

Medical Biochemistry and Chemical Pathology

Introduction

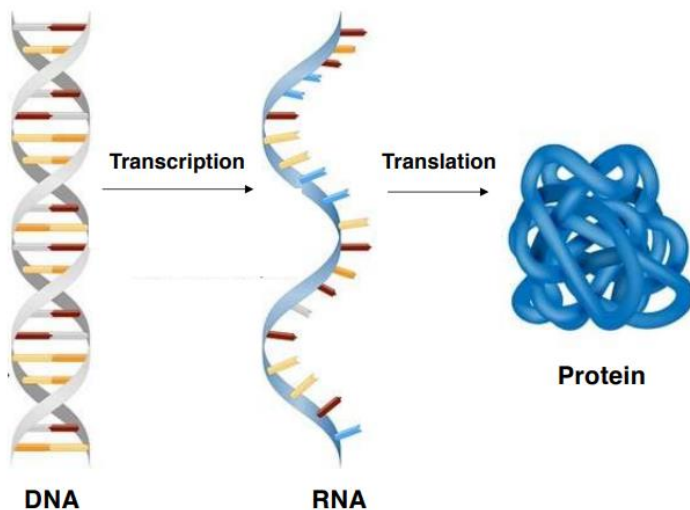
Biochemistry is a branch of science concerned with the chemical and physico-chemical processes and substances occurring in living organisms. Biochemistry generally studies the molecular nature of biomolecules while clinical biochemistry focuses on the clinical significance of these biomolecules and physicochemical processes.

The Central dogma

Each of us has enough DNA to reach from here to the sun and back, more than 300 times. However, this DNA needs to fit inside the nucleus (and inside the cell). The DNA is wound around groups of proteins called histones – these then complex to form nucleosomes. Nucleosomes fold up condensing the structure even more and the final condensed form of DNA is a chromosome (in the way we typically draw it and think of it).

The central dogma simply describes how genetic information flows from DNA to a protein

- Transcription: DNA is transcribed into RNA
- Translation: RNA is translated into protein



What does it mean to “express” a gene? -Expression just means that whatever that gene codes for is being made (the gene product is being produced).

Why is gene expression important? • The genes that a cell expresses dictate what proteins that cell will make. The proteins a given cell makes determine the cell's identity and functionality, (i.e. whether it's a muscle cell, a neuron, a liver cell, an intestinal cell, etc.)

Do all of our cells have the same DNA? We have many different cell types in our body and each cell (**for the most part) contains the same genetic information – i.e., the same set of genes and the same DNA. A muscle cell, a neuron, and an intestinal cell all make different proteins and look/do very different things but, if you looked inside each one, you would find the same set of genes (the same DNA).

How do we get different cell types then? Differential gene expression. All cells have the same set of genes/genetic information, but different cells express different genes (turn different genes “on”) which in turn produce different proteins and result in cells that look different and do different things.

What is the role of Biochemistry in Medicine

The aims, attitudes and techniques of biochemistry are as relevant to medicine or to any aspect of biology.

- To understand the true nature of diseases. All diseases have a biochemical basis.
- To give patients the necessary or appropriate dietary advice to prevent disease.
- Special relevance to medicine is areas of blood coagulation and effect of drugs and other injected substances on tissue and cells.
- To diagnose and manage disease through the analysis of blood, urine, and other body fluids.
- To carry out complex analyses on specimens of body fluids and tissues

How does a basic biochemistry laboratory look like?

All biochemical procedures and tests require special equipment in addition to basic laboratory equipment since biochemical processes take place at the cellular level. A typical biochemistry laboratory includes work benches for use by the laboratory personnel, equipment like spectrometers, microscopes, DNA sequencers, imagers, chromatographs, computers, and electrophoresis equipment along with tools and reagents for manipulation of samples.

The laboratory also has Biosafety cabinets, fume hoods and isolation boxes to protect people from hazardous substances. Storage space and specially equipped facilities like storage rooms are also necessary.

In schools especially universities, the Biochemistry laboratory is used for instruction with students obliged to spend time working on projects and getting hands-on experience while in scientific institutions, the labs are required for research and analysis of samples from suspect pathogens and even new species of organisms.

However, some Bch labs especially in a hospital setting are responsible for handling samples from patients with medical problems, analyzing these samples in the detection-diagnosis-treatment processes and interpreting the results.

The various biological samples subjected to chemical and biochemical tests include blood, urine, CSF, stool, ascetic fluid, pleural fluid, calculi, and tissues.

General safety precautions in a biochemistry laboratory

The laboratory premise should be structurally sound with a reliable water supply and the drainage from sink must be close to septic tank.

