate a 15 amp equipment from a 5 amp socket
- ✓ Poor electrical maintenance. No frayed or open/ exposed wires be ever used
- ✓ Leaving equipment switched when not in use. Out of sight is out of mind
- ✓ Deteriorated gas tubing. Leakage of gas is an open invitation to fire hazard. If you suspect gas leakage, do not operate any electrical equipment (do not ever switch on a light or a fan)

- ✓ Smoking in the laboratory
- ✓ Misusing matches. Use carbonized matches as far as possible
- ✓ Storing flammable and explosive chemicals in an ordinary refrigerator.

When a Fire Occur

- ✓ For tiny blazes; water, sand and a fire blanket can be employed to put out the fire. For larger blaze, a fire extinguisher can be used
- ✓ Never use water on an electrical fire or one caused by organic solvents (ether, alcohol, petrol, etc.). For electrical fires, use carbon dioxide fire extinguisher. For organic solvents, use sand or halon
- ✓ Escape via the fire exit route. Stay close to the floor, cover your mouth and nose with a damp cloth to filter out some of the harmful fumes
- ✓ Inform firefighting department of your area if you feel the fire can go out of hand. Medium to large fires should be reported irrespective of your preparedness to handle them.

Laboratory Equipment (Table 1.1)

- ✓ Use all laboratory equipment as per manufacturer's recommendation
- ✓ Any instrument with moving parts, such as a centrifuge, must be operated with a special regard for safety. Latch, the lid before turning it on. On turning it off, do not open the lid before it has come to a complete stop
- ✓ Autoclaves present special hazards. Strictly adhere to manufacturer's instruction to prevent explosions and burns. Use insulated gloves while removing hot items from the autoclave.

Glassware

- ✓ Use glassware that is free of chips and cracks. Damaged glassware is weakened and may break, resulting in injury
- ✓ Broken glass should be cleaned with a brush and dustpan and not with bare hands
- ✓ Glass should not be discarded into regular trashcans, but into rigid cardboard or plastic containers
- ✓ Wherever possible, replace glassware with plasticware.

Equipment Related Hazards

- ✓ Hypodermic needles: Accidental inoculation, aerosol or spillage
- ✓ Centrifuges: Aerosols, splashing and tube breakages
- ✓ Culture stirrers, shakers, agitators: Aerosols, splashing and spillage
- ✓ Refrigeration: If flammable chemicals are stored within them, the light switches, thermostats, etc. can provide sparks to ignite them
- ✓ Water baths: Provide ground for microorganismal growth

(The risk of acquiring hepatitis B from a needle stick is 30%, hepatitis C is 2 to 10% and HIV is 0.3%).

Equipment/Materials Employed to Eliminate/Reduce Hazards

- ✓ Laboratory apron: Assists in diminishing skin contacts to a certain extent

TABLE 1.1: Fire fighting equipment

Fire fighting material	*Used for*	*Contraindicated for*
Fire blanket	Clothing fire, G small blaze	Electrical fires, flammable liquids, a small blaze burning metals, alkali metal
Water	Paper, wood, fabric	Electrical fires, flammable liquids, burning metal, alkali metal
CO_2 fire extinguisher	Flammable liquids and gases, electrical fire	—
Dry powder	As above	—
Foam	Flammable liquids	—
Halon spray	All kinds of fires	—

- ✓ Biological safety cabinets: Prevent dangers arising out of aerosols and splatters
- ✓ Splatter shields: Provide protection from splatter of specimen and chemicals
- ✓ Pipetting aids (teat or electromechanical devices).
  Prevent from hazards arising out of mouth pipetting
- ✓ Goggles: Protect eyes from impacts and splashes
- ✓ Face shields: Protect the face from impacts and splashes.

Safety with Chemicals/Reagents

Excepting just a couple of reagents, almost all chemicals/ reagents used even in the most basic laboratory are lethal poisons if consumed by anyone. Even if they are splashed on the skin/eye, they can cause irreversible damage. There is an appropriate way of handling and storage of hazardous chemicals to avoid injury and damage to self and others. In our country (and other tropical nations), excessive heat can decompose many chemicals, cause explosions, or lead to the formation of toxic fumes.

Labeling of Hazardous Reagents/Chemicals

At appropriate places, display the prohibition signs; and on all dangerous reagents or chemicals, stick *Hazard* warning symbols. In the following pages, important signs and symbols as related to safety in the laboratory are given.

Incompatible Chemicals

Fair number of common laboratory chemicals react dangerously if they come in contact with specific chemicals. Ensure that you keep such chemicals away from each other. A few examples are listed below:

Acids

- ✓ Acetic acid with chromic acid, nitric acid, hydroxyl compounds, ethylene glycol,

peroxides and perman- ganates

- ✓ Chromic acid—with acetic acid, alcohol, glycerol and other flammable liquids
- ✓ Sulfuric acid—with chlorates, perchlorates, perman- ganates and water.

