A Complete Workbook on Clinical Biochemistry

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CHAPTER
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Section One

Introduction to Clinical Biochemistry

Clinical Biochemistry mainly deals with the Biochemical aspects that are involved in several clinical conditions. For a medical student it's vital to have the basic fundamental knowledge of practical Biochemistry so that it helps them to diagnose, treat , monitor prognosis and prevent different diseases when they go to clinical years. Also, it helps to know about drug monitoring, prognosis of a disease, forensic investigations and so on. Hence while attending the practical classes they should look for the clinical implications of each test.

GENERAL INSTRUCTION TO THE STUDENTS

1. Student must bring the white coat and the practical workbook to the class.
2. Students must take the help of laboratory staffs for any replacement of bench reagents or the stock reagent when they are exhausted.
3. Unnecessary wastage of the reagents is prohibited.
4. The stoppers of the reagent bottles must be replaced immediately to avoid the exchange of one stopper with another.
5. Droppers and pipettes should not be interchanged.
6. Glassware should be handled carefully to avoid breakage and injury.
7. Careful handling of corrosive and poisonous chemicals are required ; mouth pipetting should be avoided.
8. Spatulas should be used for the chemicals.
9. Working table/ bench should be kept clean and dry before leaving the laboratory.
10. Running the tap water and burning the gas unnecessarily should be prohibited.
11. Follow the first-aid instructions when needed.
12. Students should be aware of acute and chronic hazards that may be caused by different chemicals they use.

LABORATORY SAFETY MEASURES

You must know all of these rules, techniques and procedures in order to participate in any of the Lab Activities.

Before beginning any Activity

1. Know what is expected
2. Prepare a clear work environment
   3. Wait for permission to start
   4. Whenever special attention is needed in a Lab activity you will see the word Caution (This means that special care must be taken when proceeding with this activity)

General Safety Precautions

1. Work quietly and carefully.
2. Never work alone.
3. Wear appropriate clothing.
4. Wear safety equipment.
5. Inform your supervisor of health-related problems, allergies.
6. Never eat or drink in the Lab area.
7. Do not attempt Lab activities at home unless told to do so by your teacher, and only under the direct supervision of an adult.
8. Touch substances only when told to do so.
9. Smell substances using the proper technique (wafting fumes toward you).
10. Pour substances properly and safely.
11. Rinse off substances immediately that come into contact with skin or clothing.
12. Wash hands after handling substances and before leaving the Lab.
13. Clean up all spills Immediately.
14. Dispose of harmful substances by following teacher's directions.

Handling a Heat Source

Use hot plates that have thermostatic controls. If using a hot plate use a beaker of water on a hot plate to heat substances in test tubes. Use heat-resistant glass (Pyrex or Kimax) - never use cracked glass. Always keep the open end of the test tube pointed away from everyone. Never allow any container to boil dry. Use tongs or gloves to pick up hot objects. Switch off the hot plate when not in use. Report if there is damaged cord. Unplug cords by pulling on the plug, not the cord. Report and replace equipment that has frayed or damaged. Treat burns using cold water or ice.

Handling an Open Flame

1. Locate fire safety equipment before using any open flame (fire blanket, fire extinguishers, fire alarm, first-aid kit)
2. Know the proper procedures for using a Bunsen burner In the Lab
3. Remove all flammable substances from the room before lighting a flame
4. Use a test tube holder if the test tube Is being heated in an open flame
5. Point the open end away from yourself and others.
6. Gently move the test tube back and forth over the flame so that it is heated evenly.

Other Recommendations

1. Dispose of broken glassware as instructed by your teacher.
2. Report broken or damaged equipment immediately (DO NOT USE IT).

3. Clean up work area completely when you are finished.
4. Wash all glassware thoroughly and place in drying racks.
5. Report all accidents to the teacher immediately (no matter how minor).

SAFETY RULES

1. Do not perform unauthorized experiments.
2. Never work in the lab alone.
3. Report all accidents immediately to your teacher.
4. If vapors generated are toxic, use a fume hood.
5. Wear chemical splash goggles.
6. Wear a chemical resistant apron.
7. Wear chemical resistant gloves.
8. Tie back long hair.
9. Do not wear loose sleeves.
10. Do not wear shorts.
11. Do not wear sandals.
12. Do not wear contact lenses.
13. No food or beverages.
14. No gum chewing.
15. Do not leave experiments unattended.
16. Know the location of all of the science lab safety equipment, exits and telephone (safety showers, eye wash, fire blankets, fire extinguishers)
17. No running.
18. Keep aisles clear.
19. Extinguish burners when away from desk.
20. No mouth pipeting of corrosives or biological fluids.