- The overall size of the lab should be appropriate for the workload, staff number, storage and equipment.
- The floor should be well constructed with a surface that is not slippery, impermeable to liquids and resistant to the chemicals used in the lab.
- The Bench surfaces should be without crack, impervious, washable, and resistant to disinfectants and chemicals used in the lab.
- Suitable storage facility should be available including a ventilated lock store for storage of chemical and expensive equipment.
- Safe electricity supply with sufficient wall electric points
- Provision of protective safety cabinets and fume cupboards as required.
- Along with fire extinguishers, fire alarms, several buckets of sand and fire blanket are also required. There should be emergency fire exits, emergency shower and eyewash stations in the laboratory.

There are different classes of fire extinguishers and they're suitable for different classes of fires;

i. Class A - Suitable for paper, wood & textiles.

Type of fire extinguisher - Water, Foam, Dry Powder, Wet Chemical

ii. Class B - Suitable for flammable liquids.

Type of fire extinguisher - Foam, Dry Powder, Carbon Dioxide (CO₂)

iii. Class C - Suitable for flammable gasses.

Type of fire extinguisher - Dry Powder

iv. Class F - Suitable for cooking oil and fat.

Type of fire extinguisher - Wet Chemical

v. Electrical Risk - Suitable for electrical equipment.

Type of fire extinguisher - Dry Powder, Carbon Dioxide (CO₂) Fire Extinguisher Use:

Use **PASS** to handle and use the fire extinguisher:

Pull – pull the pin off the handle.

Aim – aim at the base of a fire, not the flames.

Squeeze – squeeze the handle to engage spray.

Sweep – sweep the nozzle left and right.

Only use the fire extinguisher if you feel comfortable.

LEAVE if situation becomes unsafe.

- Equipment which use gases should be checked frequently for leakages.
- Avoid pipetting with the mouth especially chemical substances.
- Proper hand hygiene before and after handling samples
- Walking barefoot and wearing open shoes are prohibited.
- Eating, chewing gum, drinking, smoking, and applying cosmetics are strictly prohibited.
- Wearing safety and protective like laboratory coats, gloves and eyewear to prevent spills, and proper care taken in handling toxic chemicals and radiation.
- Follow safety protocol and procedures provided by the laboratory.

Waste treatment and handling

Laboratory wastes usually consists of used, broken equipment which either contain or may favor growth of pathogens especially as laboratory tests are performed on people who are ill.

Infectious laboratory waste is characterized principally as waste that contains microorganisms capable of causing infection in a healthy, susceptible host. For laboratory waste to cause infection, six essential factors must be present. These factors are as follows:

- The presence of an infectious agent that is capable of invading and multiplying within a human host.
- An environment for the infectious agent that functions as a reservoir, allowing it to survive and, perhaps, to multiply.
- A mechanism for the agent to escape from the reservoir.
- A mode of transmission from the reservoir to a human host.
- A means for the agent to invade, penetrate, or enter a human host.
- A human host that is susceptible to infection by the agent

The primary responsibility for the safe handling and disposal of infectious waste resides with the generator of the waste. The major problem associated with infectious waste is the potential for occupational exposure. The disposal of infectious waste should, therefore, be performed in an effective manner that minimizes the potential for exposure of those who, by virtue of their employment, must handle the material.

Infectious waste is often decontaminated using chemicals such as chlorine bleach, phenolic disinfectants etc at the generating facility prior to its transport to a disposal site. Decontamination protects the waste transporter from the risk of infection.

Every laboratory must develop a waste management plan, such that waste is segregated and disposed properly. Laboratory waste needs to be segregated such that infectious wastes is separated from other types of waste. By so doing, the infectious wastes can be decontaminated to a certain level or packaged appropriately.

Infectious waste containers serve as primary barriers to protect the worker and to minimize the chance of environmental contamination. Typically, these containers are made from leak-resistant paper or cardboard, stainless steel, or temperature-resistant polymers.

- Solid waste can be packaged safely in sturdy bags or boxes.
- Flat trays with sealable lids are suitable for containing pipettes and other laboratory supplies during decontamination.
- Bulk liquids may be collected in leak-proof containers, decontaminated, and then safely discharged into the sewer system.
- Rigid, puncture-resistant, sealable containers are necessary for packaging "sharps," e.g., broken glass, brittle plasticware, needles, and scalpel blades.
- Wet waste should be packaged with sufficient absorbent materials to contain residual liquids and to minimize leakage. In packaging wet materials for transport, it is prudent to double-bag the waste, sealing each bag independently.
- Heavy waste such as anatomical specimens, animal bedding, and laboratory specimens need to be placed in rigid containers. Care must be taken that the weight of the waste load does not exceed the burst strength of the container.

The **physical properties** of the **container** should be compatible with the treatment process.

