Reading Material for
Medical Lab. Technician
(Clinical Pathology & Serology)

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PREFACE

This book is for the students of two years post matric teaching program of Medical Laboratory Technicians (students of Allied Health Sciences). The purpose of this reading material is to provide basic education to the paramedics about Clinical Pathology and Serology. This reading material attempts to cover almost all the basic theoretical knowledge required by students about Clinical Pathology and Serology so that they can perform their work better in Clinical Pathology and Serology laboratory.

Contents

SECTION 1

Chapter One

Introduction to Medical Laboratory Technology

Objectives

The MLT Program strives to teach the students: the knowledge, skills and professional attitude necessary to accurately and proficiently perform and evaluate clinical laboratory analyses. To provide sufficient technical information for the student to understand analytical processes, interpret analytical results and appreciate the clinical significance of analyses performed in a modern clinical laboratory.

CLINICAL PATHOLOGY

Health care delivery system involves different personnel and specialties and the caregiver must have an understanding of diagnostic evaluation. Basically, laboratory and diagnostic tests are not therapeutic but along with a history and physical examination, these tests can confirm a diagnosis or provide valuable information about a patient status and response to therapy. In addition to these, laboratory findings are useful for epidemiology and research purposes.

To effectively use the laboratory service, for disease prevention and to provide health care, every member of its work force need to:

• Understand the role of the laboratory and its contribution to the nation's health service;
• Appreciate the need to involve all members in the provision of health service;
• Follow professional ethics and code of conduct;
• Experience the job satisfaction and should have professional loyalty.

1.1 A Medical Laboratory

Overview

Medical laboratory is a place which is equipped with various biomedical instruments, equipments, materials and reagents (chemicals) for performing different laboratory investigations by using biological specimens (whole blood, serum, plasma, urine, stool, etc).

1.2 Types of Laboratories.

1.2.1 LEVEL 1 Laboratory:-

Basic laboratory level I is the simplest kind and adequate for work with organisms which have low risk to the individual laboratory personnel as well as to the members of the community. Such organisms are categorized under Risk Group I by WHO. These organisms are unlikely to cause human diseases. Example, food spoilage bacteria, common molds and yeasts.

1.2.2 Level II Laboratory:-

Basic laboratory level II is suitable for work with organisms that can cause moderate risk to the laboratory workers and a limited risk to the members of the community due to the availability of effective preventive measures and treatment. Such organisms are categorized under Risk Group II by WHO. Examples, staphylococci, streptococci.

1.2.3 laboratory (Level III)

It is used for work with infectious organisms that present a high risk to the laboratory personnel but a lower risk to the community. Such organisms are

categorized under Risk Group III by WHO. Example, Tubercle bacilli, Salmonella typhi, HIV, Yersina and others. They are easily transmitted through airborne, ingestion of contaminated food or water . Such laboratory should be a separate room with controlled access. It should also be fitted with microbial safety cabinet.

1.2.4 Level IV Laboratory

laboratory Maximum containment laboratory is intended for work with viruses, which predispose to a high risk for both laboratory personnel and the community. Such organisms are categorized under Risk Group IV by WHO. Example, Small pox, Ebola, Lassa fever and others. Most of these organisms cause serious disease and readily transmitted from on person 4 to another. These laboratories are usually a separate building with strictly controlled access.

1.4 Duties of a Medical Laboratory Staff

1- To provide health care in investigating, controlling and preventing major diseases in the region and country.
2- To Promote health care by integrated health education
3- To help in making diagnosis by Investigation through referral or testing on site, of the important diseases and health problems affecting the local community.
4- To Assist in the health care workers in deciding the severity of a patient's conditions.
5- To notify the health care providers and public health authorities at an early stage of the disease to start a treatment or prevention to save lives.
6- Screen pregnant women and children for various diseases which can become a serious threat to their lives in future.
7- Promote health cares and assists in community health education
8- Keep records, which can be used by health authorities in health planning.
9- Keep an inventory of stocks and order supplies.

1.5 Role of medical laboratory technologist

A medical laboratory technologist (MLT) is a highly skilled healthcare professional who plays a critical role in the diagnosis and treatment of diseases. MLTs work in medical laboratories and are responsible for performing a variety of diagnostic tests on patient samples, such as blood, urine, and tissue samples. They operate and maintain laboratory equipment, analyze test results, and report their findings to medical staff. MLTs work closely with other healthcare professionals, such as physicians and pathologists, to ensure accurate and timely diagnoses.

Medical laboratory technologists must be detail-oriented, analytical, and have strong communication skills. They must be able to work well under pressure and follow strict laboratory procedures to ensure accurate test results. Medical laboratory technologists

perform a variety of laboratory tests and procedures to assist physicians in diagnosing, monitoring, treating and preventing disease. These tests have a wide range of areas; blood banking, chemistry, hematology, immunology, and microbiology to name a few

• they carry out routine and specialized laboratory tests using standard laboratory procedures;

• To apply strategies to solve administrative, technical and research problems;

• They can Conduct community based researches in cooperation with other categories of health professionals;

• They can provide professional consultancy related to the establishment, renovation, upgradation and reorganization of medical laboratories.

• They must understand the role of the laboratory and its contribution to the nation's health service;
•They shall Follow professional ethics and code of conduct;
• They may experience the job satisfaction and should have professional loyalty.

A medical laboratory technologist will,

- ❖ Examine body fluids and tissues for abnormal chemical levels, cells or bacteria.
- ❖ Prepare tissue for microscopic examination by pathologists.
- ❖ Determine blood type for transfusions.
- ❖ Conduct medical research and analyses.
- ❖ Operates and maintains sophisticated instruments and equipment used in the lab and is able to properly set up, calibrate, maintain, operate and shut down such instruments.
- ❖ Maintains competence in Laboratory Information System (LIS) that is crucial to the quality operations of the laboratory and patient care.
- ❖ Actively participates in Quality Improvement Programs essential to ensure that laboratory testing is accurate, timely and meets customer needs.
- ❖ Trains and orientates new Medical Laboratory Technologists in the performance and interpretation of tests.

Medical laboratory technologists may specialize in the following areas:

Clinical chemistry - chemically analyzing blood and other body fluids.

Clinical microbiology - culturing and identifying micro-organisms and determining antibiotic sensitivities.

Hematology - studying blood cells and their formation in health and disease, and investigating coagulation factors and disorders.

Histopathology - preparing tissue specimens for microscopic examination.

Transfusion medicine - taking and testing blood to determine its suitability for transfusions and preparing

blood products.

Cytotechnology - microscopically examining prepared cellular slides for evidence of abnormality or

disease.

Clinical genetics - analyzing patient tissue to determine the presence of a pre-existing or neoplastic genetic condition.

Those who work with infectious patients and samples or hazardous chemicals must take safety

precautions to avoid infection or injury. Working hours for medical laboratory technologists vary depending on the type of laboratory in which they work. Research laboratories usually operate weekdays only

1.6 Medical Laboratory Technologist vs Medical Laboratory Technician

Medical laboratory technologists and medical laboratory technicians are both important members of the healthcare team who perform a variety of laboratory tests to aid in the diagnosis, treatment, and prevention of diseases. However, there are some key differences between the two roles.

while both medical laboratory technologists and medical laboratory technicians play important roles in laboratory testing, medical laboratory technologists typically have more education and responsibilities, including analyzing and interpreting complex test results and supervising other laboratory staff. Medical laboratory technicians, on the other hand, typically perform more routine tests and work under the supervision of medical laboratory technologists or other laboratory professionals.

1.6.1 Safety precautions: MLTs must follow strict safety protocols to prevent infection and ensure the accuracy of test results. This includes the use of personal protective equipment (PPE), such as gloves, lab coats, and face shields.

1.6.2 High-demand environment: Medical laboratory technologists must be able to work quickly and efficiently while maintaining accuracy and precision in their work. They are often under pressure to produce results quickly, which can be stressful.

1.6.3 Continual learning: Due to the ever-evolving nature of medical technology, MLTs are expected to keep up with current research and developments in their field through continuing education and professional development opportunities. The work is highly

technical and requires a great deal of attention to detail and accuracy, but it can also be very fulfilling to be able to contribute to patient care and help diagnose and treat medical conditions.

Sample Question; Describe the duties of a medical lab technician, give short note on Clinical Pathology?

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Chapter 2

Fluid Examination

Objectives;

The Students will learn to perform the body fluids in lab accurately, precisely and to interpret the results. Body fluid testing is often requested by clinicians to help evaluate abnormal fluid collections and to establish clinical diagnoses. Extravascular body fluids (EBF) analysed in the clinical laboratory comprise cerebrospinal fluid (CSF), serous fluids (pleural, peritoneal and pericardial), synovial fluid, amniotic fluid, drain fluid, semen, urine, dialysate and others.

FLUID EXAMINATION

2.1, Ascititic Fluid Examination,

Ascites is defined as pathological fluid accumulation within the abdominal cavity. The word ascites is derived from the Greek word '*askos'*, which means a bag or sack.Clinically, ascites is a consequence or complication of a number of diseases, including liver, heart, and kidney diseases, infections, and malignancy. Ascites usually carries an unfavorable prognosis. Combined analysis of laboratory data of ascitic fluid samples and clinical and pathological data is essential for establishing a differential diagnosis.

2.1, Gross appearance, The initial evaluation of the gross appearance of ascitic fluid can offer useful information in the differential diagnosis. Under normal conditions, peritoneal fluid is clear to pale yellow.

2.1.2, Ascitic fluid total protein and the serum-ascites albumin gradient (SAAG)

For many years, the ascitic total protein concentration has been used to determine whether ascitic fluid was a transudate or exudate

Lactate dehydrogenase (LDH), High levels of LDH is found in malignant effusions and low levels of LDH in non-malignant effusions.

Glucose Since glucose diffuses readily across membranes, the concentration of glucose in the ascitic fluid, under normal conditions, is similar to that in the serum.39 However, ascitic glucose concentration decreases due to consumption by bacteria, white blood cells or cancer cells in the fluid in tuberculous peritonitis, spontaneous bacterial peritonitis (SBP), and malignancy.

Amylase Amylase-rich ascitic fluid commonly occurs in cases of pancreatic duct damage or obstruction due to pancreatitis or pancreatic traum.

2.1.3, Non-biochemical tests, Includes Cell counts, bacterial culture, and polymerase chain reaction (PCR)

2.2 CSF analysis

A sample of CSF is needed. A lumbar puncture, also called a spinal tap, is the most common way to collect this sample.

What Abnormal Results Mean?

An abnormal CSF analysis result may be due to many different causes, including:

- Cancer
- Encephalitis
- Hepatic encephalopathy
- High blood sugar (hyperglycemia)
- Infection
- Inflammation
- Meningitis due to bacteria, fungus, tuberculosis, or a virus
- Multiple sclerosis (MS)
- Alzheimer disease

What is Examined?

- Antibodies and DNA of common viruses.
- Bacteria: no bacteria normally
- Cancerous cells: no cells normally
- Cell count: 0 to 5 white blood cells (all mononuclear) and 0 red blood cells
- Chloride: 110 to 125 mEq/L (110 to 125 mmol/L)
- Fungus: no fungus
- Gamma globulin: 3% to 12% of the total protein is normal
- Glucose: 50 to 80 mg/dL or 2.77 to 4.44 mmol/L (or greater than two-thirds of blood sugar level)
- Lactate dehydrogenase: less than 40 U/L
- Oligoclonal bands: 0 or 1 bands that are not present in a matched serum sample
- Protein: 15 to 60 mg/dL (0.15 to 0.6 g/L)normal
- Opening pressure: 70 to 180 mm of water normal
- Myelin basic protein (MBP): Less than 4ng/mLnormal

2.3, Pleural effusion: Collection of fluid in the intrapleural space (lung Cavities), with compression of lung tissues. Fluid collects by gravity in dependent areas of the chest. Light's criteria remains the standard for pleural fluid analysis. Light's criteria should be used to differentiate transudative from exudative effusions, at least one of the following points must be present:

- "pleural fluid protein/serum fluid protein ratio > 0.5,
- pleural fluid lactate dehydrogenase (LDH)/serum fluid LDH ratio > 0.6,
- or pleural fluid LDH > 2/3 the upper limit of normal serum LDH

2.4, Urine Examination,

2.4.1, Collection:

Early morning urine,
This is the best urine specimen for routine analysis and is collected soon after the patient awakens. It is usually concentrated and has an acid pH. Casts and cells are poorly preserved in dilute or alkaline urine and traces of dissolved substances such as protein and sugar can be missed if the urine is very dilute.

2.4.2, Random urine

This specimen is collected at any time and is convenient for the patient and is suitable for most screening purposes.

2.4.3, Timed collection specimen

Among the most commonly performed test requiring timed specimens are those measuring creatinine, urine urea nitrogen, glucose, sodium, catecholamine's etc. that are affected by diurnal variations. A timed specimen is collected to measure the concentration of these substances in urine over a specific period of time usually 8 to 24 hours. In this collection method, the bladder is emptied prior to beginning the timed collection. Then, for the duration of the designated time period, all urine is collected and pooled into a collection container, with the final collection taking place at the very end of that period. The specimen should be refrigerated during the collection period.

2.4.4, Preservative used

For routine analysis, no preservative is required but the urine is best examined fresh. Bacterial growth will ruin a specimen if analysis is delayed for more than 3 hours. Refrigeration is the best way to preserve it if analysis is delayed. Refrigeration for more than 24 hours is not recommended.

2.4.5, Container for urine collection

The container used must be thoroughly clean and free from any detergent or disinfectant residue since the oxidants contained in such cleaning agents may cause the test areas for glucose and blood to indicate false positive results. After the urine is collected, the container should preferably be sealed.

2.4.6, Urine Collection Procedure

Females**:**

Wash the genital area thoroughly with soap and water (may be omitted for urine) .

With two fingers of one hand, hold the outer folds of the vagina (labia) apart. With the other hand, rinse the area from the front to the back

with soap and running tap water.Start urination so that the stream of urine should flow without touching the skin. After a few moments, place a sterile container under the stream of urine. Remove it from the urine stream the moment that the required amount of urine is collected.

Secure and tighten the cap on the container.

Males:

Wash the genital area thoroughly with soap and

water (may be omitted for urine RE).Start urination and after a few moments, place a sterile container under the stream of urine.

Collect the required amount of urine and remove the container from urine stream.

Secure and tighten the cap.

Infants, Uncooperative and Debilitated Patients:

Plastic bags may be attached after careful and thorough washing of the genital area.

The bags should be watched so that they can be removed immediately after the patient has passed the urine.

If the patient has not voided urine within 30 minutes, the collection bag is removed.

The patient needs to be re-scrubbed and a new collection device is to be attached.

2.4.7, Transport: Storage: Laboratory Processing: Disposal

1- All urine collection or transport containers should be clean and free of particles or interfering substances.
2- The collection or transport container should have a secure lid and be leak resistant.
3- Amber colored container should be used for specimens being assayed for light sensitive analytes, such as urobilinogen and porphyrins.
4- Proper labelling should be applied to the collection container or tubes.
5- 3ml urine is required for testing in automated chemistry analyzer.
6- Boric acid is added as preservative in the container before urine collection started in 24-hour urine collection.
7- 10% bleach is added in urine container for 15 to 20 minutes
8- After 15 to 20 minutes the urine is thrown in the waste basin with continuous water flow to drain it.

2.4.8, Reference Range

Normal values are as follows:

Color – Yellow (light/pale to dark/deep amber)

Clarity/turbidity – Clear or cloudy
pH – 4.5-8
Specific gravity – 1.005-1.025
Glucose - ≤130 mg/d
Ketones – None
Nitrites – Negative
Leukocyte esterase – Negative
Bilirubin – Negative
Urobilirubin – Small amount (0.5-1 mg/dL)
Blood - ≤3 RBCs
Protein - ≤150 mg/d
RBCs - ≤2 RBCs/hpf
WBCs - ≤2-5 WBCs/hpf
Squamous epithelial cells - ≤15-20 squamous epithelial cells/hpf
Casts – 0-5 hyaline casts/lpf
Crystals – Occasionally
Bacteria – None
Yeast – None

2.5 COMMON ANALYTES

The tests in a clinical chemistry laboratory measure concentrations of biologically important ions (salts and minerals), small organic molecules and large macromolecules (primarily proteins).