Vaporizing Substances

- ✓ Acetone—with sulfuric acid and nitric acid
- ✓ Flammable liquids—with chromic acid, hydrogen peroxide, nitric acid, ammonium nitrate and halogens.

Others

- ✓ Alkali metals, e.g. calcium, potassium, sodium (these form hydroxides on coming in contact with water) and with other chlorinated hydrocarbons.
- ✓ Chlorine—with ammonia, hydrogen, benzene and other finely divided metals
- ✓ Copper—with azides, hydrogen peroxide and acetylene
- ✓ Cyanides—with all acids and alkalies
- ✓ Hydrogen peroxide—with copper, iron, chromium and most other metals
- ✓ Iodine—with acetylene and ammonia
- ✓ Sodium azide—with lead, copper and other metals.

Flammable Chemicals

These include ether, xylene, toluene, methanol, ethanol, glacial acetic acid, acetic acid, acetone, acetic anhydride, alcoholic Romanowsky stains and acid alcohol, etc.

Storage

These should be stored in a fire-proof metal box at ground level, preferably in a cool store. A container well lined with tin foil can also be used. Store only small quantities of such solvents on the shelves.

Safe Use

Ensure that there is no open flame nearby while opening a bottle containing flammable solvent. Nearest flame should be at least 10 feet away. Never heat a flammable liquid over any flame. Use a water bath or electric hot plate.

Control of Fire Caused by Flammable Chemicals

Best controlled by smothering them. Uses and, thickblanket or the now available multipurpose fire extinguishers. Pouring water on such fires will spread them. Every laboratory should be equipped with the commercially available fire extinguishers. If these are not available, there should be sand buckets in accessible places.

Corrosive Chemicals

These include strong acids, e.g. concentrated sulfuric acid, hydrochloric acid, nitric acid, glacial acetic acid, trichloroacetic acid, orthophosphoric acid, and strong alkalies like sodium hydroxide and potassium hydroxide.

Storage

Store these at low levels.

Safe Use

Never attempt mouth pipetting. Accidental swallowing can be lethal as these chemicals cause destruction of living tissue. Always pour a corrosive chemical at below eye level, slowly, and with great care to avoid splashing. Wear protective eye glasses/eye shields while opening such containers. Always add the corrosive substance to water and that too slowly. The addition of small amount of water to sulfuric acid is enough to produce sufficient heat to break a glass container.

Toxic, Harmful, and Irritating Chemicals

These are chemicals that can cause death or serious ill- health if swallowed or inhaled or if they come in contact with skin. Examples are potassium cyanide, mercuric nitrate, sodium azide, sodium nitroprusside, formaldehyde solution, chloroform, barium chloride and methanol. Iodine and sulfuric acid also fall in this category. Skin and mucous membrane irritants are xylene, formaldehyde and ammonia vapors.

Storage

Store highly toxic chemicals, e.g. potassium cyanide in a locked cupboard. Stock solutions should also be stored safely in a cupboard, not on an open shelf.

Safe Use

Always wear protective gloves and after working with them immediately lock them up. Always wash your hands after using a toxic or harmful chemical. Keep fume forming chemicals in a fume cupboard. Never mouth pipette them.