-Waste placed in stainless steel pans, waxed-lined paper bags, tempered glass, and heat-resistant plastics can all be safely processed in an autoclave.

-Metal containers have been shown to enhance the transfer of heat to the waste load during autoclaving, whereas containers made of plastic retard steam penetration.

-Most chemical disinfectants have no appreciable effect on high-strength plastics at room temperature but may be corrosive to metals.

-Liquid infectious waste is often stored in plastic carboys designed for chemical disinfection.

Cleaning the containers

-Metal receptacles can be autoclaved and recycled but are not suitable for incineration.

-Cleaning containers that are to be reused is labor intensive and increases the risk of occupational injuries and exposures to biohazards.

-Personal protection via the use of gloves and laboratory coats are recommended for all laboratory waste handling and manipulation.

Laboratory activities with a high probability of contamination caused by spills of infectious fluids, or the production of droplets, should be performed on plastic-backed absorbent bench paper.

Workers who process infectious waste in an autoclave should wear a rubber apron, sturdy shoes, asbestos-free heat-resistant gloves, and a face shield, to protect against accidents that may occur while loading or unloading the autoclave.

Steam autoclaving usually is the method of choice for decontaminating cultures, laboratory glassware, pipettes, syringes, or other small items known to be contaminated with infectious agents.

Incineration is the method of choice for treating large volumes of infectious waste, animal carcasses, and contaminated bedding materials.

Needles and other penetrating items such as surgical blades, pipettes, broken glass, and laboratory instrument sampling probes pose a physical hazard to laboratory and support service personnel and, if used to process infectious materials, may transmit infection.

All disposable "sharps" should be placed in a prominently labeled, leak- and puncture-resistant container that is consistent with the institution's waste management plan.

First aid in the laboratory

- Injuries caused by broken glass: Wash the wound immediately to remove any glass pieces. Apply mercurochrome or acriflavine ointment to the wound. Cover with gauze and adhesive tape
- Acid/Alkali splashes on the skin: Wash thoroughly; bath the affected skin with cotton wool soaked in 5% aqueous sodium carbonate if acid and 5% acetic acid or undiluted vinegar if alkali.
- Acid/Alkali splashes in the eye: Water spray from a wash bottle or rubber bulb into the medial corner of the eye. Put 4 drops of 2% Aqueous Sodium bicarbonate into the eye, if acid, and saturated solution of boric acid, if alkali.
- Swallowing acid: Make the patient drink some 5% soap solution immediately. Make him gargle with the soap solution. Give him 3 or 4 glasses of ordinary water. If the lips and tongue are burned by the acid, rinse thoroughly with water. Bathe with 2% aqueous sodium bicarbonate.
- Swallowing alkali: Make the patient drink 5% solution of acetic acid or lemon juice or dilute vinegar. Make him gargle with the same acid solution. Give him 3 or 4 glasses of ordinary water. If the lips and tongue are burned by the alkali, rinse thoroughly with water; bathe with 5% acetic acid.
- Poisoning: Send for a physician or qualified nurse, specifying the toxic substance involved. Place the victim in the open air while waiting for the physician.
- Burns caused by heat: They fall into two categories -**Severe burns**: If the victim is on fire, roll him in a blanket or overall to smoothen the flames. Inform the physician. Lay the victim on the ground. Do not remove his clothing. Cover him if he is cold. Do not apply any treatment to the burns. This must be left to the physician. -**Minor burns**: Plunge the affected part into cold water or ice-water to soothe the pain. Apply Mercurochrome or Acriflavine ointment to the burn. Apply dry gauze dressing loosely. If the burn becomes infected or does not heal, refer the patient to a physician. Never tear off the blisters that form over the burns.

Electrical hazards and its management

Electrical equipment should not be handled with wet hands, nor should electrical equipment be used after liquid has been spilled on it. The equipment must be turned off immediately and dried thoroughly.

In case of a wet or malfunctioning electrical instrument, the plug should be pulled and a note of caution should be left on the instrument. Use of extension cords is prohibited.

Bodily damage by electric shock: The symptoms are fainting and asphyxia (oxygen deprivation). Before doing anything else, put off the main switch. Send for a physician. Begin giving mouth to mouth respiration immediately.

Fire hazard and its management

Fire in the laboratory may occur due to spirit lamps, electrical appliances or other flammable reagents used in a laboratory.

All laboratories should have a fire extinguisher and easy access to safety showers and fire blankets.

For putting off the flames from the inflammable liquids, smother the fire by throwing sand over it.

Biohazards in the laboratory

This can be infectious agents themselves or items (solutions, specimens, or objects) contaminated with anything that can cause disease in humans regardless of its source.