Ions, salts and minerals,

Potassium, Sodium, Calcium, Chloride, Magnesium, Phosphorus, Lead, Iron.

Metabolites**,**
Glucose, Cholesterol, Urea, Lactic acid, Bilirubin, Creatinine, Triglycerides, Ammonia Cystatin C.

Therapeutic drugs,
Vancomycin, Theophylline, Digoxin etc.

Toxicology,
Alcohol (ethanol), Salicylate (aspirin), Acetaminophen etc..

Drugs of abuse**,**
Cocaine , Barbiturates, Amphetamines, Opiates Cannabinoids.

Transport proteins,
Albumin, Transferrin, Haptoglobin, Ferritin,Total protein

Enzymes,
Lipase, Amylase, Alanine aminotransferase (ALT,) Aspartate aminotransferase (AST)
Alkaline phosphatase (Alk Phos), Lactate dehydrogenase (LD), Creatine kinase (CK).

Electrolyte panel,
Sodium (Na**),** Potassium (K), Chloride (Cl), Calcium (total and free),
Magnesium.

Lipid profile
Total cholesterol, LDL cholesterol, VLDL cholesterol ,HDL cholesterol, Triglycerides.

Renal Tests
Urea, creatinine, BUN

Serological Tests
Hbs Ag, antiHCV, HIV.

Hormonal profile, Estrogen, Progesterone, Testosterone, Prolactin, FSH, LH, cortisol, ACTH.

T3, T4, TSH.

Sample Question; Describe the different samples of urine and the procedure of collection of urine?

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Chapter 3

Specimen Considerations

Objectives

After studying this chapter students will learn how to collect the samples accurately to avoid the pre analytical errors. Specimen collection is a common component of routine checkups in clinical chemistry laboratory. It goes hand-in-hand with lab tests, allowing medical professionals to obtain tissue or fluids to look for any changes in your health. In addition, they help physicians diagnose medical conditions, plan treatments, and monitor diseases.

SPECIMEN CONSIDERATIONS

3.1 BIOLOGIC SPECIMENS

Blood is the most common biologic fluid collected for clinical laboratory testing. Other biologic fluids (matrices) often used for testing include urine, saliva, cerebrospinal fluid (CSF), amniotic fluid, synovial fluid, pleural fluid, peritoneal fluid and pericardial fluid

Test methods that are designed for determination of an analyte in blood plasma may not be suitable for determination of that same analyte in other fluids (other matrices). When using a test method for analysis of a fluid other than blood plasma or serum, it is important to validate that the method is acceptable for the type of fluid sample being used.

Fluids typically used for clinical chemistry tests

whole blood, serum or plasma,

Urine,

Cerebrospinal fluid (CSF),

Amniotic fluid,

Saliva,

Synovial fluid (fluid that is found in joint cavities),

Pleural fluid (from the sac surrounding the lungs),

Pericardial fluid (from the sac surrounding the heart),

Peritoneal fluid (also called ascitic fluid; from the abdomen)

3.2, SPECIMEN COLLECTION AND TRANSPORT

The collection of specimens for laboratory tests from patients consists of following steps:

1:-Patient identification, Documentation/Registration

2:- Collection of specimen

3:- Dispatch of specimen to respective department

4:- Reporting the results.

Patient identification, documentation and registration

Patients or attendants or staff of the ward where the patient is admitted, report to the reception desk. The reception staff registers the patient and documents his/her identification and demographic data which includes outdoor/indoor number, Name, Age, Address and the tests to be carried out for that particular patient. Patient is allocated a specific registration number (MR no.) Patient is provided with a receipt having details of

the tests to be carried out and the tentative delivery date for the complete lab report. He is requested to sit in the waiting area to wait for his/her turn for specimen collection.

3.3, SPECIMEN COLLECTION

Blood is the most common biologic fluid collected for clinical laboratory testing. Other biologic fluids (matrices) often used for testing include urine, saliva, cerebrospinal fluid (CSF), amniotic fluid, synovial fluid, pleural fluid, peritoneal fluid and pericardial fluid. Test methods that are designed for determination of an analyte in blood plasma may not be suitable for determination of that same analyte in other fluids (other matrices).

Venous blood is deoxygenated blood that flows from tiny capillary blood vessels within the tissues into progressively larger veins to the right side of the heart.
Venous blood is the specimen of choice for most routine laboratory tests. The blood is obtained by direct puncture to a vein, most often located in the antecubital area of the arm or the back of the hand. Most laboratory reference ranges for blood analytes are based on venous blood.

3.3.1 Arterial Blood

Deoxygenated blood is pumped from the right side of the heart to the lungs, where it takes up oxygen. The now oxygenated blood is pumped through the left side of the heart through the arteries to the body tissues. The most common reason for the collection of arterial blood is the evaluation of arterial blood gases. Arterial blood may be obtained directly from the artery (most commonly, the radial artery) by personnel who are trained to perform this procedure and are knowledgeable about the complications that could occur as a result of this procedure. Arterial blood may also be obtained from a vascular access device (VAD) inserted in an artery, such as a femoral arterial line or catheter.

3.3.2 Capillary Blood

Capillary blood is obtained from capillary beds that consist of the smallest veins (venules) and arteries (arterioles) of the circulatory system. The venules and arterioles join together in capillary beds, forming a mixture of venous and arterial blood. The specimen from a dermal puncture will therefore be a mixture of arterial and venous blood along with interstitial and intracellular fluids.
Capillary blood is often the specimen of choice for infants, very young children, elderly patients with fragile veins, and severely burned patients.
Point-of-care testing is often performed using a capillary blood specimen.

3.3.3 Phlebotomy.

The act of drawing a blood sample from a blood vessel. Collecting blood from a vein is called venipuncture. The medical professional drawing the blood sample is called a phlebotomist.

Wear the Appropriate clothing and protective equipment (PPE). Phlebotomists are considered to have occupational exposure to blood borne pathogens. The performance of routine vascular access procedures by skilled phlebotomists requires, at a minimum, the use of gloves to prevent contact with blood. Airborne precautions may be considered to provide a level of safety against infectious diseases such as tuberculosis, influenza, and COVID-19. Precautions include a medical grade face mask. With risk for blood spatter a face shield provides protection. A face mask reduces risk for blood culture specimen contamination.

Observe the quality assurance issues.

Identify the patient by asking his/her particulars and compare them with the request form.

Get Informed consent of the patient about the specimen collection.

Always ask if he or she has undergone blood tests previously. In case of any history of abnormal reactions to blood collection, inform the lab incharge/manager before phlebotomy and then follow his instructions.

Thoroughly check the request form for the number and type of the investigations. Prepare proper labels and paste them on appropriate containers before obtaining specimens.

Quantity of blood required can be obtained in single prick. If multiple samples are required, or 15 ml of blood is to be collected use a butterfly needle or a cannula. Select appropriate vein (preferably antecubital) from forearm.

Cleanse the skin over the venipuncture site in a circle approximately 5cm in diameter with 70% alcohol/spirit swab, scrubbing the area vigorously.

Apply a tourniquet tight enough to obstruct venous flow only and relocate the vein to be punctured but don't touch the proposed site of needle entry or the needle itself. Ask the patient to clench the fist to make the veins prominent.

If the vein is not visible, palpate it with fingers. In case the veins of forearm are not visible or palpable, other sites such as dorsum of the hand may be selected.

Insert the needle into the vein, release the tourniquet once the needle has entered the vein and withdraw the blood till the required quantity of blood is obtained.

Do not try to withdraw the piston forcefully because it can collapse the vein and it may cause frothing and hemolysis of the blood. After that withdraw the needle and apply pressure with thumb on antiseptic swab at puncture site for 2-4 min till the blood ooze ceases.

Then patient is allowed to move away from the specimen collection chair. The antiseptic swabs should then be disposed of in the designated baskets. Now distribute the blood from syringe in to the appropriate, labelled containers.

3.4 Order of Draw

Blood samples must be drawn by phlebotomists in a specific order to avoid cross-contamination of the sample by additives found in different collection tubes. Phlebotomy order of draw is the same for specimens collected by syringe, tube holder, or into tubes pre evacuated at the time of collection. Additive carryover occurs when the needle filling a tube comes in contact with the blood/additive mixture as the tube fills, and transfers a minute amount of blood and additive into the next tube filled. This can occur with both syringe and vacuum draws. In a syringe draw, the carryover occurs with the needle of the safety transfer device. (According to OSHA, blood collected by syringe should be transferred to the tubes using a safety transfer device, not the same needle used to perform the venipuncture.)

In a tube holder draw, carryover occurs from the needle within the tube holder as tubes are exchanged.

When additives carry over into a different tube type, test results may be dramatically affected. For example:

- EDTA from a lavender-stopper tube, which is rich in potassium, carry over into a tube to be tested for potassium, the level of potassium may be falsely elevated leading to life-threatening medical mistakes;

- If a clot activator carries over into a tube to be tested for coagulation studies, the prothrombin time (PT) or activated partial thromboplastin time (aPTT) may be falsely shortened; When blood cultures are collected at the same time as other lab work and not filled first, bacteria from the non-sterile stoppers of the tubes can contaminate the bottles used for blood cultures.

  Since we know which additives adversely affect which tests, we can arrange the tubes and blood culture bottles so that any carryover is irrelevant. That arrangement is the order of draw. When tubes are filled according to the recommended order of draw, any additive carryover that may occur will have no significant impact on test results. The order is universal for glass and plastic tubes, and irrespective of whether samples are drawn with a tube holder or syringe. The recommended order is as follows:

1. Blood culture tubes (sterile)
2. Sodium citrate tubes (e.g., blue-stopper)
3. Serum tubes with or without clot activator, with or without gel separator, e.g., red
4. Heparin tubes with or without gel (e.g., green-stopper)
5. EDTA tubes (e.g., lavender-stopper)
6. Glycolytic inhibitor tubes (e.g., gray-stopper)

3.5 Biological Spills,

Biological spills outside biological-safety cabinets will generate aerosols that can be dispersed in the air throughout the laboratory. Appropriate protective equipment is particularly important in decontaminating spills involving microorganisms. This equipment includes a lab coat with long sleeves, back-fastening gown or liquid-barrier coveralls, disposable gloves, disposable shoe covers, and safety goggles and mask or full face shield. Use of this equipment will prevent contact with contaminated surfaces and protect eyes and mucous membranes from exposure to splattered materials. Consult the Pitt Biosafety Manual for additional information.

Cleaning The Spill.

- Alert people in the area of the spill to evacuate
- Remove and disinfect any material that has been splashed on you and remove/disinfect grossly contaminated clothing.
- Secure the affected area and apply biohazard-warning signs.

- . Assess the situation and wear the appropriate personal protective equipment PPE for the cleanup operation.
- Cover the spill with paper towels or other absorbent material to absorb the spill to prevent further aerosolization.
- Pour disinfectant gently over the covered spill, working from the outside inwards.
- Wait at least 15 minutes for the disinfectant to penetrate through the contained spill.
- Using the appropriate tools (i.e., shovels, forceps), remove the absorbent material and place it in a biohazard bag for autoclaving and subsequent disposal.

  Two of the most common errors that occur during specimen collection and handling are clotting and inaccurate volume. Exposure to temperature extremes may also cause specimens to be rejected for testing.

Clotting compromises the integrity of a specimen, making it unsatisfactory for testing. Overfilling collection tubes that have specific volume requirements may also contribute to clotting, and under-filling makes the volume insufficient for testing. All of these errors will cause a specimen to be rejected because the laboratory cannot ensure the accuracy of test results.

Specimen integrity may also be affected during handling and shipping. To prevent hemolysis of red blood cells, whole blood samples should never be frozen. Specimens must also be protected from high-temperature environments (such as mail vans and drop boxes), especially during the summer in order to prevent decomposition.

Phlebotomy is usually a very safe procedure, with very low chances of side effects or any risks. However, there have been rare incidents where the patients have incurred nerve damage, vasovagal reaction, and infections post the procedure.

Inform the pathologist promptly, if patient feels unwell after specimen collection, ask him to lie down on couch, reassure and give him drink. Some patients collapse when the skin is punctured or at the sight of blood. In such cases withdraw the needle immediately and ask the patient to lie down in supine position. Raise the legs of the patient. Information should be given if specimen is not drawn in first prick or if child is below the age of one year. Also, if patient is very sick or special blood specimen collection is requested

3.6 SAMPLE TRANSPORTATION

Blood should not be exposed to extreme temperatures during transportation. Extreme heat can lead to denaturation of proteins and degradation of cellular components, while extreme cold can cause crystal formation and cell damage. In general, blood samples for routine tests are typically transported at temperatures between 2°C to 8°C (36°F to 46°F). Fridge temperature (between 2°C and 10°C) or frozen (20°C or lower). These temperatures must be maintained during storage and transport.

Use insulated containers or ship the samples in temperature-controlled vehicles to maintain the appropriate temperature range for blood samples. Cleanliness of the transportation vehicle or bag or container is essential to prevent cross-contamination and maintain the integrity of blood samples. Regularly clean and disinfect the vehicle bag or carrier, and especially in the event of a spillage. Pay particularly close attention to high-touch surfaces and use appropriate disinfectants.

Agitating or shaking blood samples during transport can lead to hemolysis. Hemolysis can alter the composition of the sample and affect test results, leading to inaccurate diagnoses, poor patient care and the need to repeat blood tests. Specialised handling and transportation expertise are required, it is strongly advisable to engage a professional blood courier.

3.7 Reporting the Results,

Reporting laboratory results is a crucial part of laboratory management, as it affects the quality of patient care, clinical decision making, and public health. However, reporting results can also pose various challenges, such as ensuring accuracy, timeliness, confidentiality, and compliance with regulations and standards.

Every laboratory should have written policies and procedures for reporting results, based on the best practices and guidelines of the relevant authorities and organizations. These policies and procedures should cover aspects such as who is authorized to report and receive results, how to verify and validate results, how to handle errors and corrections, how to document and archive results, and how to comply with ethical and legal requirements.

To ensure the accuracy and integrity of the results, reliable and secure systems and technologies for reporting should be used. These may include laboratory information systems (LIS), electronic health records (EHR), or other software applications that can store, process, transmit, and display results. The results should be provided in a timely and respectful manner and try to explain the results clearly and accurately, and answer any questions. Furthermore, feedback should be taken from the recipients and use it to improve reporting practices.
To ensure the competence and professionalism of the staff involved in reporting results, the staff must be educated and trained regularly. Staff should be provided with the knowledge and skills necessary for reporting results, such as the format and mode of reporting, the reporting policies and procedures, the systems and technologies used for reporting, and the communication skills required for reporting. The performance of the reporting staff should be evaluated and provided with constructive feedback and guidance.

Failure to report test results, delay in reporting, incorrect calculation, critical results not reported or delayed, and results sent to the wrong patient are important post analytical errors.

3.8 Reporting The Critical Results,

A critical result is defined as a value or interpretation of a test that falls significantly outside the normal range and may represent a life-threatening value.
CRITICAL VALUE: A value that represents a pathophysiological state at such variance with normal as to be life-threatening unless something is done promptly and for which some corrective action could be taken.

3.8.1 - Notification: The laboratory should have procedures for immediate notification to a physician (or other clinical personnel responsible for the patient's care) when results of designated tests exceed established "alert" or "critical" values that are important for prompt patient management decisions.

3.8.2 - Read-Back of Critical Values/Critical Results: When critical values / critical results are communicated by 2. Read-Back of Critical Values/Critical Results: When critical values / critical results are communicated by number, or unique patient numeric identification) and the results is requested and documented.