WORKPLACE HAZARDOUS MATERIALS INFORMATION SYSTEM (WHMIS)

Compressed Gas

This symbol is in class A and is used to inform people of compressed gas. This category includes such things such as propane bottles, butane bottles, and acetylene bottles.

Poisonous and Infectious

This symbol belongs to class D-l and is one of the most commonly found symbols in homes. This symbol represents materials that are toxic when ingested. Most household chemicals and cleaners contain this symbol and has become known as the symbol for poison.

Oxidizing

This symbol informs people that this substance produces oxygen when burned. This specific reaction creates a high problem for combustion and has to be stored in special containers and must be transported with extreme care.

Dangerously Reactive

This symbol is found on some household products and on a large number of lab chemicals. It means that when certain chemicals are mixed they will react and produce a harmful side effect. Some chemicals that should not be mixed are bleach, drain cleaner, and ammonia because, when combined, they will form a toxic gas.

Flammable and Combustible

This symbol is the Flammable and combustible material, which is in class B and tells a person that certain substances will react with a flame and burn. Some materials that fit into this

category are gas and oil. These substances are highly flammable and ignite with little effort.

Corrosive

This symbol is the second most common symbol found in homes across North America. This symbol is most commonly found on products such as bleach and battery acid, which are highly corrosive and are able to burn organic matter.

Toxic / Infectious

This symbol belongs to class D-2 and is one of the less common symbols found in homes. It is more commonly found in Chemistry Labs. This symbol is somewhat similar to the fourth symbol, but chemicals that fit into this category cause slower effects to the body. Some examples of this are arsenic and nicotine.

Biohazardous

This symbol is often found in hospitals and is put on products that have materials that are harmful, such as viruses or bacteria.

Examples of bacteria that fall into this category are ebola and the flesh eating disease.

FIRST AID MEASURES IN THE LAB

1. Inhalational injuries are best dealt by removing the victim from the source to an open and well-ventilated area. Irritation of throat may be relieved by warm soothing drinks.
2. Chemical injuries to the eyes by splashing or vapour are treated by washing immediately with plenty of water.
3. Aspiration of acids or alkalis while pipeting needs repeated washing with water and later to relieve the irritation of mouth and esophagus sucralfate suspension is administered at frequent intervals for a day or two.
4. Chemical injuries to the skin are treated by cleansing with ample of water. Acids can be neutralized by dilute sodium carbonate or sodium bicarbonate and alkalis can be neutralized by dilute acetic acid.
5. Burn of skin is dealt by showing the affected area under continuous running water and the ointment is applied.
6. In all the above cases, after first aid treatment send the victim to the casualty for prompt medical attention.

LABORATORY WASTE DISPOSAL

The waste produced in the course of health-care activities carries a higher potential for infection and injury than any other type of waste. A medical student should be aware of the source of the biomedical wastes and their proper handling and management. According to Bio-Medical Waste (Management and Handling) Rules, 1998 of India, "Bio-Medical waste" means any waste, which is generated during the diagnosis, treatment or immunization of human beings or animals or in research activities pertaining thereto or in the production or testing of biologicals and including categories mentioned in schedule I in the following table.

Schedule-I

Categories of Bio-Medical waste in India

Option	*Waste category*	*Treatment and disposal*
Category no.1	Human anatomical waste (Human tissues, organs, body parts)	Incineration/deep burial
Category no. 2	Animal waste (Animal tissues, organs, body part carcasses, bleeding parts, fluids, blood and experimental animals used in research, waste generated by veterinary hospital colleges, discharge from hospitals, animal house)	Incineration / deep burial
Category no. 3	Microbiology and Biotechnology wastes	Local autoclaving / microwaving/ incineration
Category no. 4	Waste sharps (needles, syringes, scalpels, blades, glass etc. Includes both used and unused sharps)	Disinfection (Chemical treatment/ autoclaving/ microwaving and mutilation/shredding)
Category no. 5	Discarded medicines and cytotoxic drugs (Outdated, contaminated and discarded medicines)	Incineration, destruction and drug disposal in secured landfills
Category no. 6	Solid waste (Items contaminated with blood and fluids including cotton, dressings, solid plaster casts, linen, beddings, other material contaminated with blood)	Incineration/ autoclaving/microwaving
Category no. 7	Solid waste (Wastes generated from disposable items other than the waste sharps such as tubings, catheters, intravenous sets etc.)	Disinfection by chemical treatment/ autoclaving/ microwaving and mutilation/ shredding
Category no. 8	Liquid waste (Waste generated from laboratory and washing, cleaning, housekeeping and disinfecting activities)	Disinfection by chemical treatment and discharge into drains
Category no. 9	Incineration ash	Disposal in municipal landfill
Category no. 10	Chemicals used in production of biologicals, chemicals used in disinfection, as insecticides, etc.	Chemical treatment and discharge into drains for liquids and secured landfill for solids.