Universal Precautions generally mandate that clinical laboratories treat all human blood and other potentially infectious materials as if they were known to contain infectious pathogens.

The specifications apply to all specimens of blood, serum, plasma, blood products, vaginal secretions, semen, cerebrospinal fluid, synovial fluid and concentrated HBV or HIV viruses. Universal precautions also advise the laboratory safety precautions listed earlier.

In case of wounds caused by broken glassware containing stools, pus, etc., wash the wound immediately with antiseptic lotion. Check whether the cut is bleeding. If not, squeeze hard to make it bleed for several minutes. 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 spit it out immediately. Use a disinfectant (e.g., Diluted Dettol) for mouth washing.

If the infected material has been swallowed accidentally, forced vomiting is to be done. Ascertain the kind of infection and take advice from medical personnel.

Laboratory Ethics

Ethical practice can be defined as good technical practice accompanied by proper attitudes and behavior. In deciding what is proper, reference is often made to moral values voluntarily adhered to within the community and to standards stipulated in various codes of professional practice.

The principle of ethics of doing 'good' and causing no 'harm' is the essence of every medical code. The principles of working in the laboratory are also derived from this code and other codes. In recent times, as an aid to decision making in medicine and as starting point for discussions on medical ethics, four principles have been agreed as fundamental.

Autonomy: the right of patients to make their own decisions based on informed consent.

Beneficence: the duty or obligation to act in the best interest of the patient

Non-maleficence: the duty or obligation not to cause harm to the patient.

Justice, which involves fairness and giving what is rightfully due.

Medical laboratory practitioners are expected to comply with the laws and regulations pertaining to their professional activities. Infact, there are three main groups to which they owe responsibility.

1. Patients: Medical laboratory professionals are accountable for the quality and integrity of services provided. This includes maintaining individual competence and trying to protect the patient from incompetent or illegal practices by others.
2. Colleagues and profession: They should strive to uphold dignity and respect of the profession. Maintain a reputation for honesty, integrity and reliability. MLP should aim to contribute to the advancement of the profession by improving the body of scientific knowledge, promoting high standards of education and practice. Collaborating with colleagues and other health professionals where necessary.
3. Society: MLP have a responsibility of contributing to the general wellbeing of the society.

In this light, the laboratory has the following responsibilities

1. Collection of information- the laboratory must collect sufficient and adequate information to identify patients and patient samples. The patient should be aware of the information and the purpose for which it is collected.
2. Collection of specimens- all procedures of specimen collection require an informed consent especially for highly invasive procedures. And in a case where the patient is incompetent due to age or mental illness, an authorized person or parent could give consent.
3. Performance of tests- all laboratory work and tests must be carried out with high-level skill and competence. The tests are performed to the standards set by regulatory authorities.
4. Reporting of results- the results of a patient's test are confidential except disclosure is authorized and so they are normally reported only to the clinician who ordered the test. The laboratory is also responsible for making sure the turnaround time for the test results is reasonable considering the type of test and the patient's condition.

5. Storage and retention of medical records- laboratory must ensure information is stored so that there is reasonable safeguard against loss, unauthorized access, tampering or other misuse. Laboratories should also develop their protocol on how long different results, specimens and slides would be kept.
6. Access to medical records- normally available only to the Clinician who ordered the test, the patient, the laboratory and hospital staff if they are required for management of the patient and other authorized individuals.

Collection of specimens for use in the clinical chemistry laboratory

Laboratory test results are dependent on the quality of the specimen submitted. It is important that all specimens be properly labeled with the name of the patient, collection date, and the origin (source) of the specimen, when applicable. The amount of specimen collected depends on the number of tests to be carried out but collecting sufficient amount of sample is advised.

Some tests can be performed on more than one type of sample, however the sample used for testing is often determined by **purpose** of that test for example a blood glucose test will help diagnose diabetes and monitor the blood glucose levels in diabetics while urine glucose is one of the substances tested when a urinalysis test is performed to check for UTIs or when kidney disorder is suspected.

Blood collection- Most laboratory tests are performed on anticoagulated whole blood, plasma, or serum.

Plasma for Routine Chemistries (Preferred): The concentrations of most analytes measured, are equal in plasma and serum. However, the concentrations of potassium, LDH, total protein and phosphorus are well documented to be different in plasma and serum. Because plasma represents the more physiologic fluid (i.e., coagulation has not occurred) and because these are very commonly ordered tests, plasma is the preferred specimen for most chemistry tests.

Phosphorus averages 0.3 mg/dL higher in serum than in plasma due to the release of phosphorus during coagulation while total protein is approximately 0.3 mg/dL lower in serum than in plasma due to fibrinogen consumption during coagulation. For potassium, concentrations average 0.4 mmol/L higher in serum than in plasma due to the release of potassium from platelets during coagulation, but this difference can range from 0.0 mmol/L to 1.4 mmol/L in different patients.