3.8.3 - **Documentation**: Records (date, time, responsible laboratory individual, person (full name) notified, and test results) must be maintained showing prompt notification of the appropriate clinical individual after obtaining results in the critical range. d) A phone call or secure text is immediately placed to a responsible licensed caregiver for notification. A nurse must communicate the result directly to the licensed caregiver. If the result is sent to the practitioner by text, the practitioner must deliberately respond to the text, an automatic “read” receipt is not an acceptable response. A text may only be used as the first attempt to contact the practitioner. If a practitioner does not respond after 15 minutes, a second call is placed and a second message is left with requesting an immediate call back.
When a licensed caregiver has been notified of the critical result, document the time and the name of the caregiver to whom it was reported on the Critical Result Notification Form

or electronic documentation tool.

Sample Question; Describe the procedure and precautions of sample collection. What is order of draw?

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Chapter 4

Introduction to Management

Objectives

The goal of lab management is to guide lab personnel to deliver their assigned duties within limited time and resources. This includes acquiring grants, personnel, equipment, or the necessary tools, as well as designing the workflow, overseeing the daily operation of the lab, and training new lab personnel.

INTRODUCTION TO MANAGEMENT

4.1 Definition and General Principles of Management

Management is the process of giving direction and controlling the various activities of the people to achieve the objectives of an organization. Management is a process of planning, decision making, organizing, leading, motivation and controlling the human resources, financial, physical, and information resources of an organization to reach its goals efficiently and effectively. Management is conducted in organizations. Organizations are variously described as systems of inter-dependent human beings, a joint function of human characteristics. Organizations may be seen as combinations of resources brought together for a purpose.

Management is the act of getting people together to accomplish desired goals and objectives using available resources efficiently and effectively. Management comprises planning, organizing, staffing, leading, coordinating and controlling an organization or effort for the purpose of accomplishing a goal. Resourcing encompasses the development and manipulation of human resources, financial resources, technological resources and natural resources. Management is essential for the conduct of activities in an orderly manner. It is a vital function concerned with all aspects of working of an enterprise.

Management is needed as it occupies a unique position in the smooth functioning of a task. This suggests the need of efficient management of business enterprises. Profitable/successful business may not be possible without efficient management. Survival of a business unit in the present competitive world is possible only through efficient and competent management.

4.2 Features of Management

- Management is Goal-Oriented
- Management integrates Human, Physical and Financial Resources
- Management is Continuous
- Management is all Pervasive
- Management is a Group Activity

4.3 Management Functions

Luther Gullick has given a keyword ’POSDCORB’ where P stands for Planning, O for Organizing, S for Staffing, D for Directing, Co for Co-ordination, R for reporting & B for Budgeting. But the most widely accepted are functions of management given by KOONTZ and O’DONNEL i.e. Planning, Organizing, Staffing, Directing and Controlling.

4.3.1 Planning

It is the basic function of management. Planning is determination of courses of action to achieve desired goals. Thus, planning is a systematic thinking about ways & means for accomplishment of predetermined goals. Planning is necessary to ensure proper utilization of human & non-human resources. It is all pervasive, it is an intellectual activity and it also helps in avoiding confusion, uncertainties, risks, wastages etc.

4.3.2 Organizing

It is the process of bringing together physical, financial and human resources and developing productive relationship amongst them for achievement of organizational goals. According to Henry Fayol, “To organize a business is to provide it with everything useful or its functioning i.e. raw material, tools, capital and personnel’s”. To organize a business involves determining & providing human and nonhuman resources to the organizational structure. Organizing as a process involves:

- Identification of activities.
- Classification of grouping of activities.
- Assignment of duties.
- Delegation of authority and creation of responsibility.
- Coordinating authority and responsibility relationships.

4.3.3 Staffing

The main purpose of staffing is to put right man on right job. According to Kootz & O’Donell, “Managerial function of staffing involves manning the organization structure through proper and effective selection, appraisal & development of personnel to fill the roles designed in the structure”. Staffing involves:

Manpower planning: right person on the right job.

Recruitment, Selection & Placement.

Training and development

Promotions and transfers

4.3.4 Controlling

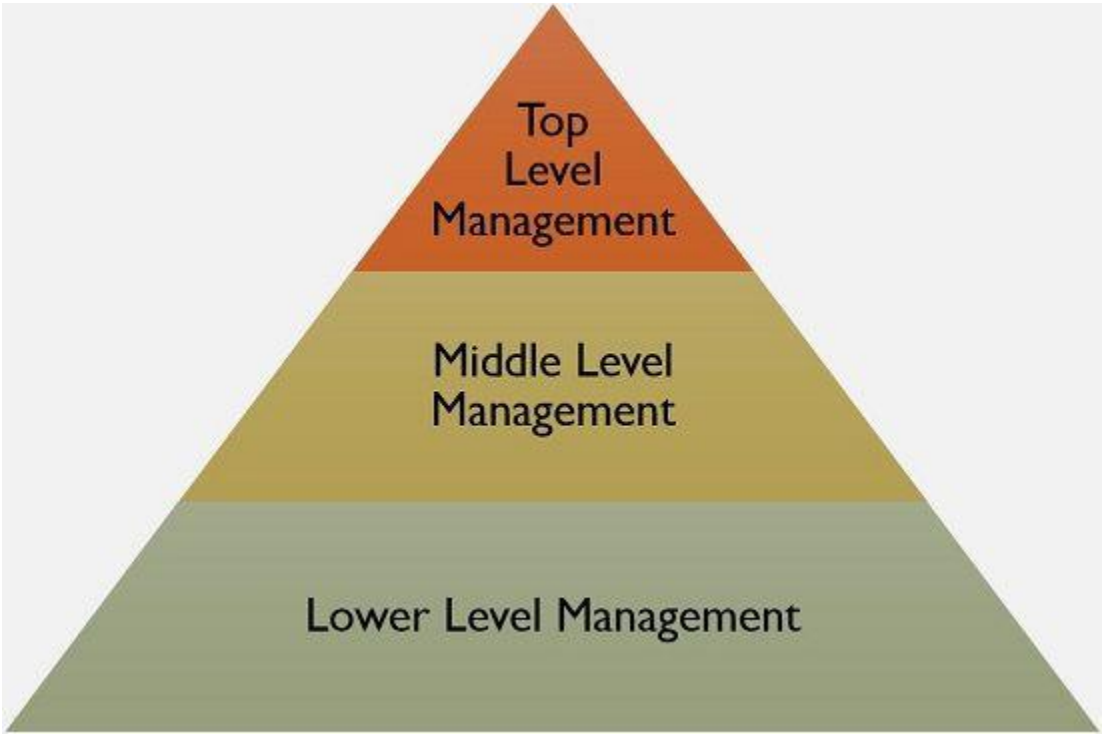
It implies measurement of accomplishment against the standards and correction of deviation if any to ensure achievement of organizational goals. The purpose of controlling is to ensure that everything occurs according to the set standards. An efficient system of control helps to predict deviations before they actually occur. According to Koontz & O’Donell “Controlling is the measurement & correction of performance activities of

subordinates in order to make sure that the enterprise objectives and plans desired to obtain them as being accomplished". Therefore controlling has following steps:

a. Establishment of standard performance.
b. Measurement of actual performance.
c. Comparison of actual performance with the standards and finding out deviation if any.
d. Corrective action.

4.4 Levels of Management

The term Levels of Management refers to a line of demarcation between various managerial positions in an organization. The number of levels in management increases when the size of the business and work force increases and vice versa. The level of management determines a chain of command, the amount of authority & status enjoyed by any managerial position. The levels of management can be classified in three broad categories:



1. Top level / Administrative level

2. Middle level / Executory.

3. Low level / Supervisory / Operative / First-line managers

4.4.1 Top level / Administrative level

Top-level management is the head of the organization also known as the organization's administrative body. They have the authority and responsibility relating to maintaining the overall function, image, direction, and performance of the organization. As the head of the family (organization), top-managers does make formal long-term plans and strategies, bring coordination to the organization, manage organizational resources, and guide & control other lower managerial positions. Top managers are keen on their qualifications and performing given tasks. They are appointed by such as shareholders and management committees. And, are accountable to them for the overall performance of the firm.

Some major functions of top-level management include:

- Top-level managers set the overall direction and objective of the organization.
- Makes plans, policies, and strategies aligning organizational resources and middle-lower managerial competencies to best carry out them.
- Direct, coordinate, and control all the organizational members.
- Evaluate and review the overall performance of the organization so as to make plans for implementation in the right direction and achievement of expected goals and objectives.
- Represent and maintain the organization's image in the outside world.

4.4.2 Middle-Level Management

Middle-level management is also called the executory level. Middle-level managers are responsible to execute plans and policies given by top managers. They are also given adequate authority and responsibility to execute the given tasks. Middle-level management consists of functional heads of different departments and is responsible for preparing and implementing departmental plans. Middle-level managers are different departmental heads of the organization. They are the largest group of managers found in the organization. This level is also known as the backbone of the organization. They subordinate to top management and direct lower-level management. They have a significant role in the execution of plans formulated by top managers as they play a mediator role between top management and lower management in terms of plan execution, progress report submission, and controlling lower managers who actually perform the tasks.

This level of management can also be of two types in some big organizations such as junior and senior middle-level management. The department heads come under the senior level and branch heads are considered junior level.

The tasks of middle-level managers include:

- Middle managers play a mediator role between the top and lower management.

- Execute the plans and strategies according to the instruction given by top management.
- Make plans and policies for departmental goals.
- Control and train lower-level managers.
- Submit progress reports and provide valuable suggestions to top managers.
- Conduct different training programs and inspire junior levels to work with ease and efficiency.

4.4.3 Lower-Level Management

Lower-level management is also called the operating level which is directly involved in the implementation of plans. Lower managers have direct relations with operative employees. They are responsible for the implementation of works laid down by senior levels management mainly by the middle level. Lower-level managers are strongly responsible for effectively and efficiently conducting of day to day activities which ensures short-term goals achievement. They are given the necessary authority and responsibility by the middle managers to command operative employees. They further train, motivate, control, and inspire working employees. They communicate the problems faced by workers to senior managers. They are mainly responsible to manage working employees and strengthen the workforce of the organization to effectively and efficiently execute given tasks. They further manage the necessary tools and equipment to successfully work to be done.

4.5 Principles of management,

4.5.1 Simple: Objective should be clear and understandable to the health team.

4.5.2 Measurable: To monitor the day to day activity (assessment of performance) and to help evaluate the activities (for the future course of action)

4.5.3 Achievable: Feasibility is an important criterion for good planning. Objective should be should be realistic.

4.5.4 Relevant: All action plans should be directed to the major problems of the community. The objective should address the real problems of the community.

4.6 Management Skills

1) Technical skills are job-specific knowledge and techniques needed to proficiently perform specific tasks.

2) Human skills are the ability to work well with other people individually and in group

3) Conceptual skills are the ability to think and to conceptualize about abstract and complex situations

Effectiveness is the degree to which a stated objective is being achieved; it is something that management tries to improve.

4.7 Principles of Management.

4.7.1 Division of Work – This principle of management is based on the theory that if workers are given a specialized task to do, they will become skillful and more efficient in it than if they had a broader range of tasks. Therefore, a process where everyone has a specialized role will be an efficient one.

4.7.2 Authority – This principle looks at how authority is necessary in order to ensure that managerial commands are carried out. If managers did not have authority, then they would lack the ability to get work carried out. Managers should use their authority responsibly and ethically.

4.7.3 Discipline – Discipline is needed within an organization for it to run effectively. Organizational rules, philosophies, and structures need to be met. In order to have disciplined workers, managers must build a culture of mutual respect and motivation.

4.7.4 Unity of command – There should be a clear chain of command in place within an organization. An employee should know exactly whose instructions to follow. **Unity of direction** – Work should be organized in a way that means employees are working in harmony toward a shared objective or goal using a shared method or procedure.

4.7.5 Subordination individual interests to the collective interests – The interests of the organization as a whole should take precedence over the interests of any individual employee or group of employees. This encourages a team spirit and collective mentality of all for one and one for all.

4.5.6 Remuneration – In order to motivate and be fair to employees, they should be paid a reasonable rate for the work they carry out. An organization that underpays will struggle to attract quality workers who are motivated.

4.5.7 Centralization – This principle relates to whether decisions should be made centrally, as in from the top down, or in a more democratic way, from the bottom up. Different decision making processes are appropriate for different types of decisions.

4.7.8 Scalar chain – This relates to the principle of a clear chain of communication existing between employees and superiors. The chain should be respected, unless speedy communication is vital, in which case the chain may be bypassed if all parties consent.

4.7.9 Order – This relates to the proper use of resources and their effective deployment in a structured fashion.

4.7.10 Equity – Managers should behave ethically towards those they manage. Almost every organization in the modern world will have a written set of policies and procedures which will outline exactly what is expected from staff at all levels.

4.7.11 Stability of tenure of personnel – It is seen as desirable within an organization to have a low staff turnover rate. This is due to the benefits that come with having experienced staff and the time and expense needed to train new ones. There should be a clear and efficient method of filling any staff vacancies that arise.

4.7.12 Initiative – Employees that have an input as to how to best do their job are likely to feel more motivated and respected. Many organizations place a great deal of emphasis on listening to the concerns of staff.

Morale – Keeping a high level of morale and team spirit is an essential part of having the most productive organization possible. Happy and motivated employees are far more likely to be productive and less absent.

At the end of all the activities **achievement** is compared with the objective, i.e. evaluation is done. This helps us to judge how effective our management was and to plan for the future course of action.

4.8 Management In A Clinical Chemistry Laboratory

Equipment and Supplies

In any laboratory there are two main types of items.

Expendable, which are also called **consumables**. Materials that are used until exhausted are expendable items and may include matches, cotton, wool, laboratory stains, disposable syringes, glass wares, etc.

Non-Expendable also called **capital.** These are instruments/equipment that are used for several years and need care and maintenance. These instruments or equipment include microscopes, autoclaves, centrifuges, water baths, incubators, spectrophotometers, balances, etc.

Procedures in the Management of Laboratory Items

Ordering: obtaining lab items from stores or procuring items from suppliers or manufacturers.

Storing**:** Keeping the Record of every item received on a stock register, labeling them and holding these items in a stock or storeroom.

Issuing**:** Dispensing the required items to be used in different laboratory works and by the staff and keeping the record in the dispense book.

Maintenance: Maintenance of the equipment is necessary which may be time bound at different intervals. Maintenance of the instruments like autoanalyzers, centrifuges is required to get proper results and to follow the quality assurance principles. This includes controlling expendable items and maintaining and repairing equipment/instruments.

4.9 Purchase of Lab Items

Only senior staffs are authorized to order lab items. Laboratory supplies are ordered regularly before stocks are depleted. Stock for one year must be present at the beginning of each year. The shelf life and the expiry date of the reagents, controls and calibrators must be observed. Expired and shelf life of the stock is very important for proper results. Check the prices of items from other hospitals which have purchased these items before ordering to ensure that laboratory expenditures do not exceed the budget. One has to follow government guidelines (PVMS and PPRA rules) carefully when ordering supplies.

The following skills are needed to order equipment:

Enlist all the requirements with relevant and clear specifications.

Balance the requirements with available resources and make cost estimates.

Use a catalogue book to make correct lists of items

Complete an order form or requisition form while in government set ups the procurement is done through the tenders in which different companies participate to supply the required items on purchase or reagent rental basis (RRB). The companies must be registered with international accreditation organizations like FDA (US Food and Drug Authority), CE (marks European Commission), PNAC (Pakistan national Accreditation council) etc.

It is important that in procurement of items there should be certificate of origin of each required item and expiry date clearly labeled to have more than 80% laps of time before this date.

4.9.1 Making Lists

Make a list of laboratory items with appropriate date of purchase, date of installation, the company name from which the item is purchased, the model of the equipment and their maintenance. Maintain the log book of all machines. For each item write down the exact type required (specification of items is very essential). For example, microscope, binocular 220v.