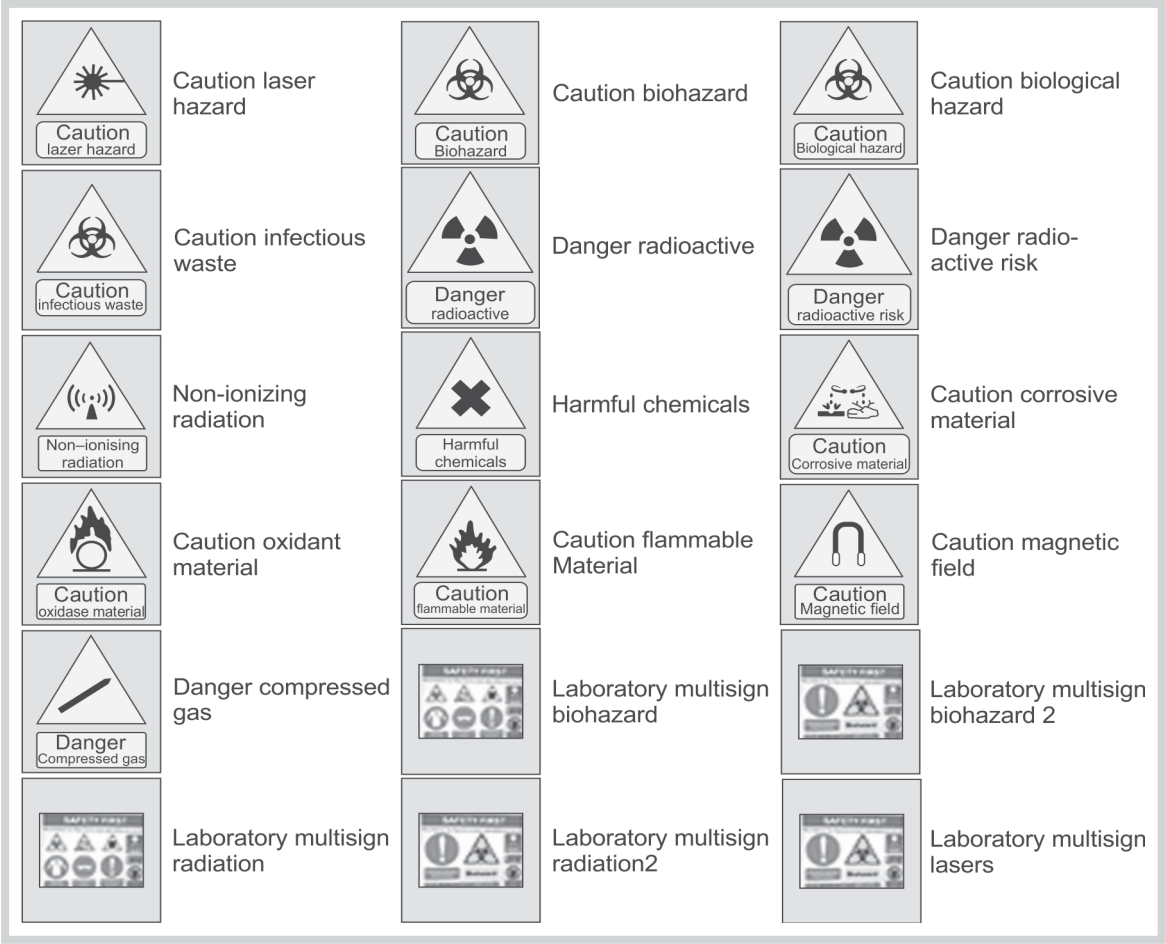
Oxidizing Chemicals

These include chlorates, perchlorates, strong peroxides, potassium dichromate, and chromic acid.

Storage

Keep these away from organic materials and reducing agents. They can produce much heat when in contact with other chemicals, especially flammable chemicals.

SIGNS FOR MEDICAL LABORATORIES



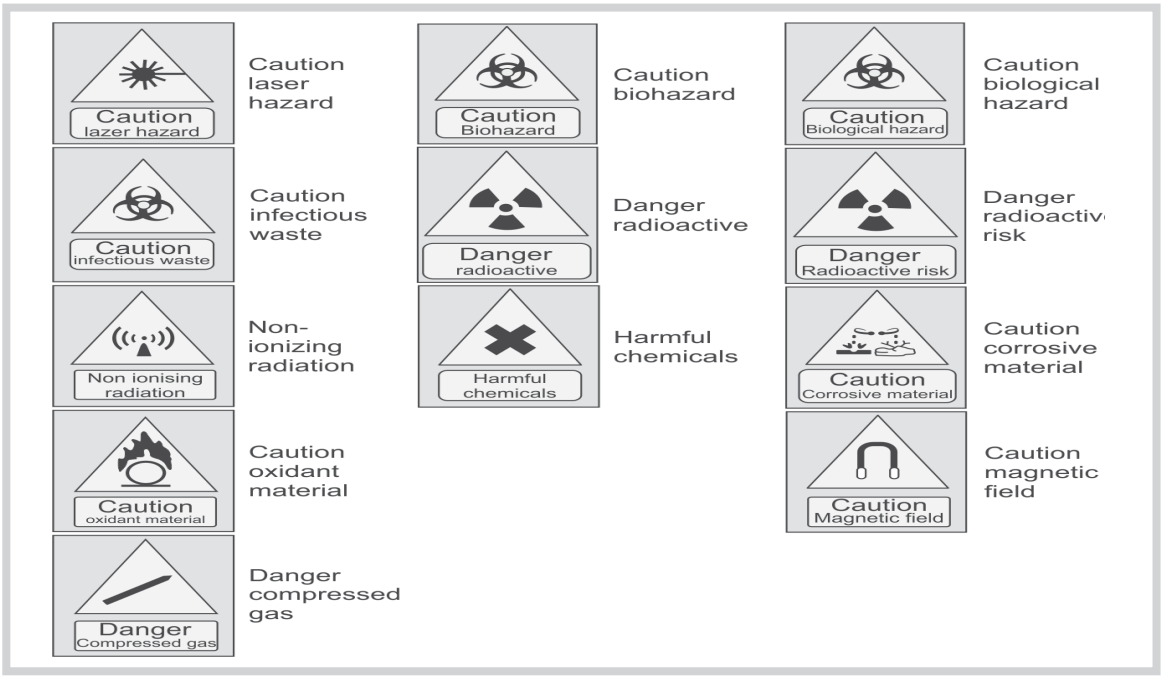


FIG. : General laboratory

FIG. : Laboratory cautionary

Safe Use

Handle them with utmost care. Most of them are dangerous to skin and eyes and when in contact with reducing agents.