The difference between plasma and serum plasma concentrations has been shown to directly correlate with platelet count. Thus, the primary clinical reason for plasma being the preferred specimen for chemistry testing is to assure a physiologic and accurate potassium value.

The type of anticoagulant in plasma collection tubes is also vital as anticoagulants like heparin will chelate minerals like calcium, citrate dilutes the sample and so these will interfere with the results.

Plasma also provides the advantage of faster turnaround time as specimens can be centrifuged immediately upon receipt rather than waiting for serum to form which can be lengthy in patients receiving anticoagulant therapy.

Blood gases: blood must be collected anaerobically and into a stoppered tube (to prevent CO₂ escaping) and placed on ice to prevent lactic acid production.

Peptide hormones are susceptible to protease degradation and require addition of protease inhibitors.

Whole blood samples can be stored at 4-8°C for up to 24 hours before the serum can be separated but it must not be frozen.

Cerebrospinal fluid- it is usually collected into numbered plastic tubes. CSF is usually obtained for gram stain, cell count, protein, glucose, and aerobic culture. It is obtained by lumbar puncture often called a spinal tap. It is performed while the person is lying on their side in a curled up, fetal position or sometimes in a sitting position. The back is cleaned, and a local anesthetic is injected under the skin. A special needle is inserted through the skin, between the two vertebrae and into the spinal canal. After collection, a sterile dressing and pressure is applied to the incision site and the patient is asked to lie quietly in a flat position without lifting their head for one or more hours to avoid potential post-test spinal headache.

Other fluids like peritoneal fluid, pleural fluid and pericardial fluid are collected using similar procedures to CSF collection.

The preferred order of collection of CSF for testing is:

1. Tube #1: Chemistry (and Immunology)
2. Tube #2: Microbiology
3. Tube #3: Hematology
4. Tube #4: Specimen control where specimen can be stored for 30 days at -80°C unless 4°C or -20°C is needed for a particular test

Tissue Biopsy- could be obtained from a number of different body sites like breast, lung, skin etc. Needle biopsy involves inserting a needle into the site and obtaining cells and/or fluid. An excisional biopsy is a minor surgical procedure in which an excision is made and a portion or all of the tissue is cut from the site.

Urine Collection: collection is done in leak-tight containers and properly labelled. Random or 24-hour urine collections usually need a special bottle containing the correct additive. Azide or toluene are often used to prevent bacterial growth. For urine Ca²⁺, Mg²⁺ and phosphate (Pi) the bottle

must contain acid (HCl) since Ca^{2+} and Mg^{2+} form an insoluble precipitate with Pi at alkaline pH.

Urine samples can be sealed in plastic bag and stored in refrigerator at around 4°C for up to 24 hours.

Sputum- the patient is instructed to cough from as far down in the lungs as possible. It is best accomplished first thing in the morning before eating or drinking, by taking several deep breaths before coughing into the collection cup. Sputum should be relatively thick and not as watery as saliva.

Stool- patients collect these samples themselves following instructions to prevent contamination from other materials in the toilet. Remember to wash hands well after collecting the specimen.

Semen- male patients ejaculate into a specimen container avoiding lubricants, condoms or any other potential contaminating material. The specimen must not be refrigerated but kept as close to body temperature as possible and delivered to the laboratory within 60 minutes.

Saliva- could be obtained via swab or if a larger volume is required the patients spits into the container without generating sputum.

Oral fluid- this is saliva plus oral mucosal transudate. A special device is used to swab around their outer gums.

Sweat- collected using a special sweat stimulation procedure that is painless and allows sweat to be collected into a plastic coil of tubing or a piece of gauze or filter paper. Usually for analysis of amount of chloride in sweat.

Some samples like throat, nasal, vaginal and superficial wound cultures are generally obtained by simply running a swab over the affected area.

Post office regulation in posting pathological specimens.

Specimens to be transported must be properly packaged and sealed. It is extremely important to close the specimen containers tightly and securely ensuring that the seal is not broken to avoid leakages. There are several stages to packaging the samples.

- i. Primary packaging- the sample is contained in a primary receptacle like blood tubes and other sample containers. The container must be leak proof, appropriate for the sample and tightly sealed.
- ii. Secondary packaging- where the container is placed in water tight and leak proof secondary container. An example is a round leak proof mailing container or plastic leak

proof mailing bag. Absorbent material must be placed between the primary and



secondary container.

- iii. Outer packaging- the secondary mailing container must be placed in an outer packaging. Either the secondary container or outer packaging must be rigid. The minimum dimensions on the two sides must be 100mm x 100mm. there should be



cushioning between the secondary and outer packaging.