Before disposal of Bio-medical wastes they are collected in different colour coded containers. The colour coding and type of container for disposal of bio-Medical wastes are as follows.

Schedule-II

Colour coding and type of container for disposal of bio-medical wastes

Colour coding Schedule-I	*Type of container*	*Waste category*	*Treatment options as per*
Yellow	Plastic bag	Cat.1,2,3 and 6	Incineration / deep burial
Red	Disinfected container/ plastic bag	Cat. 3,6 and 7	Autoclave/ microwave/ Chemical treatment
Blue/ White translucent	Plastic bag/puncture proof container	Cat. 4 and 7	Autoclave/ microwave/ Chemical treatment and destruction/Shredding
Black	Plastic bag	Cat. 5,9 and 10 (solid)	Disposal in secured landfill

WHEN WE ENTER A LABORATORY

1. When the students enter into the laboratory it should be mandatory to use a white coat. According to the experiment extra apron may be worn.
2. Safety gears such as glasses, visors, surgical gloves etc should be worn.
3. The student should check the cleanliness of the working bench, all reagents are in proper place, burners and other equipments provided are in working order.
4. The student should ensure all glass wares provided are properly cleaned, rinsed with distilled water and dried.
5. The care should be taken to check the spillage of reagents on the bench or floor.
6. Any leaking bottles, leaking water taps and drainage pipes and leakage of gas should be immediately be reported to the supervisor.
7. Any abnormal odour of gas leakage, electrical burning, ammonia or any strong reagent which gives a pungent or obnoxious smell should be immediately be reported to the supervisor.
8. Student should be aware of availability of fire extinguisher device in the laboratory.
9. **Arrangement of chemicals**
   Chemicals are arranged in the laboratory as **bench reagent** or **side shelf reagent**.
   Bench reagents include the commonly used reagents including the dilute acids and alkalies.
   Side shelf reagents include the concentrated acids, alkalies and rarely used reagents.
10. **Arrangement of instruments**
    Like reagents some of the instruments are also arranged in the benches or on the side shelves.

On the bench the instruments arranged are-

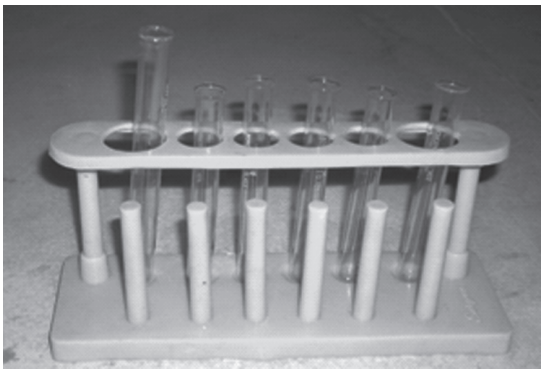
- Bunsen Burner
- Tripod stand
- Test tube holder
- Test tubes in stand
- Wash bottle
- Dispensing bottle
- Burette stand
- Pipettes in stand

On the side shelf the instruments arranged are-

- Centrifuge
- Colorimeter/ Spectrophotometer
- Hot air oven
- pH meter
- Water bath
- Vortex mixer
- Incubator
- Microscope

Pipettes

Micropipettes



Test tubes with stand

Bench reagents

SAMPLE COLLECTION

Different specimen collected for various biochemical analyses are whole blood, serum, plasma, urine, saliva, sweat, pleural fluid, CSF, peritoneal fluid, gastric juice, calculi etc. Out of these most commonly used sample is blood and urine.

Majority of the tests done in any biochemistry laboratory is on blood. Detail discussion of other specimen is out of the scope of this book.

Blood

Site: Blood can be obtained from veins, arteries or capillaries.

Mode of collection: Venipuncture or phlebotomy

Out of these mostly blood is collected by Venipuncture.

Venipuncture: Before collection of sample, patient's identity is verified.

- He is made to sit comfortably or asked to lie down at least for 20 minutes before collection.
- Most common vein is median cubital vein inside the bend of the elbow known as antecubital fossa.
- Clean the skin with 70% ethanol and allow to dry.
- Tie a tourniquet 4-6 inches above the site of puncture.
- Usually a 19-22 gauge needle is used.
- Insert the needle at about 15^0 to the plane of arm.
- When the needle enters the vein, gently pull the plunger and as blood appears release the tourniquet.
- When the desired amount of blood is

withdrawn, syringe is pulled out and a cotton pad soaked in spirit is placed at the puncture site and hold it firmly for a few minutes.

- If serum is required then blood is collected in test tube and allowed to clot .
- If plasma is required blood is transferred to a tube containing anticoagulant.