Estimate the quantity of each item. For example, 5 packs of applicator stick for a year. The quantity of an item used depends on the number of people using it and can be estimated from the last year use of that item and little increase in the number for next year. Since resources are always limited it is important that consumable items be used immediately.

4.9.2 Balance the Requirements and Resources: Health services all over the world face lack of resources. Therefore, requirements must always be balanced against resources. Sometimes one can obtain more resources, e.g. if a donation is made, equipment/instruments may be ordered without affecting the regular budget like on reagent rental basis (RR). In any event, a cost estimate must be made before completing the order form.

4.9.3 Making a Cost Estimate Draw a columned sheet of paper and list the items, quantity, and price per unit and total price.

4.9.4 Use a Catalogue book, A catalogue is a book that contains a list of articles available for purchase from a certain place. A catalogue is used wherever things are purchased at a distance. A catalogue may be published by a government store or by a private firm, manufacturer or shop. The disadvantage of catalogue purchasing is that the purchaser does not see the articles (equipment) he/she is buying often there are several types of the same item e.g., there may be six different kinds of centrifuges.

Therefore, the catalogue must be read with greater care and the exact item number, description and price carefully identified. There must also be a need to request suppliers to submit certificate of origin which is required by our government policy.

4.9.5 Completing an Order-form or Requisition Form, An order form or requisition form is usually supplied together with the catalogue. If not supplied the person responsible should prepare his/her own order form. An order-form for example should have a column for each of the following:

- Item number
- Name of article (material)
- Specifications in detail/ Type
- Quantity required - Unit and total price

4.9.6 Supplying the order

A well-organized laboratory should submit its supply need to the central supply store according to the time and schedule set by the authorities or management.

To draw up order, check the stock stock register one by one. An ideal stock register should provide information about the availability of all the items

4.10 Shelf Life and Expiration Date

Shelf life is defined as the length of time a product may be stored without becoming unsuitable for use or consumption. Shelf life depends on the degradation mechanism of the specific product.

“Shelf life” and “expiration dates” are often used interchangeably, but if you want to get technical, these terms are distinctly different. *Shelf life* refers specifically to the amount of time a *properly sealed and stored* reagent will last without degrading in quality. *Expiration Date* refers to the amount of time an *opened* reagent will last before needing to be disposed of. Tracking and adhering to both of these dates can help guarantee the standard of your laboratory’s data, and can help ensure all reagents meet safety and testing standards.

Imagine an *unopened* container of a reagent or control is acquired, which has a *shelf life* of one years. It is currently January 2024, which would mean that our hypothetical reagent will reach the end of its *shelf life* in January of 2025.

FACT: Shelf Life does NOT mean expiration date.
FACT: A standard's, expiration date should *never* exceed 1 year.
FACT: A standard's expiration date should *never* exceed 1 year.

4.10.1 Standards and Controls

Standards are used as the basis for the comparison of other solutions in the clinical laboratory. They must be suitable for qualitative and quantitative analysis and contain a known amount of analytically pure substance (99.95%) in a specific volume.

4.10.2 Certified Reference Material– is a reference solution or test solution used for assessment of the performance of an analytical procedure. A rigorously tested and high quality known analyte at a certain concentration is known as a Certified Reference Material (CRM). CRMs are generally governed by ISO Guide 34:2009. NIST makes its own service marked brand of CRMs named Standard Reference Materials (SRMs). CRMs and SRMs should have statements of calibration (precision) and boas (accuracy).

4.10.3 Calibrator– Often it is in the beginning of a run. What makes a standard or control a calibrator is that it is placed before the unknowns are tested. The calibrators are run in a series over the hoped for linear dynamic range. The response from the testing of the calibrators is plotted on a signal versus concentration y-x axis graph. A line is drawn along the data points to determine if the response is linear. Calibrators are used to construct the calibration curve. It is the Quality Control (QC) of a quantitative process. A calibrator is a solution with a known amount (concentration) of analyte of interest that is hopefully pure and only contains that analyte of interest. It is placed within the batch of the run as part of the QC procedures to insure that the analytical instrument is detecting the known within an established range of values.

4.10.4 Controls, Used to monitor the precision of chemistry assays and instruments, clinical chemistry controls are available in assayed and unassayed versions and in liquid or lyophilized format to suit each laboratory's requirements. Performing **Laboratory quality control** is designed to detect, reduce, and correct deficiencies in a laboratory's internal analytical process prior to the release of patient results, in order to improve the quality of the results reported by the laboratory. Quality control (QC) is a measure of precision or how well the measurement system reproduces the same result over time and under varying operating conditions. Laboratory quality control material is usually run at the beginning of each shift, after an instrument is serviced, when reagent lots are changed, after equipment calibration, and whenever patient results seem inappropriate. Quality control material should approximate the same matrix as patient specimens, taking into account properties such as viscosity, turbidity, composition, and color. It should be simple to use, with minimal vial-to-vial variability, because variability could be misinterpreted as systematic error in the method or instrument. It should be stable for long periods of time, and available in large enough

quantities for a single batch to last at least one year. Liquid controls are more convenient than lyophilized, (freeze-dried) controls because they do not have to be reconstituted, minimizing pipetting error. Dried Tube Specimen (DTS) is slightly cumbersome as a QC material but it is very low-cost, stable over long periods and efficient.

4.11 Laboratory Storage

Laboratory storage refers to the collection of containers, cabinets, and other storage units used to organize, store, and protect equipment, samples, chemicals, and other materials used in a laboratory setting. The goal of laboratory storage is to maintain the integrity of the stored items and to ensure that they remain safe and accessible.

Most chemical reagents should be stored in a cool, dry, dark place to maximize shelf life and prevent premature degradation. Room temperature is suitable for the majority of chemical reagents, though some may require specialized storage like refrigeration. Storing quality control materials properly in a medical laboratory is essential to ensure their integrity and accuracy.

Some quality control materials, reagents and standards, especially those containing biological components, might require refrigeration or even freezing and some may be stored at room temperature. Follow the manufacturer's recommendations for the appropriate storage temperature.

A moderate humidity level in the storage area to prevent damage to the materials is required. Excessive humidity can lead to deterioration or contamination.

Quality control materials and reagents should be kept away from exposure to direct sunlight or bright artificial light. Light exposure can degrade certain components and affect the stability of the materials.

Keep track of the expiration dates and shelf lives of quality control materials and reagents. Do not use materials that have expired, as they may no longer provide accurate results.

Clearly label each container with the material's name, lot number, expiration date, and storage requirements. This helps prevent mix-ups and ensures traceability.

Ensure that containers and vials are tightly sealed to prevent contamination and evaporation. Use sealing caps or covers provided by the manufacturer.

Inventory control System: Implement a "first-in, first-out" (FIFO) system to ensure that older quality control materials are used before newer ones. This practice helps maintain the integrity of the materials and prevents the use of expired products. Conduct regular inventory checks to monitor the availability and condition of quality control materials. Replace any materials that are close to their expiration dates.

Keep different types of quality control materials, reagents and standards separate from one another. Group them according to their intended use and application to avoid mix-ups. Separate quality control materials from patient samples and reagents to prevent any cross-contamination.

Control access to the quality storage area to prevent unauthorized personnel from handling the materials. This helps maintain the integrity of the quality control process.

When handling quality control materials, reagents or standards, follow the manufacturer's instructions carefully. Avoid exposing them to extreme temperatures or direct sunlight during transportation.

Perform regular checks of the quality control materials' condition, including visual inspections for signs of deterioration or contamination. If any issues are detected, take appropriate actions, such as contacting the supplier or discarding the affected materials.

Maintain proper documentation of the chemicals (quality control material, reagents and standards) usage, storage, and any incidents related to their integrity. This documentation is essential for auditing and accreditation purposes.

There are several types of laboratory storage available, each designed to meet specific needs and requirements. Some common types of laboratory storage include:

4.11.1 Refrigerators and freezers: Used to store perishable materials and samples that require low temperatures.

4.11.2 Chemical storage cabinets: Used to store chemicals, these cabinets are typically made of materials that are resistant to chemicals and can be sealed to prevent spills.

4.11.3 Glassware storage cabinets: Designed to store glassware, such as test tubes and beakers, these cabinets typically feature shelves or drawers that can be adjusted to accommodate different sizes of glassware.

4.11.4 Centrifuge and microscope storage cabinets: Used to store centrifuges and microscopes, these cabinets are typically designed to be sturdy and to provide protection from dust, moisture, and other contaminants.

4.11.5 storage cabinets: Designed to store flammable liquids and chemicals, these cabinets typically feature fire-resistant materials and ventilation systems to prevent fires.

The following steps must be taken into account,

- Store supplies under the right conditions as indicated by the supplier (room temperature/5°C/-20°C/-70°C/etc.)
    - Flammable supplies are stored in a fire-resistant cabinet.
    - Corrosive substances are stored in corrosive resistant cabinets/trays/containers.

- Liquid chemicals are stored in the lower part of safety cabinets, solid chemicals in the upper par.
- Access to all storage areas must be restricted to authorized personnel only.
- Hazard symbols must be placed visibly on each storage area.
- Provide Material Safety Data Sheets (MSDS Sheets) for all the materials stored in each area. Keep these near the location where the items described in the MSDS Sheets are stored so that they are easily accessible for staff members

When supplies are received in the store following information should be recorded:

- Name of the supply
- Preparation date/date of receipt
- Expiry date (can often be found on internet if not visible on supply package)

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4.12 Labelling and Storing lab Equipments

Lab equipment is essential for conducting experiments, tests, and analyses in various fields of science. However, improper labeling and storage of lab equipment can lead to damage, contamination, loss, or confusion. To avoid these problems, you need to follow some basic guidelines on how to label and store your lab equipment safely and efficiently.

4.12.1 Labeling lab equipment

The first step to avoid damage is to label lab equipment clearly and consistently. Labeling helps identify the equipment, its contents and its purpose. Different methods of labeling can be used, such as stickers, tags, markers, or barcodes, depending on the type and size of the equipment. However, always use durable, waterproof, and legible labels that can withstand the conditions of the lab and follow the standard naming conventions and symbols for the equipment and the substances it contains. For example, use the International System of Units (SI) for measurements, for chemical hazards and the Biological Safety Level (BSL) for biological risks.

4.12.2 Storing lab equipment

The second step to avoid damage is to store your lab equipment properly and securely. Storing helps protect the equipment from physical, chemical, or biological harm, as well as theft or misuse. Different types of storage, such as cabinets, shelves, drawers, racks, or containers, depending on the shape and function of the equipment can be used. However, always use clean, dry, and stable storage that can prevent the equipment from falling, breaking, leaking, or corroding and also follow the standard safety and quality rules for the equipment and the materials it holds. For example, store flammable, explosive, or toxic substances in fireproof, ventilated, and locked cabinets, and store temperature-sensitive or perishable substances in refrigerators, freezers, or incubators.

4.13 Educating lab users

The next step is to educate the lab users about the proper labeling and storage of the lab equipment. Education helps inform, train, and remind the lab users of the rules, responsibilities, and consequences of handling the lab equipment. You can use different methods of educating, However, you should always use effective, interactive, and ongoing methods that can enhance the knowledge, skills, and attitude of the lab users. Te standard policies and regulations for the equipment and the lab environment must be followed. For example, the lab users must be instructed about the labeling and storage guidelines and procedures. Lab users must be supervised and provided with feedback and correction when needed.

Equipment is stored in a main Store or reserve store. It is important that a record-book (stock-book) is kept during reception and issuing of new articles Separate ledger should be used for expendable & nonexpendable equipment. A ledger balance should be kept, for example:

Date	Item	Received from	Invoice	No Received	No Issued	Balance in Stock
	X			120	29	91
	Y			40	7	33
	Z			21	16	5

4.14 Issuing the Equipment

After equipment has been ordered, and then received and recorded in the stock-book (ledger), it is issued for use when it is needed. There are three paper procedures used in issuing equipment.

1. **A ledger record:** i.e. writing the issue in the stock ledger.
2. **Issuing a voucher**: to be signed
3. **An inventory**: record of the section or lab receiving the equipment and using it.

4.14.1 A ledger Record

When an issue is written in the stock register, the balance remaining in stock can be found by subtracting the amount issued from the total in stock. When the balance is getting low, it is time to order new equipment. It is important to record issues in the stock ledger and to calculate the balance of stock remaining.

4.14.2 Issue Voucher

There is an official form on which are recorded: -

Date of issuance of the equipment or item

What is issued, and how much?

Where it is to be used?

Who is responsible (usually head of the lab section)?

Signature of person responsible for its use.

The person who signs the issue voucher takes responsibility for the care of the apparatus or equipment. Issue files must be registered and kept in the store. Duplicate copies are given to the section that receives the equipment.

4.14.3 Inventory

An inventory is a list of items that are kept at a certain place. New equipment issued must be added to the inventory. This inventory is used to check stocks of equipment in use, at intervals.

- To detect discrepancies and explain them

Convince and teach the staff to clean the equipment and keep it in good condition, to prevent transmission of infection, e.g. by a dirty or untidy instrument, dirty or damp equipment deteriorates more rapidly than equipment that is kept clean and dry

Inspection Checklist should be made to see what is present and checking it against the inventory. How often equipment should be checked depends on whether it is consumable or long lasting and whether it is liable to bark down.

Sample Question; Define Standards, Calibrators, Controls and the standard reference materials. Describe the process of issuing lab items.

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Chapter 5

Different Types of Laboratory Glassware and Plastic Ware

Objectives

This chapter is meant for teaching the students to learn about Laboratory apparatus which will allow students to control and manipulate variables, ensuring precise measurements and observations. Without the right apparatus, experiments would be prone to errors and inconsistencies, making it difficult to draw valid conclusions.

Types of Laboratory Glassware and Plastic Ware

Glassware is found in abundance in laboratories and comes in all shapes and sizes. Plastic ware are cheaper, more durable and less fragile materials. Some substances and experiments or applications still require the use of glassware. The reasons are firstly, glass is relatively inert, meaning it will not react with the chemicals or substances placed inside and thereby upset the results. It is also transparent, allowing for easy monitoring, and heat-resistant, allowing for high temperatures. Furthermore, it is easy to shape and mould into any form required.

5.1 Different types of glass instruments used in laboratories:

5.1.1 Bulb and graduated pipettes. These are used to transport specific amounts of fluids from one place to another.

5.1.2 Burettes. These are used to dispense exact quantities of liquid into another vessel.

5.1.3 Beakers. Simple containers used to hold samples and reagents.

5.1.4 Volumetric flasks. Similar to beakers, these are used to hold samples, but usually come in a conical or spherical shape with a tapering neck.

5.1.5 Condensers. Specifically used to cool heated liquid or gas.

5.1.6 Funnels. The tapered neck of a funnel allows easy pouring of a liquid into a narrow orifice.

5.1.7 Petri dishes. Shallow dishes used to culture living cells.

5.1.8 Graduated Cylinders. Similar to beakers, these cylindrical vessels have volumetric markings to allow for monitoring of volume.

5.1.9 Vials. Small bottles used to store samples or reagents.

5.1.10 Slides. Used to hold items under a microscope for inspection and study.

5.1.11 Stirring Rods. Used to mix solvents and samples together.

5.1.12 Desiccators. A container designed to absorb moisture from a substance.

With such an extensive range of glassware in the workplace and maximum precision required, it is necessary that the equipment is kept in top-quality condition. Though glass is resistant to high temperatures and most chemicals, prolonged use over a protracted period of time will inevitably lead to degradation.