The outer package must be labelled to include the name and address of the sender including relevant telephone numbers in case of emergency. The name and address of the receiving laboratory must also be included.

Large boxes may be used for deliveries by the couriers and the boxes must be made of smooth impervious material such as plastic or metal which can easily be disinfected or cleaned. The boxes should be secured with a fastenable lid and retain liquids in the event of spillage. It should be clearly labeled with 'Biological substance, Category B' with identity of sender plus relevant telephone numbers in case of emergency.



Anticoagulants

Anticoagulants are the Chemical substances that prevent the blood from clotting when mixed with inappropriate concentration with the Blood Specimen.

Some of these agents are used to treat thrombotic and thromboembolic diseases such as Stroke, Myocardial Infarction, and Deep Vein Thrombosis.

- **Thrombus:** A Thrombus is a fibrinous clot that forms in and obstructs the blood vessel, or that forms in one of the Chambers of the heart (It stays in one place).
- **Embolism:** An Embolism is a mass, such as an air bubble, a detached blood clot, or a foreign body, that travels to the bloodstream and lodges so as to obstruct a blood vessel (it is a mobile thrombus).

Anticoagulant is used in the hematological laboratory where whole blood or plasma is required which depends upon the tests to be done and then the type of anticoagulant is decided. Most of the anticoagulants prevent the clotting by removing calcium or iron which is necessary for clotting process. Every anticoagulant is added in fixed proportion to blood.

Working principle of anticoagulants

Most of the anticoagulant commonly used acts by removing the calcium ions present in the blood which is required for coagulation process. The anticoagulant binds with the calcium and thus prevent blood from clotting. Also, some anticoagulants are used for specific assays due to their unique properties such as fluoride which inhibits the activity of Phosphorylase enzymes and provides precise results in Blood Sugar Estimation and Citrate in specific concentrations used for the Coagulation studies etc.

The following Anticoagulants are most commonly used in almost every laboratory for routine test Assays –

EDTA- Ethylene diamine tetra acetic acid

It is widely used chemical anticoagulant in the laboratory. EDTA is colorless, water soluble and acts as a chelating agent that is it has the ability to bind to metal ions like Calcium. It is used in the concentration of 1.25 to 1.75 milligram per ml of blood.

Advantages of EDTA

It gives better preservation to the cellular morphology of blood cells when observed even after 3 hours of blood collection.

It can be used for platelets counting as it inhibits the clumping of platelets.

Disadvantages of EDTA

Excess of EDTA in the blood may lead to shrinkage of RBCs & WBCs. It may cause degenerative changes in the blood cells.

It activates naturally occurring anti-platelet auto-antibodies which cause the platelet adherence to Neutrophils.

Uses of EDTA

Following tests are commonly done by using EDTA as an anticoagulant –

- Complete Blood Count (CBC)
- Hemoglobin estimation
- Hematocrit or Packed Cell Volume estimation
- ESR by wintrobe's method
- HbA1C test
- Platelet count
- Red cell Indices
- Differential Leukocyte Count

Tri-Sodium Citrate

It was first used as an anticoagulant in blood transfusion in the earlier 20th century. Its usefulness continues till today in blood collection tubes for the ESR and Coagulation studies as well as for the preservation of blood in blood banks.

Mechanism of Action

The action of Citrate ions is to form calcium citrate complexes which disrupt the blood clotting mechanism by chelating or binding with the calcium ion in the blood and inhibits the coagulation of blood.

Preparation of Tri-Sodium Citrate

3.8% solution of Tri-sodium citrate is commonly used which can be easily prepared in laboratories as follows:

- Tri-sodium Citrate – 3.8gm
- Distilled Water – 100ml

Dissolve the Tri-Sodium Citrate in Distilled water and mix well. 0.4ml of this anticoagulant is sufficient for the 2ml of blood.

Uses of Tri-Sodium Citrate

- ESR estimation by Westergren Method – for 1 volume of citrate, 4 volume of blood is added.
- Coagulation studies – for 1 volume of citrate, 9 volume of blood is added.

Citrated blood cannot be used for Packed Cell Volume (PCV), Hemoglobin (Hb) Estimation, Total Leukocyte Count TLC, and Differential Leukocyte Count (DLC) because citrate is used as a solution and it alters the concentration of blood.

Oxalates

They can be used as Single oxalates such as Sodium Oxalate or Potassium Oxalate or Ammonium oxalate but are commonly used as Double Oxalates because when used alone for example, Potassium oxalate, when used at a concentration of 2mg/ml of blood causes the Shrinkage of Red Blood Cells (RBCs) whereas the Ammonium oxalate may cause the Swelling of Red blood cells when used at concentration of 2mg/ml.