Explosive Chemicals

These chemicals can explode on being heated or on getting exposed to flame or friction. A good example is picric acid, which must be stored under water. If picric acid is allowed to dry, it can explode.

Carcinogens

These chemicals can cause cancer by ingestion, inhalation, or by skin contact. Such chemicals include benzidine, O-toluidine, O-dianisidine, α and β naphthylamine, nitro-samines, nitrosophenols, nitronaphthalenes, and selenite. The carcinogenic risk is directly proportional to the length and frequency of exposure and the concentration of the chemical.

Storage

Label their containers "CARCINOGENIC" and handle with special precautions.

Safe Use

Must wear protective plastic or rubber gloves, a facemask and eyeshields when handling carcinogenic chemicals. Do not let them come in contact with skin. After handling a carcinogen, wash well in cold water all the apparatus, bench, bottles and protective gloves (before removing them) and change your overall. Rinse your hands in cold running water before using soap. Should a carcinogen come in contact with skin, wash the affected part in cold running water for 5 minutes.

ACCIDENTS IN THE LABORATORY

They may be caused by:

1. Acids } Splashes on the skin, Splashes in the eye
2. Alkalis } • Swallowing
3. Toxic substances
4. Heat
   - Open flames
   - Hot liquids
   - Inflammable liquids
   - Explosions
5. Broken glass
6. Contamination by infected material
7. Electric shock.

A suggested list of first aid equipment is given later in the chapter. The items should be readily available in the laboratory. They must not be kept in a locked cupboard.

FIG. : General prohibition

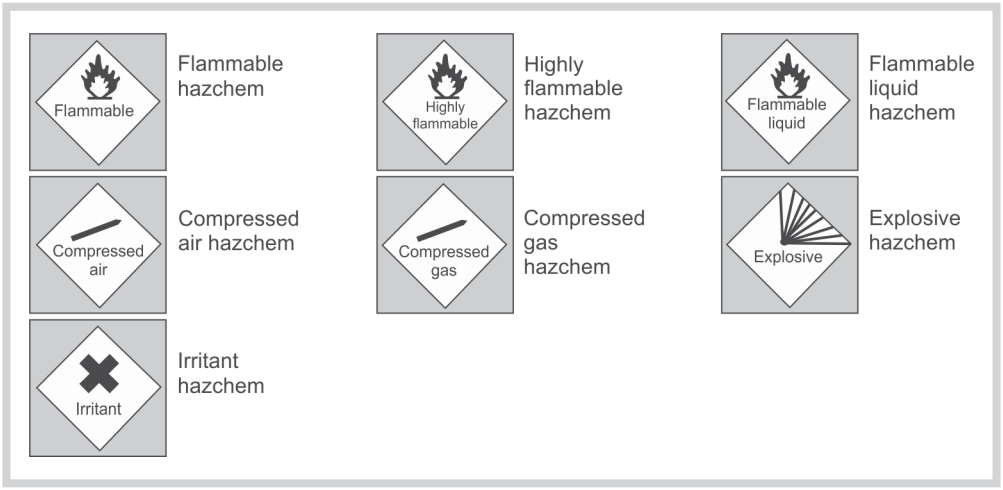


FIG. : General laboratory

First Aid in Laboratory Accidents

Acid Burns

Nitric, sulfuric, hydrochloric and trichloroacetic acids.

In all cases: Wash immediately with large quantities of water.

Acid Splashes on the Skin

a. Wash thoroughly and repeatedly with water.
b. Bathe the affected skin with cotton wool soaked in 5% aqueous sodium carbonate.

Acid Splashes in the Eye

a. Wash the eye immediately with large quantities of water sprayed from a wash bottle or rubber bulb. Squirt the water into the corner of the eye near the nose (Figs 1.4 and 1.5).
b. After washing, put 4 drops of 2% aqueous sodium bicarbonate into the eye.
c. Refer the patient to a physician. Continue to apply bicarbonate solution to the eye while waiting for the doctor. Alternatively, hold the eye under the running tap.

FIG. 1.4: Eye washing upright

FIG. 1.5: Eye wash lying

Swallowing Acids

Accidental swallowing while using a pipette:

a. Call a physician.

b. Make the patient drink some 5% soap solution immediately. Alternatively, give him two whites of egg mixed with 500 mL of water or milk. If neither of these is available, he should drink ordinary water.
c. Make him gargle with the soap solution.
d. Give him 3 or 4 glasses of ordinary water.
e. If the lips and tongue are burned by the acid:
   - Rinse thoroughly with water
   - Bathe with 2% aqueous sodium bicarbonate.