As plastic material are refined and made available to manufacturers, plastic has been increasingly used to make laboratory utensils. Vessels holding or transferring liquid are designed either to contain (TC) or to deliver (TD) a specified volume. As the names imply, TC devices do not deliver that same volume when the liquid is transferred into a container, whereas the TD designation means that the lab ware will deliver that amount.
Whenever possible, routinely used clinical chemistry glassware should consist of high thermal borosilicate or aluminosilicate glass and meet the Class A tolerances. Class A Borosilicate volumetric glassware has superior thermal and chemical resistance and is better suited to glassware for storage of solutions, e.g. flasks. The use of Class A borosilicate glass for volumetric glassware means accuracy is retained over a longer

working lifetime. Glassware that does not meet Type A specifications may have twice the tolerance range despite its appearance being identical to a piece of Type A glassware.

5.2 The Different Types of Glass in the Lab

5.2.1 Borosilicate Glasses is among the most common type of glass found in laboratories and is used in beakers, vials, test tubes, flasks etc. This material has a low expansion that makes it suitable for a wide variety of laboratory applications. It has a high resistance to chemical attacks and a very low coefficient of expansion. However, there are some substances that this glass material is not inert to, like hydrofluoric acid, phosphoric acid.

5.2.2 Silica glass or Ouartz is another name for labware made from this material. These are among the most uncompromising equipment found in a laboratory. They are created at high temperatures of 2,000C by melting sand. This is normally transparent with superior thermal and optical properties.

If conducting experiments with wide temperature differences, then this is the ideal glass to be used in laboratory. There is a wide range of laboratory ware available in this material including joints, tubes, flasks, beakers and cuvettes. It can also be used to hold and store samples or chemicals. Another area of functionality is mixing chemicals for experiments and preparing solutions and in lab processes such as spectrophotometry, distillation, contained chemical reactions and chromatography.

5.2.3 Actinic Glass is tinted dark brown or amber. These can be created from any material and are named after the colour. This is done to protect light-sensitive chemical compounds from getting altered by infrared radiation, visible light and ultraviolet radiation. Amber glass is ideal for light-sensitive applications. Generally, tinted glass is used only in bottles to store chemicals in solution or powder form.

Soda Lime Glass. This glass is extremely fragile and has a low melting point. It is almost impossible to repair but it is a lower cost and can be easily made. Hence, it is used for lab equipment that is required in abundance such as pipettes, measuring cylinders, disposable test tubes and volumetric flasks.

5.2.4 A volumetric flask is calibrated to hold one exact volume of liquid (TC). The flask has a round, lower portion with a flat bottom and a long, thin neck with an etched calibration line. Volumetric flasks are used to bring a given reagent to its final volume with the prescribed diluent and should be Class A quality. When bringing the bottom of the meniscus to the calibration mark, a pipet should be used.

5.2.5 Erlenmeyer flasks and Griffin beakers are designed to hold different volumes rather than one exact amount. Because Erlenmeyer flasks and Griffin beakers are often used in reagent preparation, flask size, chemical inertness, and thermal stability should be considered. The Erlenmeyer flask has a wide bottom that gradually evolves into a smaller, short neck. The Griffin beaker has a flat bottom, straight sides, and an opening as wide as the flat base, with a small spout in the lip held upright by an octagonal or circular base. The cylinder has calibration marks along its length and is used to measure volumes of liquids.

5.2.6 Graduated cylinders do not have the accuracy of volumetric glassware. The sizes routinely used are 10, 25, 50, 100, 500, 1,000, and 2,000 mL. All laboratory utensils should be Class A whenever possible to maximize accuracy and precision and thus decrease calibration time.

5.2.7 Pipets are glass or plastic utensils used to transfer liquids; they may be reusable or disposable. Although pipets may transfer any volume, they are usually used for volumes of 20 mL or less; larger volumes are usually transferred or dispensed using automated pipetting devices or jar-style pipetting apparatus.

5.3 Classification of pipets

5.3.1 Design

A. To contain (TC)
B. To deliver (TD)

5.3.2 Drainage characteristics
A. Blowout
B. Self-draining

5.5.3 Measuring or graduated
1. Serologic
2. Mohr
3. Bacteriologic
4. Ball, Kolmer, or Kahn
5. Micropipet

5.5.4 Transfer
1. Volumetric
2. Ostwald-Folin
3. Pasteur pipets
4. Automatic macropipets or micropipets

Similar to many laboratory utensils, pipets are designed to contain (TC) or to deliver (TD) a particular volume of liquid. The major difference is the amount of liquid needed to wet the interior surface of the ware and the amount of any residual liquid left in the pipet tip. Most manufacturers stamp TC or TD near the top of the pipet to alert the user as to the type of pipet. Like other TC-designated labware, a TC pipet holds or contains a particular volume but does not dispense that exact volume, whereas a TD pipet will dispense the volume indicated.

When using a pipet, the tip must be immersed in the liquid to be transferred to a level that will allow it to remain in solution after the volume of liquid has entered the pipet without touching the vessel walls. The pipet is held upright, not at an angle. A slight suction is applied to the opposite end until the liquid enters the pipet and the meniscus is brought above the desired graduation line, suction is then stopped. While the meniscus level is held in place, the pipet tip is raised slightly out of the solution and wiped with a laboratory tissue of any adhering liquid.

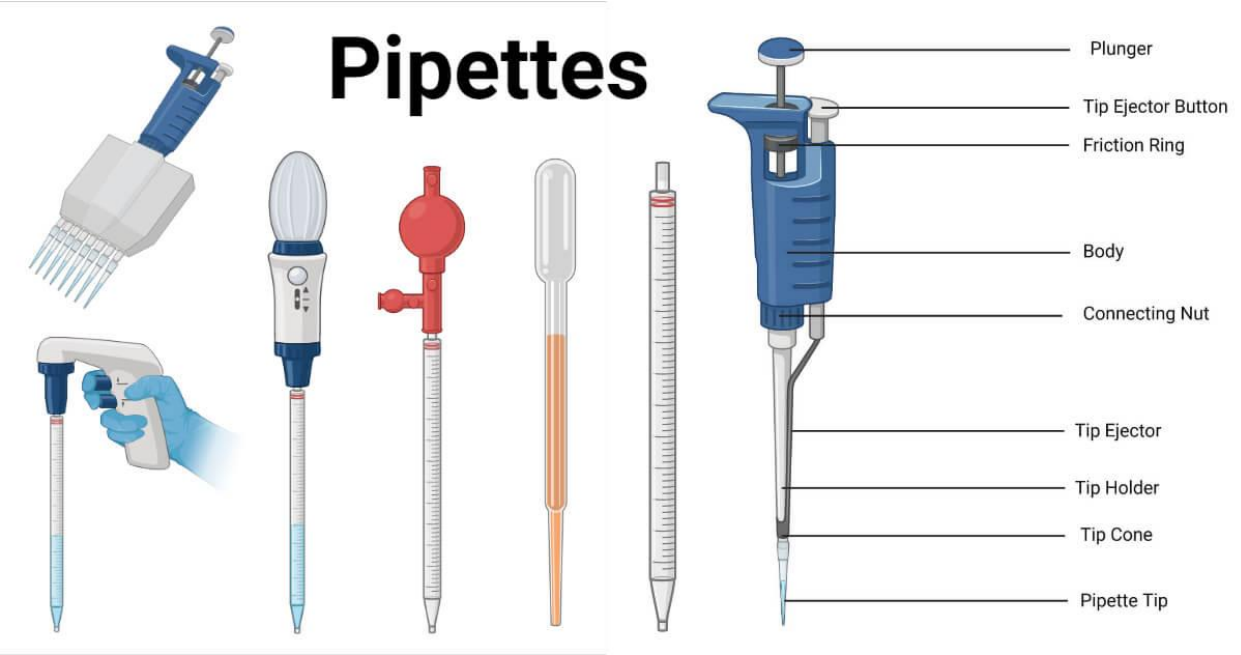
The liquid is allowed to drain until the bottom of the meniscus touches the desired calibration mark.

5.6 Types of pipettes,

5.6.1 Transfer Pipettes/Pasteur Pipettes transfer small quantities of liquids. Plastic bulb pipettes are typically not precise enough to be used for exact measurements but their glass counterparts are more precise. Generally, this type of pipette is considered inexpensive enough to be disposable. However, as long as the glass point is not chipped, the Pasteur pipette may be washed and reused. The volumes are usually marked on the stem, though the markings are not particularly accurate.

5.6.2 Serological Pipettes have a plain, narrow tube which extends to a tip and graduates uniformly along its length.

5.6.3 Mohr pipette, calibration marks may be confined to the stem.



Pipette and parts

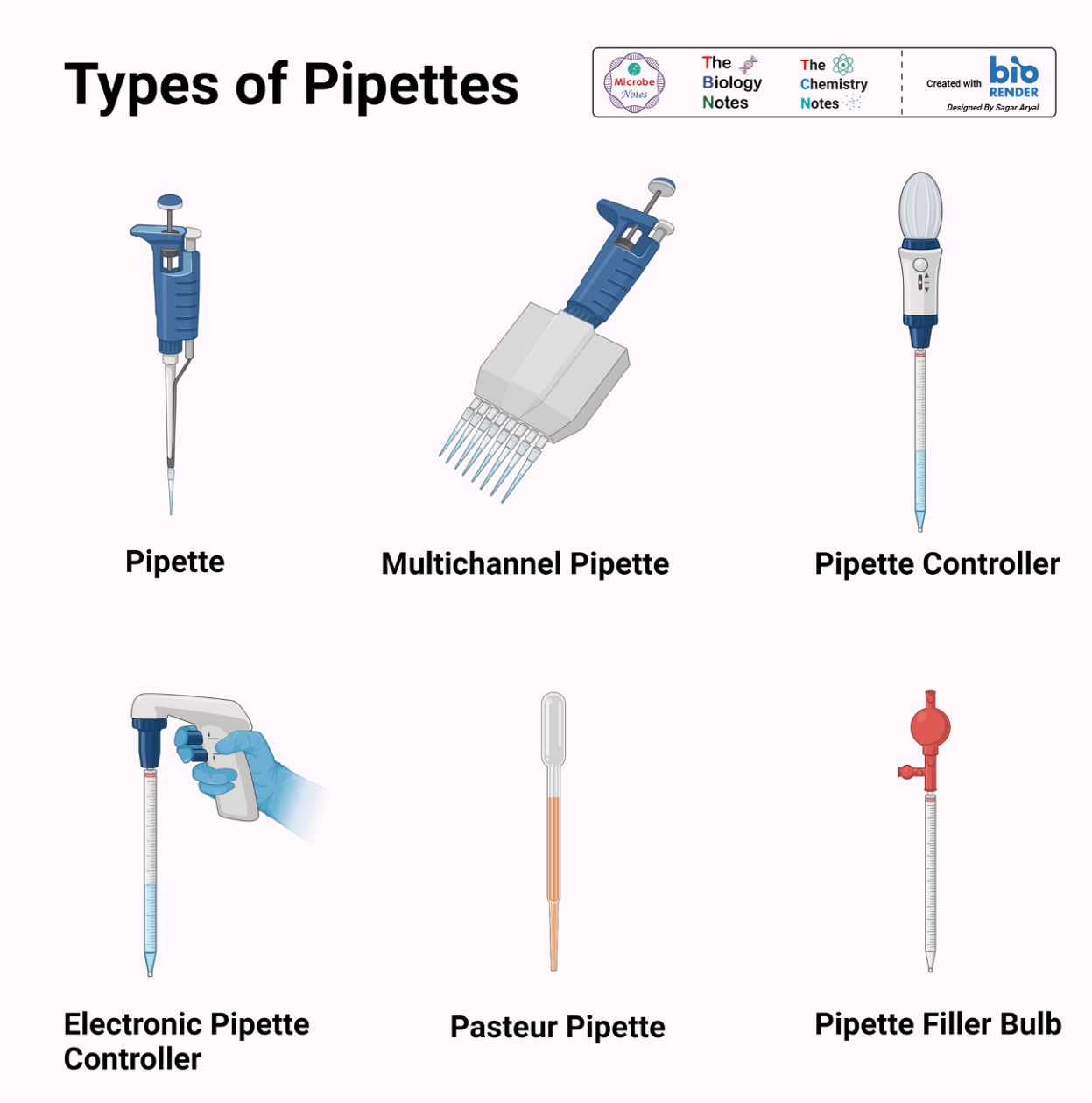
5.6.4 Volumetric Pipettes offer very accurate volume measurement of a solution. They have a large bulb with a long narrow portion. This portion has a single graduation mark, as it is calibrated for a single volume such as 10, 25, and 50 mL. These pipettes are commonly used in analytical chemistry to make laboratory solutions from a base stock as well as prepare solutions for titration.

5.6.5 Pipetting Aids are used with disposable pipettes. These aids range from a basic rubber bulb to electronic controllers that help ease the repetitive nature of some pipetting applications.

5.6.6 Ostwald-Folin Pipette is designed for the measurement of viscous fluids, e.g., whole blood, and is typically used in medical laboratory settings.

5.6.7 Graduated pipettes with volume marked in well-defined increments along the side of the tube, allowing for a high degree of precision (though slightly less than

volumetric types) when measuring out solutions. Smaller pipettes can work with smaller quantities, while larger capacities are also available for more voluminous materials.



5.6.8 Air Displacement Pipettes are highly accurate pipettes that will deliver measured volumes of liquid according to the user's adjustments. They are operated

through piston-driven air displacement mechanisms. As one depresses the plunger, a ceramic or metal piston sheathed within an airtight sleeve will rise, creating a vacuum that suctions up the target solution, and this can then be raised, moved, and released as needed.

5.6.9 Positive displacement pipettes are like air displacement types. The main difference is that they use a disposable plastic micro syringe at their tips that comprise the movable piston and capillary. These pipettes are used in instances where contamination needs to be avoided at all costs, such as when handling DNA specimens or where highly viscous or volatile substances need to be handled in small volumes.

Sample Question; Describe the types and parts of a pipette.

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Chapter 6

Water for Clinical Chemistry

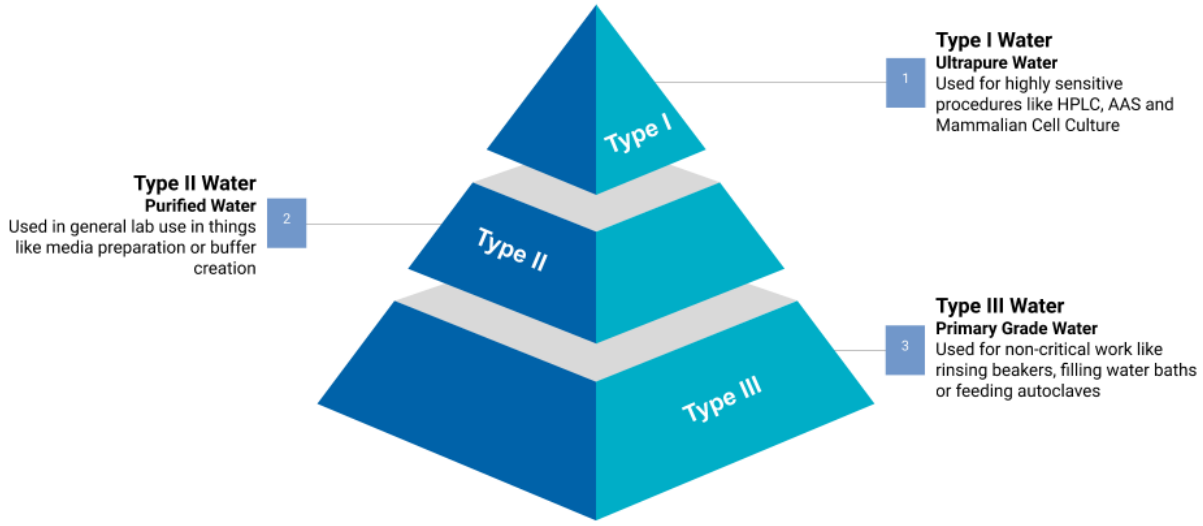
Objectives;

After studying this chapter, students will learn two significant objectives for the importance of water in the clinical setting: the need to comply with norms or guidelines (those of the Clinical and Laboratory Standards Institute, CLSI) and the sensitivity of the chemistries themselves to water quality.