Anticoagulants

Various anticoagulants are used to preserve the volume of red cells. Gentle mixing with the anticoagulant is needed whenever used. Different anticoagulants used are- Heparin (Lithium heparin), EDTA (Ethylenediaminetetra-acetic acid), Sodium fluoride and potassium oxalate mixture (ratio of 1:3), sodium or potassium oxalate or their mixture, sodium citrate.

Sample Preservation, Storage and Transport

Always the fresh sample is the best sample. Immediately the sample should be delivered ideally. With passage of time the concentration of different constituents may fall. If delay is inevitable then serum is separated and kept at 4^0c or if longer periods it may be frozen. Sample for estimation of plasma glucose is collected in a mixture of sodium fluoride and potassium oxalate mixture which prevents glycolysis in the red cells. Sample for blood gas analysis is collected in heparinised syringe and the needle is bent and vacutainers are used to collect blood from patients having HIV or Hepatitis B infectious diseases. Sample for bilirubin should be kept away for light as UV radiation and daylight as they destroy bilirubin. Enzymes should be immediately assayed and haemolysed samples should be avoided. Serum for calcium estimation should be kept in glass.

Urine

There are three methods of collection of urine-

a. *Random collection*- collected at any point of time, suitable for few biochemical tests
b. *Timed collection*- collected at a specific phase of time
c. *24 hours collection*- Collected for full 24 hours after discarding the first urine sample

Preservation of Urine Sample

Usually acidification below pH 3.0 is done by adding hydrochloric acid (15 ml), boric acid (15 g) or glacial acetic acid. Thymol and chloroform also may be used for preservation. Sodium bicarbonate is used to preserve porphyrin and urobilinogen. Light sensitive compounds should be protected by collecting in amber coloured bottle or by covering with aluminum foil or dark coloured paper.

POCT- AN INTRODUCTION FOR MBBS STUDENTS

Quite often the physiscian is in need of clinico-pathological information on urine, excreta and other body fluids to establish a diagnosis or monitor the progress of the illness or treatment. Some of these information need to be available instantly at the time of clinical examination of the patient for correlation. In

earlier days the physicians looked at the colour of urine, pus or exuding fluids or odour of breath, sweat and feaces for gathering such information. From these quest evolved a battery of tests, such as reaction of urine towards litmus paper or testing for ketone bodies or sugar in urine. These tests are mostly biochemical tests which are constantly being developed and modified and began to be known as bed-side tests. As on today most of the biochemical investigation can be performed with ease and swiftness at patient bed-side or in the physician office under the name of "**POCT- Point Of Care Testing"** or "Alternative site" or "ancillary" or "off-site" testing.

Mostly these instruments or modalities were in vitro type but with advent of medical science many in vivo or minimally invasive devices have also been designed.

In vitro Devices

1. Single use, qualitative/ semi quantitative cartridge/ strip test
2. Single use quantitative cartridge/ strip tests with reader device
3. Multiple use quantitative cartridge/ bench top devices- multiple parameters assayed.

In vivo Devices

Used in continuous monitoring

Transcutaneous or closed loop models- Monitor blood glucose, pH, blood gases, electrolytes etc.

The principles involved in such devices are based on dry reagent chemistry, immunochemistry, molecular diagnostics or multi testing platform. *Some of the examples are—*

1. Reagent strips in the name of dipstick or Multistix either for single tests or multiple tests—done for ph, specific gravity, urinary glucose, protein, ketone bodies, nitrites etc.
2. Immunosensors— serum tested for cardiac Troponin T, H.pylori, and HCG for pregnancy test etc.
3. Monitoring devices—Most common example is a Glucometer. They can be hand –held, portable, remote operated or bench top devices measuring multiple parameters.
4. In vivo devices- The sensor is kept in a canula fed in an indwelling line in radial artery and calibration is done outside the body. They help in continuous monitoring of different parameters.
5. Non-invasive devices- no need to collect blood by Venipuncture or needle stick injury. These devices use newer techniques such as reverse iontophoresis, coupled to potentiometry, amperometry and optical methods. Examples are **Glucowatch** which measures glucose in interstitial fluid through transcutaneous route. Another is BiliCheck system which monitors the bilirubin level in newborns with hyperbilirubinemia.

These devices are used outside the central biochemistry laboratory in ICUs, wards, physician office, at home by the patients or less qualified paramedics. These are single step procedures, give a fast result, prove life-saving sometimes, portable and the result accuracy and precision are comparable to the central laboratory. High cost; quality assurance dependence on the manufacturer and operator capability, inadequate data

storage and lack of scope to store operator or patient ID limits their rampant use. But in critical health care situations where time is the crucial factor in spite their high cost the POCT devices prove as life saving equipments.

POCT DEVICE

GLUCOWATCH BIOGRAPHER