Alkali Burns

Sodium, potassium and ammonium hydroxide.

<u>*In all cases*</u>: Wash immediately with large quantities of water. *Important*: Alkali burns are as serious as, and often more serious than, acid burns.

Alkali Splashes on the Skin

a. Wash thoroughly and repeatedly with water.

Bathe the affected skin with cotton soaked in 5% acetic acid (or undiluted vinegar).

Alkali Splashes in the Eye

a. Wash immediately with large quantities of water sprayed from a wash bottle or rubber bulb. Squirt the water into the corner of the eye near the nose.
b. After washing with water, wash the eye with a saturated solution of boric acid (apply drops repeatedly).
c. Refer the patient to a physician at once.

Swallowing Alkalis

Accidental swallowing while using a pipette:

a. Send for a physician.
b. Make the patient drink at once:
   - A 5% solution of acetic acid or lemon juice or dilute vinegar (1 part vinegar to 3 parts water).
c. Make him gargle with the same acid solution.
d. Give him 3 or 4 glasses of ordinary water.
e. If the lips and tongue are burned by the alkali:
   - Rinse thoroughly with water
   - Bathe with 5% acetic acid.

Poisoning

This can be caused by:

✓ Inhaling toxic vapors or gases (e.g. chloroform)
✓ Accidental swallowing while pipetting a poisonous solution.

In all cases

a. Send for a physician or qualified nurse, specifying the toxic substance involved
b. Place the victim in the open air while waiting for the physician.

Burns Caused by Heat

They fall into two categories:

- ✓ Severe burns—affecting large areas of skin, e.g. burns caused when burning ether or boiling water is spilled over the victim
- ✓ Minor burns—affecting a small area of skin, e.g. burns caused by hot glassware or a Bunsen flame.

Severe Burns

a. If the victim is on fire, e.g. if splashed with burning ether or other inflammable solvent, roll him in a blanket or overall to smother the flames.
b. Inform the physician on duty immediately.
c. Lay the victim on the ground. Do not remove his clothing. Cover him if he is cold.
d. Do not apply any treatment to the burns. This must be left to the physician.

Minor Burns

a. Plunge the affected part into cold water or ice-water to soothe the pain.
b. Apply mercurochrome or acriflavine ointment to the burn.
c. Apply a dry gauze dressing loosely.
d. If the burn becomes infected or does not heal, refer the patient to a physician.

Note: Never tear off the blisters that form over the burns.

Injuries Caused by Broken Glass

These are caused by broken test tubes, syringes or other glassware.

a. Wash the wound immediately to remove any glass pieces.
b. Apply mercurochrome or acriflavine ointment to the wound.
c. Cover with gauze and adhesive tape.
d. If the cut bleeds profusely, stop the bleeding by pressing down on it with a compress. Refer the patient to a physician.
e. If the cut bleeds heavily with the blood spurting out at intervals, try to stop the bleeding with a compress and call a physician or qualified nurse.
f. Continue to press on the wound while awaiting the physician's or nurse's arrival. He or she will decide whether a tourniquet should be applied.

Contamination by Infected Material

Wounds caused by broken glassware containing stools, pus, etc.

a. Wash the wound immediately.
b. Check whether the cut is bleeding. If not, squeeze hard to make it bleed for several minutes.
c. Bathe the whole area, i.e. the edges of the cut and inside the cut, with antiseptic lotion.
d. Wash thoroughly with soapy water.

e. Bathe again with antiseptic lotion.
f. Refer the patient to a physician, if the material involved is known to be very infective, e.g. pus.

If infected material is accidentally sucked into the mouth:

a. Spit it out immediately.
b. Wash out the mouth with diluted antiseptic lotion.
c. Wash out the mouth thoroughly with large amounts of clean water.

Bodily Damage by Electric Shock

A low-voltage alternating electric current (220 V) is usually used in the laboratory and electric shocks are rare. They may occur when faulty equipment is being handled, particularly with wet hands. The symptoms are fainting and asphyxia.

a. Before doing anything else, put off the main switch.
b. Send for a physician.
c. Begin giving mouth-to-mouth respiration immediately if required (Fig. 1.6).