6.1 Water for Clinical Chemistry

In the clinical laboratory, water is a key factor. Clinical and Laboratory Standards Institute (CLSI) guideline was written to ensure the use of a basic level of water purity so that clinical chemistry assays could be run safely. It should be noted that basic water requirements in the clinical laboratory include:

- Removing particulates, which can clog needles and interfere with spectroscopic detection.
- Checking silica levels to prevent the formation of deposits in the needles, which can modify the volumes dispensed
- Reducing the level of organics and other molecules which have high UV absorbance and fluorescence properties and can interfere in analysis.



6.2 Types of water

6.2.1 Type 1 Water (Ultrapure Water),

Type I grade water or Ultrapure Water, is the purest form of water and is used for the most critical applications and advanced analytical procedures. It is used for,

• Cell and Tissue Cultures

• Liquid Chromatography, including High Performance Liquid Chromatography (HPLC), • Gas Chromatography

• Molecular Biology

Type I can also be used in applications that require Type II water. This is quite a common practice that can help to avoid the generation of by-products during applications.

6.2.2 Type II Water

Type II water grade doesn't have the same pureness of Type I, but still maintains high levels of purity. It is a good water for clinical analyzers as the calcium build-up is reduced with this water type. It can be used in,

• General Lab Practices

• Microbiological Analysis and preparation

• Electrochemistry

• General Spectrophotometry

It can also be used as feed water for Type I water production.

6.2.3 Type III Water (RO Water)

Type III grade water, also known as RO water, is water produced through reverse osmosis. Of all the pure water types it has the lowest level of purity, but is typically the starting point for basic lab applications, such as cleaning glassware, heating baths or media preparation. It can also be used as a feed water for Type I water production.

6.2.4 Type IV & Feedwater

The last type of pure water is the feed water which is normally called distilled water. This type of pure water is produced through a **laboratory water purifier** for experimental works.

Type IV has less importance than the above three types of pure water. This type of water is used in various types of small-scale lab experiments as well as helpful in producing pure water of types I and II. Type IV pure water is produced through the reverse osmosis of tap water. **Lab water purification systems are** all prevalent in the market to get this type of pure water.

6.3 PURIFICATION TECHNOLOGIES

A combination of purification technologies is used in the clinical laboratories. The techniques reduce contaminant levels and also ensure that the water dispensed to the clinical analyzer is of constant quality. A description of these purification techniques is as follows,

6.3.1 General **filtration** reduces the incoming particle load.

6.3.2 **Activated carbon** is used to eliminate the oxidative agents (chlorine, chloramines, fluorine) that are present in tap water to avoid the development of microorganisms.

6.3.3 **Reverse osmosis (RO)**, a membrane-based technology that has become a standard pretreatment filtration technique, is used to decrease the load of ions, organics, colloids and particulates. However, RO rejects a percentage of the contaminants. Therefore, the quality of RO water is susceptible to variations, depending on daily and seasonal variations in tap water quality.

6.3.4 **Electrodeionization** (EDI) removes ions (inorganic and organic). This technology uses semi-permeable membranes and ion exchange (IEX) resins.. No maintenance is required for this technology.

6.3.5 **Bacteria control** can be achieved in water purification systems and clinical analyzers with a variety of means, including screen membrane filtration (0.22 µM), germicidal UV 254 nm and chemical sanitization (peracetic acid, bleach, chlorine dioxide).

6.3.6 **Ultrafiltration** has been proposed as a method of eliminating bacteria by products (alkaline phosphatase, endotoxins) in immunoassays.

Laboratory requirements generally call for **reagent grade water** that, according to the Clinical and Laboratory Standards Institute (CLSI), is classified into six categories. These categories include

clinical laboratory reagent water (CLRW)

special reagent water (SRW),

instrument feed water,

water supplied by method manufacturer,

autoclave and wash water, and commercially bottled purified water.

Laboratories need to assess whether the water meets the specifications needed for its application. Most water-monitoring parameters include at least microbiological count, pH, resistivity, silicate, particulate matter, and organics. Each category has a specific acceptable limit.

Sample Question, Describe the types of water and the procedures of purification of water.

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Chapter 7

Quality Assurance

Objectives;

The objectives of teaching this chapter to students are given below,

1. Ensuring that the results provided in the lab meets established quality standards and customer requirements.
2. Ensure the result is fit for its intended purpose to diagnose the disease.
3. Ensure the product or service meets or exceeds customer expectations.
4. Quality assurance involves planned and systematic activities.

7.1 QUALITY ASSURANCE

The quality assurance system in clinical chemistry allows for the identification of errors and control actions to correct them. It is well known that laboratory errors can be classified as: **pre-analytical**, **analytical** and **post-analytical**. While pre-analytical and post-analytical errors are very difficult to identify, the analytical variability (both imprecision and inaccuracy) can be monitored with internal quality control (IQC) programs and external quality assessment (EQA) schemes. The purpose of IQC is mainly to verify the stability of laboratory estimates with time and therefore it is essentially a control of imprecision. IQC programs are based on the use of control samples which are analyzed in each analytical series. As for the criteria according to which an analytical series should be accepted or rejected, the use of practical control rules is widely spread in laboratories. Participation in EQA schemes allows the laboratory to have a retrospective estimate of its performance in terms of both imprecision and inaccuracy. In case of lack of definitive or reference methods, mean can be derived from the data obtained by all the participants.

The purpose of quality assurance (QA) is to provide tests that are relevant and affordable, and test results that are reliable, timely, and performed correctly in patients and community health care. Staff must be adequately trained and the competence of staff must be monitored. Well written and implemented Standard Operating Procedures (SOPs) covering the pre-analytical, analytical and post-analytical stages of testing are key to ensuring that results are verified and reported within the target turnaround time, using a standardized format.

QA encompasses all aspects of laboratory testing, starting with test ordering and ending with delivery of the rest result.

7.2 External Quality Assessment (EQA)

The term external quality assessment (EQA) is used to describe a method that allows for comparison of a laboratory's testing to a source outside the laboratory. This comparison can be made to the performance of a group of laboratories or to the performance of a reference laboratory. The term EQA is sometimes used interchangeably with **proficiency testing**; EQA is defined as a system for objectively checking the laboratory's performance using an external agency or facility. External Quality Assessment (EQA) / Proficiency Testing (PT) allows for a comparison of a laboratory's testing procedures to other laboratories.

EQA provides assurance to both staff and customers that testing taking place in the laboratory provides accurate and reliable results. Problems can be identified early on and corrective action can be untaken. The reliability of methods, materials, and equipment can be evaluated and training can be developed and its impact monitored. EQA participation is often a requirement for accreditation, gaining accreditation to run a laboratory.

EQA / PT alone cannot provide a complete evaluation alone; it is important to also run third party controls regularly. EQA results can also be affected by variables not relating to patient samples, including preparation, clerical functions, matrix effects, and selection of method.
If possible, every laboratory should participate in an EQA scheme that covers all testing procedures. Laboratories need to develop a management process with the objective to assure that EQA samples are treated appropriately and in the same manner. This includes, sample handling, sample analysis, record keeping, investigating deficiencies, taking corrective actions, and communicating results with laboratory staff and management. • EQA provides valuable resources and data to effectively maintain accurate and reliable results and should be seen as educational.

The External Quality Assurance Assessment Scheme (EQAAS) is a scheme organized and coordinated by WHO with a view to evaluate the technical performance and quality control of laboratories.

7.3 POINT OF CARE TESTING (POCT) AND QUALITY ASSURANCE

Point of care testing (POCT) refers to testing that is performed near or at the site of a patient with the result leading to a possible change in the care of the patient. The popularity and demand for POCT has recently seen rapid growth, this comes from the advantages including the added convenience of being able to obtain a rapid result at the patient's bedside, thus allowing immediate action, saving time and improving the potential outcome for the patient. Although there are many benefits of using POCT devices in terms of their convenience, these benefits are only true if the results produced are both accurate and reliable. Ensuring accuracy and reliability is the primary responsibility of Quality Control. EQA is strongly recommended for all point of care devices and is recommended by ISO 22870, which provides specific requirements applicable to point-of-care testing and is intended to be used in conjunction with ISO 15189.

The following features of POCT are ubiquitous:

- POCT should be simple to use.
- Reagents and consumables should have durable resistance during storage and use.
- POCT results should align with established laboratory methods.
- POCT results should align with established laboratory methods.

7.4 POCT Vs LABORATORY TESTING

POC testing can also be performed by people who have not had formal laboratory training. This includes nurses, doctors, paramedics and testing by patients themselves. There are many kinds of near point testing, including malaria antigen testing, pregnancy tests, blood glucose monitoring, urinalysis and many more.

The main advantage is the shorter time it takes to obtain a result. Typically, results may also be presented in a way that is easier to understand, but this is not always the case and results may still require a healthcare professional to interpret them safely.

These tests often require relatively easy sample collection such as body fluids (e.g. saliva or urine) or finger-prick blood. Together with other portable medical equipment, such as thermometers or blood pressure devices, they can facilitate rapid and convenient medical assessment.

POC approaches can also be more costly than laboratory based testing. There are hidden costs that may often be overlooked, such as those associated with a quality control program or equipment upkeep. However, other kinds of hidden costs such as buildings, staff and overheads can apply to laboratory testing as well.

Nonetheless, the immediacy and convenience of POC testing can balance the increased costs. Rapid results can allow a treatment plan to be put into effect quickly, and where time is critical for better care, this can make a big difference.

Even where time is not critical and is more a matter of convenience, being able to move on with diagnosis and treatment is almost always of benefit to the patient. In some circumstances, a rapid result can help to allow a safe medical discharge from hospital, shortening the length of stay and helping to reduce costs of care.

7.5 IQC, Internal Quality Control

The trueness and precision of clinical laboratory results are ensured through total quality management systems (TQM), which primarily include internal quality control (IQC) practices. Laboratory quality control (QC) ensures that the lab processes and operations run efficiently and guarantees the production of accurate and reproducible results. In addition, the QC measures developed in a lab are the building blocks for the process of certification and accreditation. Failure to integrate quality control in a laboratory can lead to several negative consequences, including the following:

- Unreliable results, which will impact the integrity of the lab and consequently any funding options and certification/accreditation process.
    - Budget implications, as more reagents are needed to carry out repeat tests and experiments.
    - Loss of customer loyalty and satisfaction.
    - Delayed diagnosis or unnecessary treatments for patients.

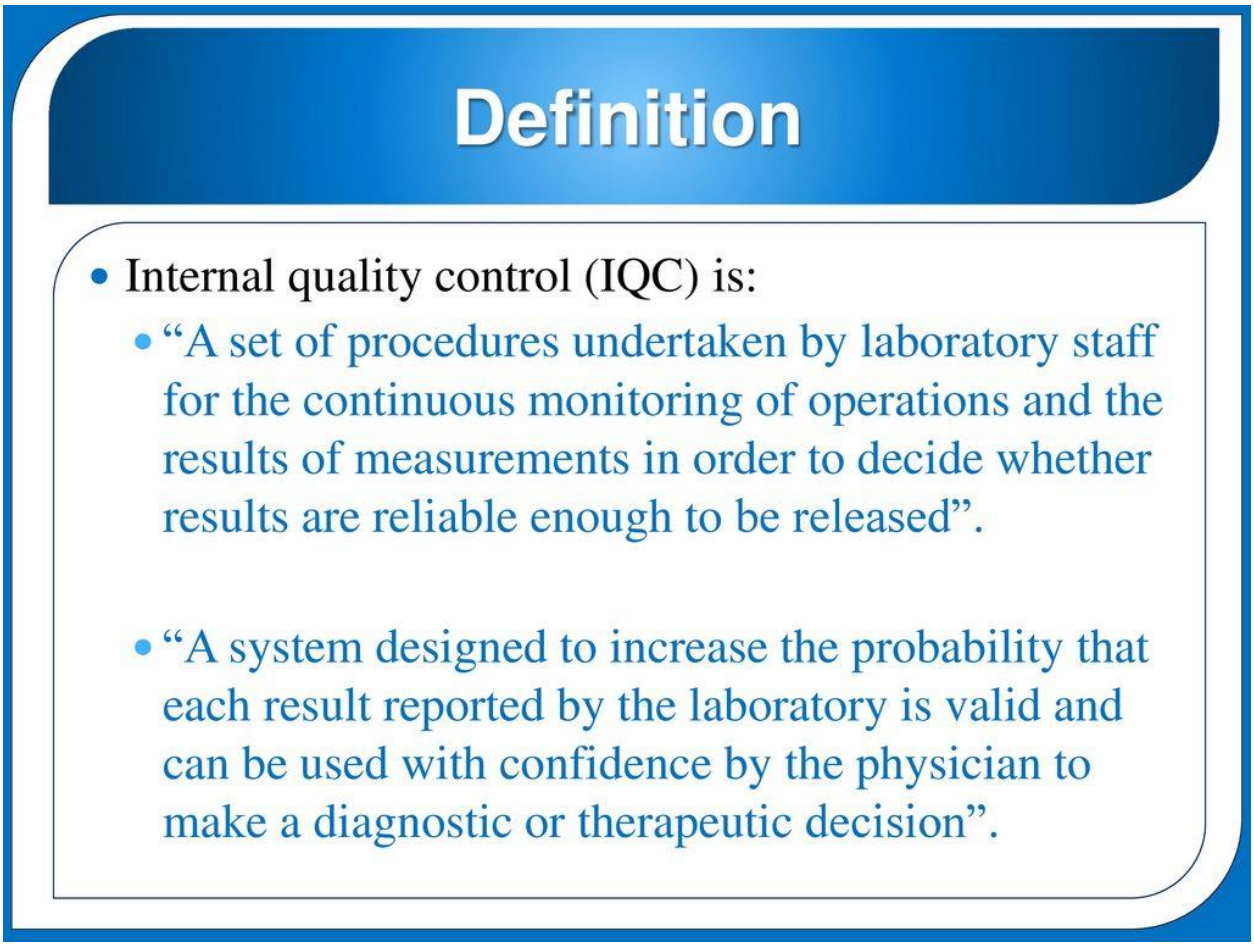
7.6 Detecting and Minimizing Laboratory Errors

In the ***pre-analytical stage***, the error occurs when test samples or materials are mishandled prior to analysis. This may include errors like sample mix-up, mislabeling, improper storage or transportation, and unsuitable sample collection methods.

To minimize pre-analytical errors, ensure that:

- Proper sample collection methods are followed by adhering to the standard operating procedure.

- Samples and test materials are clearly labeled using name or lab codes, date of collection, source, test to be done.
- Test materials are transported to the lab in proper containers and at the recommended temperature. For any potentially infectious or toxic sample, triple packaging rules outlined by the International Air Transport Association (IATA) regulations should be followed and proper warning labels attached.
- Samples and test material are properly stored to avoid negative impacts on the quality of results. Samples that might undergo degradation could lead to false results.
- It is advisable to always carry out sub-sampling. Storing aliquots of test material provides backup in cases of errors in downstream processes.



In the ***analytical stage***, errors arise during the process of testing. This could be due to the use of the wrong test reagents, the use of defective and non-calibrated equipment,

the use of the wrong proportions of reagents, and general non-adherence to standard operating procedures (SOPs).

These errors can be minimized by ensuring that:

- All laboratory equipment is well maintained and calibrated.
- A proper inventory is in place, outlining all reagents and their validity to ensure no expired reagents are in use.
- All standard operating procedures are documented and accessible.
- Actions are taken on staff that is continually non-compliant with the SOPs use.

Errors can be introduced in the ***post-analytical*** **stage** through incorrect calculations, recording, and interpretation of results.

To minimize these types of errors:

- Avoid manual calculations.
- Ensure that only well-trained personnel interpret and record results.