Mechanism of Action

It acts as a chelating agent and binds with the calcium ions present in the blood and forms insoluble precipitates of Calcium Oxalates.

Preparation of Double Oxalate

The double oxalate consist of

- Ammonium Oxalate – 1.2 mg (3 parts)
- Potassium Oxalate – 0.8 mg (2 parts)
- Water – 100 ml

It is prepared as the 20 mg/ml concentrated oxalate solution that means 2mg/0.1 ml of oxalate. 0.5 ml of this solution is poured into the container and dries, which is sufficient for 5 ml of blood.

Remember that the Potassium oxalate and Ammonium Oxalate should be used in a ratio 2:3 and at a concentration of 2mg/ml of blood.

Uses of Oxalates

It can be used for the Blood chemistry, Packed cell volume (PCV), Erythrocyte Sedimentation Rate (ESR), Total Leukocyte Count (TLC), Specific gravity etc.

Advantages of Double oxalates

Double oxalate is preferred as it prevents the swelling effect of Ammonium oxalate & shrinking effect of Potassium oxalate on the RBCs.

Disadvantages of Oxalates

The morphology of the White Blood Cells (WBCs) is not preserved well so it is not useful for making Peripheral Blood Smear.

The Calcium Oxalate precipitate that forms in the blood is harmful as it is a toxic agent & it is not used as a preservative in blood banks.

Sodium Fluoride

It is the anticoagulant of choice for the estimation of blood sugar. It binds with the calcium ions present in the blood and forms Calcium fluoride. Also, it is the competitive inhibitor of Phosphorylase enzymes and prevents glycolysis by blocking its activity.

Preparation of Sodium Fluoride

It is commonly used at the concentration of 6mg/ml of blood and can easily be prepared in the laboratory as follows –

- Sodium Fluoride – 3gm
- Distilled water – 100 ml

Dissolve the content and mix well. It gives the solution at a concentration of 30mg/ml. 1 ml of this solution is poured into a container and allowed to dry, which is sufficient for 5 ml of blood as 6mg/ml is used.

Uses of Sodium Fluoride

It is commonly used for the estimation of Blood Sugar and other biochemical tests.

Biological / Natural Anticoagulant – Heparin

Heparin is a natural anticoagulant which cannot be prepared in the laboratory. It is obtained from the leech. It is a good anticoagulant and well preserves the morphology of the Red Blood Cells (RBCs).

It is used in the concentration of 10-15 units equivalent to 0.1-0.2 mg/ml of Blood. The anticoagulant is first sterilized and then taken in the container or blood collection vial, afterward blood is added to it in an appropriate concentration, mixed gently by inverting it 10-15 times or shaking it gently.

Heparin inhibits the thromboplastin formation and destroys the thrombin (Anti-thrombin activity) and hence disrupts the clotting mechanism.

The heparinized blood specimen is commonly **used** to determine the blood gases especially the Arterial Blood Gas Analysis. It can be used for Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Osmotic Fragility Test (OFT), Immunophenotyping and other Hematological tests.

Cleaning and storage of glasswares

It is generally good practice to rinse glassware with tap water as soon as possible after use. When material is left to dry on glassware surfaces, it is much more difficult to remove.

Water-soluble substances are typically removed by soaking or scrubbing with a 2% phosphate-free laboratory detergent solution. The detergent is removed by rinsing with warm tap water.

Acid-soluble residue is removed by soaking or rinsing with a 10% or 25% (v/v) hydrochloric acid solution followed by a tap water rinse. The glassware is then rinsed at least three times with distilled deionized water. Special cleaning procedures for volumetric glassware, glassware used in trace element work, and difficult to clean glassware are also included.

The methods discussed here pertain to most commonly used laboratory glassware and routine laboratory analysis. The actual cleaning procedures used should be tailored to the substances that are to be removed, the determinations to be made, and to the type of glassware.

GENERAL CLEANING

-As mentioned before, it is good to rinse glassware with tap water immediately after use, even if it will be properly cleaned later. Remove all markings, tape, etc. from glassware prior to cleaning. Scrape off thick deposits, dirt, adhesive, etc. with knife, scoop, or razor blade. Use a wipe or towel soaked in acetone or ethanol to remove ink.

-Completely immerse object in working (2%) detergent solution. Remove air bubbles to ensure total contact between surfaces and solution. Larger objects may be cleaned by applying detergent solution from a squeeze bottle and using a brush.

An alternative method of cleaning larger objects is to use 1-2 pumps of working solution from the pump bottle next to the sink, filling the large object with tap water, and allowing to soak.

Soaking times vary depending on the contaminants to be removed. In general, most glassware can be cleaned by soaking for 1 to 24 hours. Avoid longer soaking times, as it may cause etching, removal of painted markings, and formation of detergent residues.