FIG. 1.6: Mouth-to-mouth respiration

Precautions for the Avoidance of Accidents

1. Handling acids and alkalis
   a. Diluting sulfuric acid with water: Always add the sulfuric acid to the water drop by drop, stirring the mixture after each drop. Do this preferably in a sink. Never pour water into sulfuric acid (because of the danger of splashing).
   b. Bottles of acids and alkalis: Keep them on the lower shelves of the cupboards. When you take one out, hold it firmly upright with a dry hand. Do not keep acids and alkalis in bottles with ground glass stoppers as they may get stuck.
   c. Pipetting: Where possible, use small measuring cylinders for measuring acids and alkalis. If more accurate measurement is required, use a pipette plugged with non-absorbent cotton wool or with a rubber tube attached. Pipette slowly, watching the level of the liquid.

2. Heating glassware and liquids
   a. Test tubes: Never heat the bottom of a test tube. The liquid inside might sputter. Heat the middle of the tube, shaking gently. The mouth of the tube should be facing away from the worker and any other person, towards an empty space or a sink.
   b. Ordinary glass and Pyrex: Only Pyrex glassware and porcelain receptacles can be heated over a Bunsen flame. Ordinary glass will break.
   c. Inflammable liquids: Only small quantities of in- flammable liquids such as ether, ethanol, acetone, benzene, toluene and carbon disulfide should be kept in the laboratory.

      Warning: Ether will ignite at a distance of several meters from a flame. Never place a bottle of ether on a workbench where there is an open flame (Bunsen burner, spirit lamp, etc.). Carbon disul- fide is even more dangerous.
   d. Butane gas: When lighting a gas burner, always light the match and hold it to the burner before turning on the gas tap. Turn off the main valves of all butane gas cylinders every evening. Replace the rubber connecting pipes once a year.
3. Do not use broken, cracked or chipped laboratory glassware.
4. Put clear labels on poisons. Keep them in a locked cupboard.
5. Do not use nylon clothes while working as these are easily inflammable. Always use a laboratory apron.
6. Always ensure that electrical wiring and electrical appliances are in good condition.

Suggested List of First Aid Equipment for Laboratory

1. 5% aqueous sodium carbonate
2. 2% aqueous sodium bicarbonate in an eye drop bottle
3. 5% acetic acid
4. Saturated solution of boric acid in an eye drop bottle
5. Soap powder solution (5 g per liter of water)
6. Acriflavine ointment
7. Mercurochrome 2%
8. Antiseptic lotion
9. Cotton wool
10. Gauze
11. Roller bandage
12. Adhesive tape
13. Scissors.

Contamination from Infective Material

If contamination has occurred, then:

1. Disinfect the part with the disinfectant available in the laboratory. Thoroughly clean the affected area with a stream of running water.
2. Sucking the contaminated material: Spit out all that has been sucked. Use a disinfectant liquid (e.g. diluted dettol) for mouth washing. If the infected material has been swallowed accidentally, forced vomiting to be done, ascertain the kind of infection and take advise from a medical person.

3. If skin is infected by highly virulent organisms, touch the involved part with pure carbolic acid.

Precautionary Measures

1. A fire extinguisher should always be handy.
2. Keep sand bucket in the laboratory.
3. Take measures to prevent electrical short circuiting.
4. No smoking in the working zone of the laboratory.
5. Breakable items should be kept in proper racks and never at the edge of the working table.
6. Do not suck anything with the mouth, use rubber teats and bulbs for sucking.
7. Do not place eatables on the working bench.
8. Keep fingernails short.
9. At the end of the day, clean all working benches with a disinfectant. See that nothing except the required electrical appliance is on.
10. Dispose all infected material properly. Can put such material in hypochlorite solution or in an acidic solution, e.g. diluted sulfuric acid (25%). Burn off all dried contaminated articles, e.g. filter papers.
11. The glassware should be disinfected with a suitable disinfectant and be cleaned thoroughly with running water.
12. Use rubber gloves and a nose mask while working with infective samples, e.g. serum of viral hepatitis patient.

UNIVERSAL WORK PRECAUTIONS (UWP) FOR LABORATORY PERSONNEL (ESPECIALLY IN RELATION TO HIV TRANSMISSION)

Introduction

Healthcare personnel (HCP) can acquire certain illnesses beyond those acquired by all others who live and work in our society, by virtue of their profession. HCPs are at risk of acquiring any of the whole gamut of infections from patients/specimens, which may be viral, bacterial, parasitic or fungal. However, this risk due to occupational exposure can be minimized if not obliterated altogether, if we follow universal work precautions (UWP).

Today, with the WHO estimates of above 5 million HIV positive persons in India, there is an urgent need to review UWP. Besides HIV, there is the very real danger of acquiring Hepatitis B and Hepatitis C in exactly the same way as HIV and could also be fatal. Hepatitis B is 100 times more infectious than HIV. Besides, Hepatitis B is also far more prevalent in India in comparison to HIV with estimated carriers being between 30 and 40 million, a considerable number being infectious. However, fortunately, effective vaccination is available for hepatitis B; therefore, it is strongly recommended for all levels of healthcare workers. Much of the contamination in the laboratory occurs as a result of penetrating injuries caused by sharp objects and the spilling and splashing of specimen materials.