Quality control is designed to detect, reduce, and correct deficiencies in a laboratory's internal analytical process prior to the release of patient results. Quality control samples are special specimens used in the testing process and treated as if they were patient samples by being exposed to the same operating conditions. The purpose of including quality control samples in analytical runs is to evaluate the reliability of a method by assaying a stable material that resembles patient samples. Quality control is a measure of precision or how well the measurement system reproduces the same result over time and under varying operating conditions. Pathologists need to be involved in development of quality control protocols, the selection of quality control materials, long term review of quality control data and decisions about repeating patient samples after large runs are rejected. These quality control activities play an important part in assuring the quality of laboratory tests.

Quality control material is usually run;

at the beginning of each shift,

after an instrument is serviced,

when reagent lots are changed, after calibration,

when patient results seem inappropriate

A quality control scheme must be developed that minimizes reporting of erroneous results, but does not result in excessive repetition of analytical runs. The manufacturer

should recommend in their product labeling the period of time within which the accuracy and precision of the instruments and reagents are expected to be stable.

Each laboratory should use this information to determine; their analytical run length, considering the sample stability, reporting intervals of patient results, cost of reanalysis, work flow patterns, and operator characteristics. Quality control samples must be analyzed at least once during each analytical run. Manufacturers should recommend the nature of quality control specimens and their placement within the run. Random placement of quality control samples yields a more valid estimate of analytical imprecision of patient data than fixed placement and is preferable.

Quality control materials should have the following characteristics;

They should have the same matrix as patient specimens, including viscosity, turbidity, composition, and color For example, a method that assays serum samples should be controlled with human serum based controls.

Quality control material should be simple to use because complicated reconstitution procedures increase the chance of error. Liquid controls are more convenient than lyophilized controls because they do not have to be reconstituted.

Controls should have minimal vial to vial variability, because variability could be misinterpreted as systematic error in the method or instrument.

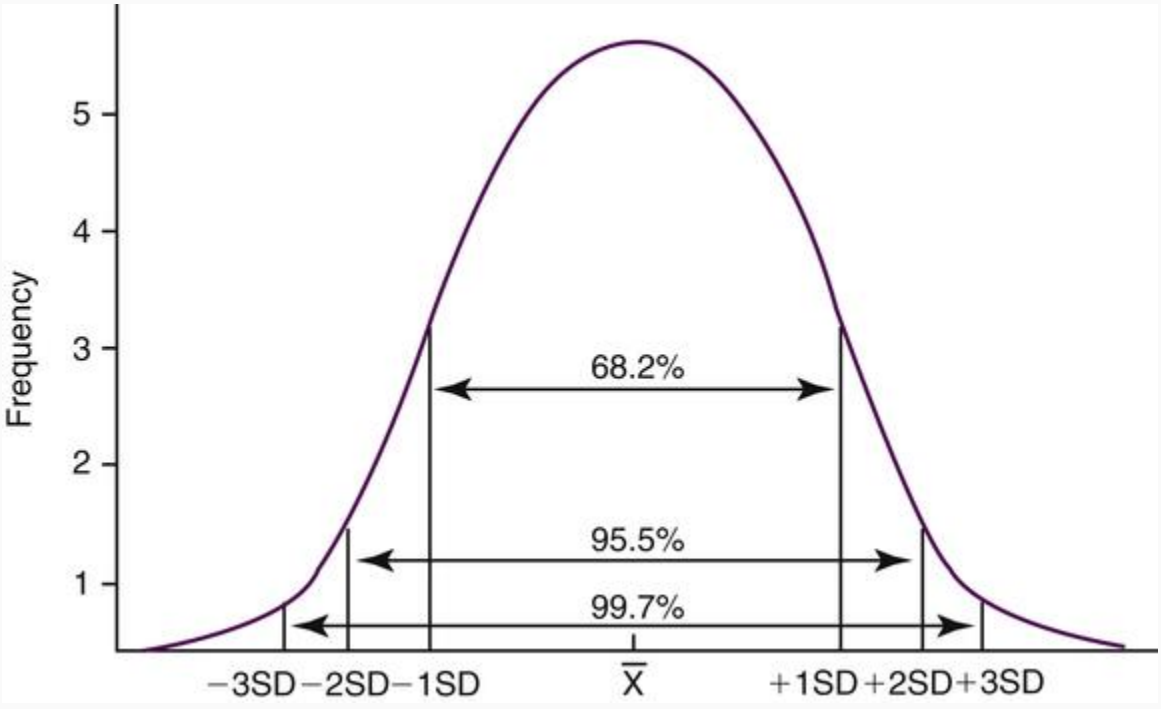
Quality control materials should be stable for long periods of time. Controls with short shelf lives necessitate frequent reordering and verification against the outgoing material, creating more unnecessary work.

Quality control material should be available in large enough quantities to last at least one year. Purchasing a large batch decreases the number of times that control ranges have to be established.

Controls should have target values that are closer to medical decision points. Quantitative tests should include a minimum of one control with a target value in the healthy person reference interval and a second control with a target value that would be seen in a sick patient .Examples include sodium controls of 140 and 115 mEq/L and glucose controls of 75 and 225 mg /dL. If three control levels are run, an abnormally low patient range should be included. Quality control levels for therapeutic drug monitoring should mirror therapeutic, toxic, and trough values. If a test is qualitative, giving either negative or positive results, a negative control and a weak positive control with a concentration at the lowest detectable level are recommended. Semi-quantitative tests should have controls at each graded level , trace, 1+, 2+, etc.

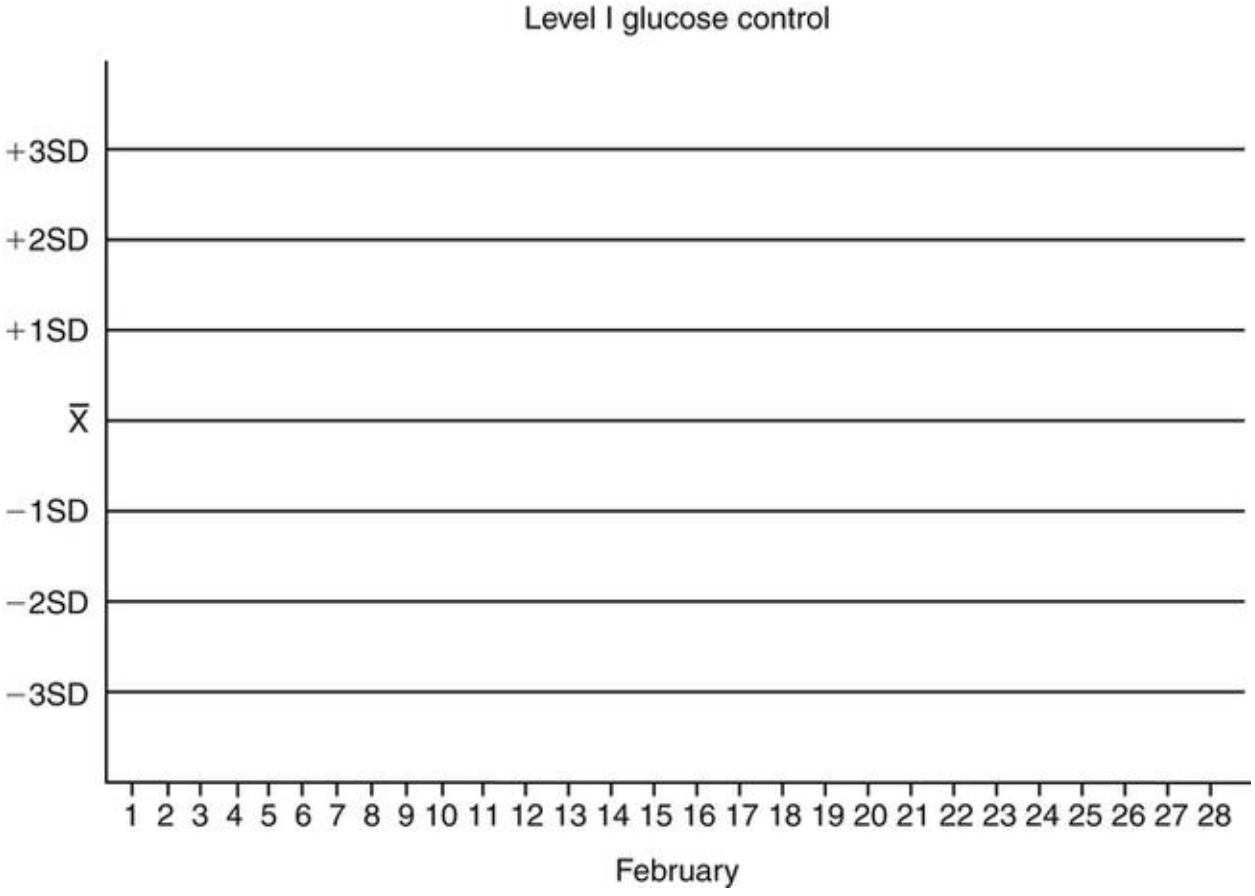
Both assayed and unassayed control material are available.Assayed controls are measured by a reference method and sold with published target values. They are more expensive than unassayed controls. .Assayed controls are recommended for physician office laboratories. Unassayed controls must be analyzed by the laboratory to determine the target value and acceptable range.Comparison studies need to be run between the current and new unassayed control materials.If the new control material is from the same manufacturer, only five samples of the new control material need to be run to establish a mean. If the mean is close to the mean of the outgoing quality control material, the new control material can be accepted. The standard deviation of the outgoing controls is adopted for use until enough data points are collected for calculation.

Interpretation of quality control data involves both graphical and statistical methods. Quality control data is most easily visualized using a **Levey-Jennings control chart**. The **dates** of analyses are plotted along the **X-axis** and **control values** are plotted on the Y-axis. The mean and one, two and three standard deviation limits are also marked on the **Y-axis**. Inspecting the pattern of plotted points provides a simple way to detect increased random error and shifts or trends in calibration. With a correctly operating system, repeat testing of the same control sample should produce a Gaussian distribution. That is, approximately 66% of values should fall between the +/- 1 s ranges and be evenly distributed on either side of mean. Ninety five percent of values should lie between the +/- 2 s ranges and 99% between the +/- 3 s limits. This means that 1 data point in 20 should fall between either of the 2 s and 3 s limits and 1 data point in 100 will fall outside the 3 s limits in a correctly operating system. In general, the +/- 2 s limits are considered to be warning limits. Values falling between 2 s and 3 s indicates the analysis should be repeated. The +/-3 s limits are rejection limits. When a value falls outside of these limits the analysis should be stopped and patient results held, and the test system investigated.



7.7 The Levey Jennings Charts

CLIA'88 and good laboratory practice require the use of at least two quality control materials per day for each non waived method to ensure accurate and reliable patient results. The results of the quality control material must be analyzed to determine if the method is "in control" before patient results are reported. One mechanism for quickly analyzing each control value is to plot the value on an individual Levey-Jennings control chart. In Levey-Jennings chart, the mean and SD ranges are plotted on the *y* axis, and the days of the month are plotted on the *x* axis. Although CLIA'88 requires the use of two levels of quality control material for automated hematology analyzers each day, many laboratories use three levels instead and spread them out over the three shifts. If the analyte was creatinin, the hemoglobin results obtained from the three different levels of controls would be plotted on three different Levey-Jennings charts, one for each level of control.



Where X is mean of the measure values. SD is the standard Deviation.

7.8 Mean and Standard Deviation,

Mean can be defined as the average of the data points. All the measured values are added and then divided by the number of values and is denoted by X.
Standard deviation (SD) is a measure of imprecision. It indicates the variability or dispersion around the mean. The distance of the measured value to the mean, it may be +1SD, +2SD, +3SD or -1SD, -2SD, -3SD.
Together, mean and SD determine acceptable ranges for a lot of control material. New control values must be calculated and acceptable ranges established for each new lot of control materials. Ideally, at least 20 samples should be tested over time for good statistical data.
The mean is calculated by adding all of the values, and dividing by the number of values. The formula is

$$\overline{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

The standard deviation (abbreviated s or SD) is calculated according to the following formula:

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \overline{x})^2}{n-1}}$$

The mean value characterizes the "central tendency" or "location" of the data. Although the mean is the value most likely to be observed, many of the actual values are different than the mean. When assaying control materials, it is obvious that technologists will not achieve the mean value each and every time a control is analyzed. The values observed will show a dispersion or distribution about the mean, and this distribution needs to be characterized to set a range of acceptable control values.

7.9 Co-efficient of Variation (CV)

The co-efficient of variation (CV) is a statistical measure of the dispersion of data points in a data series around the mean. The co-efficient of variation represents the ratio of the standard deviation to the mean, and it is a useful statistic for comparing the degree of variation from one data series to another, even if the means are drastically different from one another. The co-efficient of variation shows the extent of variability of data in a sample in relation to the mean of the population.

$CV = s/x * 100$

s = standard deviation

$\bar{x}$ = mean

7.10 Variance

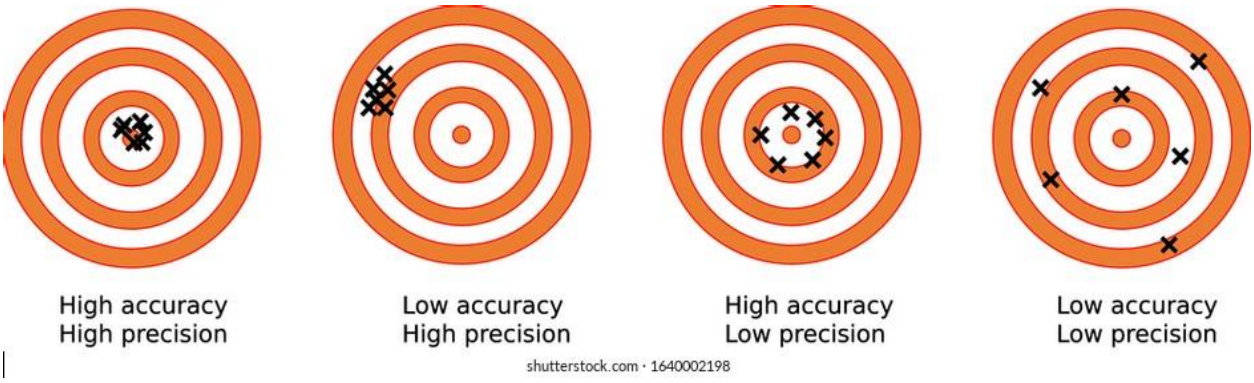
It is the square value of the standard deviation from the mean and is calculated by:

Variance = SD^2

7.11 Accuracy and Precision

The accuracy of an analytical measurement is how close a result comes to the true value. It is the degree of agreement between observed and true value of a constituent in the specimen. Determining the accuracy of a measurement usually requires calibration of the analytical method with a known standard.

Precision is defined as the degree of closeness between replicate measurements of a constituent in a specimen. It is the measure of reproducibility of test results.



7.11.1 Sensitivity It is the ability of an analytical method to produce a change in signal relative to a change in quantity, concentration or property of the analyte. I is ability of the test to identify the patients having disease.

7.11.2 is the ability of the test to correctly identify those patients without the disease. Therefore, a test with 100% specificity correctly identifies all patients without the disease.

7.11.3 Confidence Limits This is defined as percentage certainty with which values in a series will lie within a given range. This is usually expressed as mean ±2SD. A single SD value gives 68% confidence limit while ±2SD gives about 95% confidence limit.

7.11.4 Interference; The term interference describes the effect that a compound or group of compounds other than the analyte in question has on the accuracy of measurement of an analyte. Detection limit It is the ability of the method to detect the lowest concentration of a constituent in a specimen.

7.11.5 Distribution; Raw data is given on an interval scale showing the occurrence of data at each interval. This is frequency of distribution. If the same is made on the graph paper and upper ends of each line are joined a distribution curve is formed. If the curve is bell shaped it is called normal or **Gaussian distribution**. In this curve the series is symmetrically distributed on either side of the mean value.

7.11.6 Westgard rules are used for laboratory quality control using “runs” consisting of measurements of multiple samples (typically 2-4 samples), called multi-rule.