-Rinse object immediately and thoroughly after removal from detergent solution using warm tap water.

For chloride determination, glassware can be cleaned using only the detergent soaking followed by deionized water rinses.

-The acid rinse step is to be used for removing acid-soluble contaminants from glassware. It should not be used for glassware that will be used for chloride determinations. The only pieces of glassware that should be routinely acid rinsed are the 125 mL Erlenmeyer flasks. If in doubt, however, it is best to employ the acid rinse as a precaution.

-Rinse or soak glassware with a 10% (v/v) hydrochloric acid solution immediately after rinsing detergent solution off with warm tap water. Soak glassware in acid bath for 20 minutes. Be sure all traces of detergent are thoroughly removed prior to using acid. Use a plastic wash bottle for applying acid and collect used acid in a container for disposal. Do not allow acid solutions to drain into any sink in the building. It is most convenient and safest to perform acid rinsing in or near the acid sink (Rm 205) only.

-For the glassware to be used in trace metal determinations, use a 20% (v/v) nitric acid solution for rinsing. Keep this glassware separate, however, and do not use glassware cleaned with nitric acid for any other purposes.

-Rinse acid from glassware using warm tap water.

- Final rinsing is accomplished with distilled deionized water. Rinse glassware with DDW at least 4 times immediately after the tap water rinse. Use a large wash bottle for rapid rinsing. Clean glassware is indicated when water drains uniformly in a thin film from the surface. Water droplets indicate glassware is not completely cleaned and must be rewashed. It is not necessary to completely fill container with DDW, rather fill to ~10% capacity, shake, and empty.

-Clean glassware can be air-dried by inverting on a rack or other clean surface so that water may drain and air will circulate. Be sure that air-dried glassware does not become contaminated with dust or other air-borne materials. Return clean and dried glassware to the appropriate storage location. Do not leave dried glassware in a drying area indefinitely.

- Volumetric flasks can be cleaned using the detergent washing and distilled deionized water rinsing procedures described above.

The inside of the flasks can be soaked by putting 1-2 pumps of working solution in first with the pump bottle next to the sink before filling with tap water. Follow soaking times as if you were soaking the flask inside a Citronox bath. Vigorous shaking of the detergent solution should aid in cleaning the inside of the flask. Acid rinses may be used if the solutions prepared are likely to resist detergent cleaning. Do not allow alkaline solutions to be stored for long periods of time in volumetric flasks as they may become damaged. After cleaning a volumetric flask, fill with deionized water before returning it to storage. Discard water before using flask again.

-Rinse volumetric pipettes immediately after use and place in a pipette storage jar filled with 2% Citronox acid cleaning solution. When full, the basket of the storage jar is transferred to a pipette washer/rinser. Cycle warm tap water through the pipette washer approximately 10-12 times. After the detergent has been completely rinsed from the washer, drain tap water from the washer and fill with distilled water. Raise and lower the basket several times to cause agitation and flush out the inside of the pipettes.

-Allow pipettes to soak in distilled water for 10-15 minutes. Drain washer and refill with fresh distilled water. Raise and lower basket, agitating and flushing out the inside of the pipettes. Repeat distilled water flush at least 3 times, allowing the pipettes to soak in distilled water between cycles. After the last rinse cycle, remove basket and drain excess water. Remove each

pipette from the storage basket and rinse briefly with distilled deionized water using a wash bottle. Transfer to drying rack with tips up. Fill pipette storage jar with fresh 2% Citronox acid detergent before returning to the emptied basket.

NOCHROMIX (OR DICHROMATE) SOLUTION FOR RESISTANT RESIDUES

-Stubborn contaminants may be removed using a sodium dichromate cleaning solution or a commercial equivalent formulation such as Nochromix.

Use the chromic acid substitute when available. This cleaner is prepared from concentrated sulfuric acid and should be handled with the utmost caution.

Treat glassware in fume hood and wear eye protection, gloves, and apron. Nochromix (or dichromate) should only be used when nothing else will remove the contaminants.

Remove excess water from the object to be cleaned, as water will diminish the effectiveness of this cleaning solution.

Allow surface to soak for at least 15 minutes in the acid cleaner.

Remove and flush thoroughly with warm tap water. Proceed to distilled deionized water rinse. Return cleaner to container for reuse.

Dispose used Nochromix in acid sink after neutralization and follow it with abundant amounts of tap water.

GLASSWARE STORAGE

All laboratory users are responsible for returning cleaned and dried glassware to the storage location where it was obtained. The cleaning process is not complete until the items are returned. If the glassware was cleaned for a particular purpose, it should be noted and stored separately from other glassware.