Components of UWP

1. Handwashing.
2. Barrier precautions (mask, cap, plastic apron and protection of feet).
3. Careful handling of all kinds of sharps and needles.
4. Effective infection.
5. Sterilization.
6. Correct disposal of different kinds of wastes generated in a health care facility.

Guidelines of Basic Practices and Procedures

- ✓ Prevention of puncture wounds, cuts and abrasions and protection of existing wounds, skin lesions, conjunctiva and mucosal surfaces
- ✓ Application of simple protective measures designed to prevent contamination of the person and his/her clothing
- ✓ Good basic hygiene practices, including regular handwashing
- ✓ Control of surface contamination by containment and disinfection procedures
- ✓ Safe disposal of contaminated waste.

Biosafety Regulations for Laboratory Procedures

- ✓ Wear gloves when handling infectious materials or where there is a possibility of exposure to blood and other body fluids. All laboratories that work with material that is potentially infected with HIV require a generous supply of good quality gloves.
- ✓ Discard gloves whenever they are thought to have become contaminated or perforated, wash your hands and put on new gloves. Alternatively, where there are economic constraints, wash gloved hands whenever they get contaminated with blood/body fluids before collecting further samples
- ✓ Do not touch your eye, nose, or other exposed membranes or skin with your gloved hands.

Sterilization (for Nondisposable Items)

- ✓ For sharps, reusable blades, cystoscopy instruments, endoscopyinstruments, use CIDEX(2% glutaraldehyde) or 5% Korsolex. Disinfection usually occurs in 30 minutes
- ✓ Use autoclaving for other reusable items (e.g. needle holders, gowns, etc.)
- ✓ Wherever, autoclaving is not possible, boiling must be for 30 minutes at the least.

Waste Disposal

Divide waste into three parts at source.

i. Household type noninfectious waste:
   - Not to bedecontaminated
   - To be disposed off as such.

ii. Infected sharp waste disposables (needles/surgical instruments):
   - Place in puncture-proof container containing disinfectant (1% bleach prepared every morn- ing). Needles should ideally be burnt (machines are available that operate on electricity)
   - Final disposal.

iii. Infected nonsharp waste:
   - Is to be decontaminated
   - Placed in disinfectant 5 to 10% bleach as the case may be (left over blood, tissues, etc.).

Final Disposal

- ✓ Purchase of needle destroyer if resources permit
- ✓ Incineration of all infected waste
- ✓ Deep burial in controlled land fill sites (protected from all sides)
- ✓ Shredding of disposable plasticware waste.

Postexposure Care

- ✓ Minor bleed with percutaneous inoculation, open skin wound, breached skin, exposed mucous membranes.

First Aid

- ✓ Allow to bleed by squeezing
- ✓ Wash with water
- ✓ Antiseptic.

Report

- ✓ Employee identification, date, time with place of accident
- ✓ Circumstances around accident
- ✓ Action taken.

Initial Consultation

- ✓ Easy access to medical advice with counseling. Consult, physician for AZT prophylaxis regime if medication available.

Laboratory Testing

- ✓ After consent with counseling within 2 weeks, 5 weeks, 12 weeks, or 24 weeks.

Clinical Follow-up

- ✓ For fever, pharyngitis, rash, malaise, lymphadenopathy, myalgia and arthralgia within 6 months
- ✓ Do not leave the workplace or walk around the laboratory while wearing gloves
- ✓ Wash hands with soap and water immediately after any contamination and after work is finished. If gloves are worn, wash your hands with soap and water after removing the gloves. This is a vital and simple precaution that is often overlooked
- ✓ Wear a laboratory gown or uniform when in the laboratory. Wrap-around gowns are preferable. Remove this protective clothing before leaving the laboratory
- ✓ When work with material that is potentially infected with HIV is in progress, close the laboratory door and restrict access to the laboratory. The door should have a sign BIOHAZARD: NO ADMITTANCE

- ✓ Keep the laboratory clean, neat and free from extraneous materials and equipment
- ✓ Disinfect work surfaces when procedures are completed at the end of each working day. An effective all-purpose disinfectant is a hypochlorite solution with a concentration of at least 0.1% available chlorine (1 g/L, 1000 ppm)
- ✓ Whenever possible, avoid using needles and other sharp instruments. Place used needles, syringes and other sharp instruments and objects in a puncture resistant container. Do not recap used needles and do not reuse needles from syringes for disposal
- ✓ Never pipette by mouth
- ✓ Perform all technical procedures in a way that minimizes the risk of creating aerosols, droplets, splashes or spills
- ✓ Use a biosafety cabinet while working on aerosolizing specimen
- ✓ Do not eat, drink, smoke, apply cosmetics or store food or personal items in the laboratory
- ✓ Make sure that there is an effective insect and rodent control program
- ✓ If a laboratory personnel has lesions on hand and feet, then:
  - If superficial, he or she should wear protective dressing and wear gloves over it
  - If wound is deep or raw then the concerned person should not handle samples till the wound heals.
- ✓ If there is a pregnant healthcare worker then in view of the occupational risk to the woman and the developing fetus, on compassionate grounds, where possible she should be involved in clerical tasks or stay away from work for the duration of her pregnancy.

Containing Spills

- ✓ Cover the spill immediately with absorbent material to avoid aerosolization
- ✓ Soak the material by pouring disinfectant on it
- ✓ Leave the area for 30 minutes
- ✓ Mop with more adsorbent material after wearing gown, mask and gloves
- ✓ Place material in appropriate bin for disposal (autocl- aving or incineration).

Collection of Specimen

- ✓ Always keep labeled bottle ready on the bedside
- ✓ Wear disposable gloves
- ✓ Keep adequate cotton with spirit at collection site
- ✓ Keep a bucket full of disinfectant [CIDEX (glutaraldehyde)], one for at the most 5 beds.

Transport of Specimen

- ✓ Specimens should be collected in plastic; screw-capped containers prelabeled with patient identification data, should be packaged and transported in puncture resistant containers in upright position with the sign of biohazard on the container.

MEDICOLEGAL ASPECTS OF CLINICAL PRACTICE

Under the Consumer Protection Act (CPA), India, 1986; any patient, registered consumer organization, state or central government or patient's legal heirs can sue the undermentioned persons for shortcomings in "service" provided by them.

- ✓ A technician, microbiologist, biochemist or pathologist running a laboratory
- ✓ Any private polyclinic, nursing home or hospital, registered or otherwise
- ✓ As government hospitals provide service without consideration (free of cost), they cannot be held responsible under CPA 1986
- ✓ Doctors appointed by the government, however, can be held accountable under other civil and criminal laws for proven negligence
- ✓ Medical practitioners delivering new service without any consideration in a charitable hospital or medical camps are exempted from the provisions under CPA
- ✓ As per clause 2(d) (ii) of the CPA 1986, a consumer implies any person who hires or avails of any service for a consideration, which has been paid or promised, or partly paid and partly promised, or under any system of deferred payment, and includes any beneficiary of such services other than the person who hires or avails of the service for consideration paid or promised or partly paid and partly promised or under any system of deferred payment, when such services are availed of with the approval of the first mentioned person
- ✓ The time limit stipulated for filing a complaint is 2 years from the date of alleged negligence
- ✓ Patients can be dealt with severely if they file frivolous and false complaints just to harass the medical practitioner Free services provided are exempted under CPA
- ✓ A laboratorian is also a consumer as he buys various instruments, equipment, diagnostic kits/reagents/ devices. He too can file a complaint under CPA for any defect or deficiency in service related to that purpose
- ✓ Ignorance is not held as an excuse as an established legal principle. Concurrently, law does not expect a very high degree of knowledge but expects only average knowledge from a medical practitioner
- ✓ Medical negligence is a civil wrongdoing classified as 'tort', where a medical practitioner fails to take proper care in respect of examination, diagnosis, investigation, treatment, etc. resulting in injury or mortality
- ✓ Laboratorians are expected to keep all reports confidential (legally and ethically). The reports can be divulged to the referring clinician or to the patient or the relatives of the patient (with patient's onsent). Reports pertaining to sexually transmitted diseases or HIV/AIDS should be handed over only to the patient
- ✓ Legally, only authorized or registered blood banks can supply units of blood. All mandatory information must be clearly mentioned on the bottle label legibly
- ✓ These days doctors have 'Malpractice Insurance Covers'. In case a legal notice is received by such a doctor, he should immediately notify the insurance company. The insurance company must take all necessary actions in such a case. The company should appoint a lawyer to give reply or to take legal steps and inform the doctor about it. The doctor, by permission of the company, can appoint a lawyer of his choice
- ✓ What constitutes a legal notice? Any letter received by a medical practitioner from a patient or a voluntary registered organization or an advocate, demanding explanation about treatment given or demanding some explanation about treatment for alleged injury or death constitutes a legal notice
- ✓ Section 27 of the Civil Procedure Code provides that when a suit has been duly

instituted, a summon may be issued to the defendant to answer the claim, and such summon is to be served in the prescribed manner. When a complaint is lodged before the commission or the forum, the defendant practitioner is informed by a registered letter by the office, which is called a summon in legal parlance. In this summon, time for the reply and date of hearing is mentioned. Usually, the time given for filing the reply is 30 days.

Reference:

1. Concise Book of Medical Laboratory Technology: Methods and Interpretations- **Ramnik Sood.**