Rule	Criteria
1_{2S}	One measurement exceeds 2 standard deviations either above or below the mean of the reference range.
1_{3S}	One measurement exceeds 3 standard deviations either above or below the mean of the reference range.
2_{2S}	2 consecutive measurements exceed 2 standard deviations of the reference range, and on the same side of the mean.
R_{4S}	Two measurements in the same run have a 4 standard deviation difference (such as one exceeding 2 standard deviations above the mean, and another exceeding 2 standard deviations below the mean).
4_{1S}	4 consecutive measurements exceed 1 standard deviation on the same side of the mean.
$10_{\bar{X}}$	10 consecutive measurements are on the same side of the mean.

Sample Question; What is Quality assurance and describe the Levey Jennings charts and Westgard Rules?

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Chapter 8

STANDARD OPERATING PROCEDURES (SOPs) FOR BLOOD SPECIMENS COLLECTION

Objectives

Objectives of standard operating procedures for blood specimens collection is to obtain laboratory results that provide prevalence estimates of disease, risk factors for exam components, and baseline information on health and nutritional status of the population.

STANDARD OPERATING PROCEDURES (SOPs) FOR BLOOD SPECIMENS COLLECTION

8.1 Common SOPs

1- Correct identification of the patient and sample required.

2- Instruct the patient/attendant what is going on, explain the procedure to the patient/attendant.

3Transport the sample with a request form separately.

4- Taking care of the patient and the staff from any bio-hazard like, spills etc.

5-Completely filled request form with history to interpret results correctly.
6- Proper sample collection, clean the site of for the needle insertion.
7- Must be prepared if any complication occurs to the patient or to staff and ready for any biohazard.

8- Sample must be transported in proper boxes.

9-Place in appropriate receptacle and then into bag with a request form separately.

10-During transportation the sample should be avoided from extremes of temperatures.

11-Specimens to be avoided from shock to avoid hemolysis.

12-Avoid taking sample from the side with i/v line or to puncture twice the same vein.

8.2 ABGs (Arterial Blood Gases)

8.2.1 Transport, Processing,

1- Sample is taken in Green Vacutainer (heparinized)/heparinized syringe.
2- Take the sample from artery.
3- Sample must be transported on Ice in proper containers with ice.
4- Place in green vacutainer (Heparinized)/Heparinized syringe and then into bag with a request form separately.
5- During transportation the sample should be avoided from extremes of temperature.
6- 5 CC heparinized Blood is required in green vacutainer (heparinized)/heparinized syringe.
7- Result must be provided urgently and before 30 minutes for proper results.
8- Vacutainer is collected in yellow bags and handed over to hospital waste management department.
9- Heparinized syringe is wasted in yellow basket and handed over to hospital waste management department.
10- Which is further processed and disposed by waste management company.

8.2.2 Methods and Principles

Air bubbles that mix and equilibrate with arterial blood will shift the Pa_{O_2} toward 150 mm Hg and will lower the Pa_{CO_2}. Heparin must be added to the syringe as an anticoagulant. excess heparin can alter all three ABG measurements. After flushing the syringe with heparin, a sufficient amount usually remains in the dead space of the syringe and needle for anticoagulation without distortion of the ABG determination.

If a delay of more than 10 minutes is anticipated, the specimen must be immersed in an ice bath. Leukocytes and platelets continue to consume oxygen in the sample after it is drawn and can cause a significant fall in Pa_{O_2} Cooling will prevent any clinically important effect for at least 1 hour by decreasing the metabolic activity of these cells.

ABGs are measured with an automated analyzer. The basic components of such a unit are three electrodes, one each for determining pH, P_{CO_2}, and P_{O_2}. The pH electrode measures the potential difference between a measuring electrode (which contains the sample in contact with a special glass membrane permeable only to H^+ ions) and a reference electrode (which has a known, stable pH). From the voltage across these electrodes, the sample pH is calculated. The P_{CO_2} electrode employs an adaptation of the pH measurement. Carbon dioxide from the blood sample equilibrates across a gas-permeable membrane with a bicarbonate solution in a reaction that generates H^+ ions. The P_{CO_2} of the sample is determined indirectly by sensing the pH change in this solution. The P_{O_2} electrode determines P_{O_2} amperometrically. Oxygen from the blood sample diffuses across a semipermeable membrane and is reduced at the cathode of a polarographic electrode. This reaction produces a measurable current that is directly proportional to the sample P_{O_2}.

8.3 Serum Electrolyte's

8.3.1 Transport, Processing,

1- Sample is taken in Green Vacutainer(heparinized).
2- Sample must be transported on ice in proper bags.
3- 5 CC heparinized Blood is required in Green vacutainer (heparinized).
4- Centrifuged within 30 minutes after taking sample.
5- Keep the samples after centrifugation at 2-8 degree Celsius if not used immediately.
6- Vacutainer is collected in yellow bags and handed over to hospital waste management department.
7- Which is further processed and disposed by waste management company.

8.4 Lipid Profile

8.4.1 Transport, Processing,

1- Sample is taken in Red Vacutainer (clot activator).
2- The patient is advised to have fasting for 8 to 12 hours, should avoid eating, only can drink water.
3- 5 CC Clotted Blood is required in red vacutainer (clot activator).
4- Sample is centrifuged and processed in automated chemistry analyzer.
5- Keep the sample at 2-8 degree Celsius if not used for 72 hours after centrifugation.
6- Sample is centrifuged and processed in automated chemistry analyzer.
7- Keep the sample at 2-8 degree Celsius if not used for 72 hours after centrifugation.

8.4.2 Methods and Principles

Electrolye analyzers are used in the quantitative measurement of sodium, potassium, and chloride in whole blood, serum, or plasma. The most common method of electrolyte analysis is ion-selective electrode (ISE). The ISE method of electrolyte measurement is based on the principle of potentiometry in which the voltage that develops between the inner and outer surfaces of an ISE is measured.

$Ca^{2+,}$ Na, K, Cl^{-} are measured by ISEs.

8.5 Renal Function Test (RFTs)

No special preparation is required for the panel to test.

5 CC Clotted Blood is required in red vacutainer (clot activator).

8.5.1 Creatinine methods

There are two automated creatinine methods based on Jaffe and enzymatic principle.

Jaffe creatinine method is based on alkaline picrate. At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in the absorbance at 500 nm because of the formation of this complex is directly proportional to the concentration of creatinine in the sample.

Creatini Enzymatic principle. Creatinine in the sample is hydrolyzed by creatininase to creatine. Creatine is in turn hydrolyzed by creatinase to sarcosine and urea. Sarcosine from this reaction is oxidized by sarcosine oxidase to glycine and formaldehyde, with the concomitant production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and N-ethyl-N-sulfopropyl-m-toluidine in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 548 nm is proportional to the creatinine concentration in the sample.

8.5.2 Urea measurement principle,

Urease catalyses the breakdown of urea into ammonia and carbondioxide. The test organism is cultured in a medium containing urea and the indicator phenol red. Urease, the enzyme will hydrolyse urea to give ammonia and carbondioxide. With the release of ammonia, the medium become alkaline shown by change in color of indicator to reddish pink.

Urea + H2O————urease enzyme———>Ammonia + CO2

The basic principle of this test is to check the color change of indicator phenol red used in the media caused due to the degradation of urea and formation of basic product i.e NH3. The change in the color of the medium from orange to pink red due to the rise in pH indicates the positive test, while the unchanged color of media indicates negative test.

8.6 Liver Function Tests (LFTs)

1- Sample is taken in Red Vacutainer (clot activator).
2- Specimens to be avoided from light for **bilirubin** and shock to avoid hemolysis.
3- Sample is processed and tested as early as possible otherwise the activity of liver enzymes is reduced with time.

Mmethods for the assay of total bilirubin in serum or plasma are based on the azocoupling of bilirubin and diazonium ions. Basically, bilirubin reacts with diazotized sulfanilic acid under formation of an azopigment and hydroxypyrromethene-carbinol which reacts with a further molecule of diazotized sulfanilic acid, resulting in formation of azopigment. This last reaction produces two diazotized isomers from unconjugated bilirubin and two different glucuronidized azopigments plus two monomers. The azopigments produced serve as indicators. Although direct bilirubin (i.e., bound to glucuronic acid) reacts quickly when the diazo reagent is added to the specimen, to produce azobilirubin, indirect

bilirubin (i.e., unconjugated) still produces azobilirubin, but reacts much more slowly (supplementation of ethanol can accelerate the reaction). Caffeine and sodium benzoate are used as accelerators, and diazotized sulfanilic acid is used as the diazo component. After the completion of the reaction, ascorbic acid is added to eliminate the excess diazopigment, thereby stopping the azocoupling. In a neutral medium, the reaction producces red color at a maximum absorption of 530 nm. The addition of alkaline tartrate further leads to a shift in the absorption maximum to a 598-nm wavelength, at which the solution shift to a blue color. The bilirubin content is then assessed by photometric quantification of the color change.

8.6.1 Aminotransferses,

Hemolysis can cause spuriously increased activity because of enrichment by erythrocyte aminotransaminase. Heparinized, EDTA, citrated, or oxalated plasma are generally acceptable but may cause problems with specific reagent-instrument systems. Serum transaminase activity is stable at room temperature for several hours or up to 3 days at 4°C. Freezing may result in loss of activity and is not recommended.

8.6.2 Determination of ALT values

It is based on a transamination reaction that is coupled with an indication reaction. The donor of the amino group in the transamination reaction is alanine. For the indication reaction, we use the enzyme lactate dehydrogenase (LD), which also performs the function of an enzyme ensuring the reduction of endogenous oxoacids.

In the first reaction catalyzed by ALT, pyruvate is formed from alanine, which is subsequently reduced to lactate in the indicative reaction catalyzed by LD, which is added to the reaction mixture together with NADH.

The reduction of pyruvate to lactate is accompanied by a decrease in NADH, which is manifested by a decrease in absorbance at 334, 340 or 365 nm. The catalytic concentration of ALT is proportional to the decrease in absorbance.

8.6.3 Determination of AST values

Similar to ALT, it is based on a transamination reaction coupled with an indicator reaction. The donor of the amino group in the transamination reaction is aspartate, and the enzyme for the indicator reaction is malate dehydrogenase (MD).

- *In the first enzyme reaction, catalyzed by AST, oxaloacetate is formed. The latter is reduced to malate by malate dehydrogenase in the next indicative reaction, with simultaneous oxidation of NADH to NAD+.*
- *Based on the decrease in NADH, we will determine the activity of AST (proportional to the decrease in absorbance at 334, 340 or 365 nm).*
- *Pyridoxal-5'-phosphate is present in the reaction mixture, which ensures sufficient AST saturation and thus full enzyme activity. The presence of lactate dehydrogenase is necessary to ensure the reduction of endogenous pyruvate (preventing falsely higher results).*

8.7 THYROID PROFILE

1. 3CC Clotted Blood is required in red vacutainer (clot activator).
2. Sample is centrifuged and processed in automated chemistry analyzer.
3. Keep the sample at 2-8 degree Celsius if not used for 72 hours after centrifugation.
4. Time of taking sample may be considered as hormones in morning are raised.

Thyroid Hormones are measured by using the technique of chemiluminescnce (CLISA) which is described in serology section.

8.8 Glucose Testing

Blood glucose tests fall into several categories, including:

fasting, For a fasting blood glucose test, can't eat anything for 8 to 12 hours before the test but can drink only water. Drinking water before blood test may make it easier to take blood.

random (non-fasting) taking blood sample for testing glucose any time in 24 hours and don’t require to not eat or drink before the test.

- oral glucose tolerance test (OGTT), a 2-hour test
- post-prandial, which means testing after consuming food or drink

Medications that can affect blood glucose levels include:

Steroids, birth control pills, diuretics, aspirin, antipsychotic drugs, etc.

Severe stress can also cause a temporary increase in your blood glucose, like

Major surgery, trauma, heart attack, stroke.

Finger-prick tests (POCT) It is used to monitor glucose levels at home using a blood glucose meter or a continuous glucose monitor (CGM).

Sample is collected in grey top tube. This tube contains potassium oxalate as an anticoagulant and sodium fluoride as a preservative – used to preserve glucose in whole blood and for some special chemistry tests.

8.8.1 A glucose challenge test is used to test for gestational diabetes in pregnancy. If blood glucose level is higher than normal, then gestational diabetes is diagnosed. You'll need an oral glucose tolerance test (OGTT) to get a diagnosis. This test may be done to check whether a woman has developed diabetes associated with pregnancy (gestational diabetes).

8.8.2 An oral glucose tolerance test (OGTT) is used to diagnose gestational diabetes, and type 2 diabetes and prediabetes in people who aren't pregnant. A blood sample will be taken before a sugary drink and then again, every hour for the next 2 or 3 hours. It is sometimes also done if it is thought that the body doesn't control glucose levels normally but not badly enough to be called diabetes. This is referred to as pre-diabetes (also called impaired glucose tolerance or non-diabetic hyperglycaemia). The oral glucose tolerance test is not now usually used to diagnose diabetes.

- Additional diabetes tests, such as the HbA1c test, may be needed if your doctor thinks you may have diabetes. HbA1c level may be done every 2-6 months. This test measures recent average blood sugar (glucose) level. Because it is an average measurement, no need to fast on the day of the test. The test measures a part of the red blood cells. Glucose in the blood attaches to part of the red blood cells. This part can be measured and gives a good indication of average blood glucose over the previous 2-3 months. HbA1c results used to be expressed in percentages in line with the Diabetes Control and Complications Trial (DCCT). The non-diabetic 'normal' range is 4-6%.

Sample Question; Describe three SOPs for collection of blood specimens. Describe methods of analysis of creatinine?

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Chapter 9

Urine Examination

Objectives,

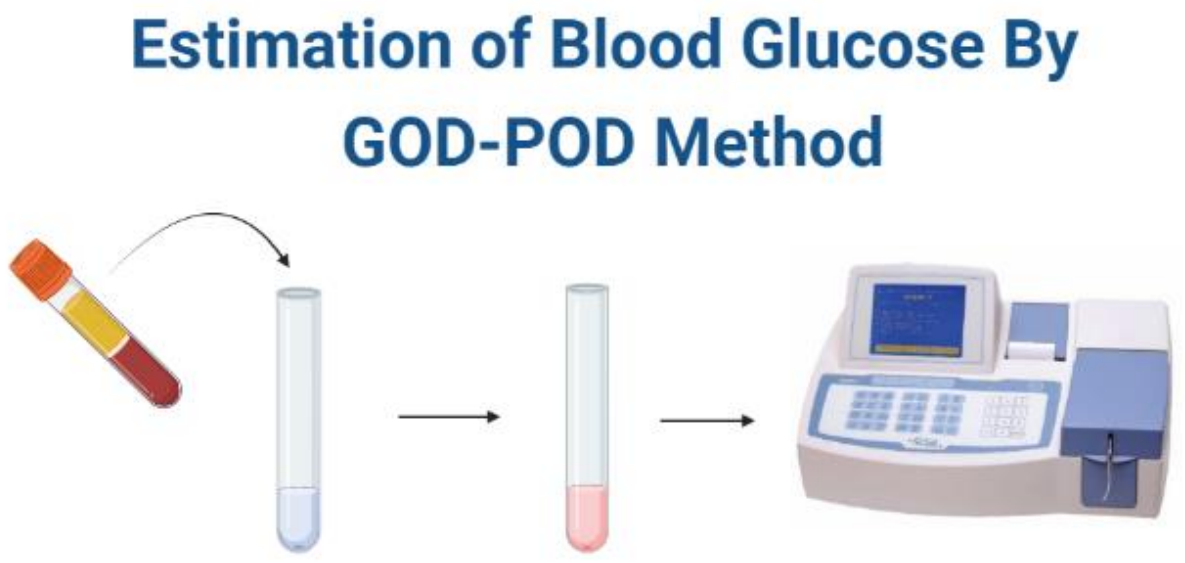
After studying this chapter students will learn to detect and manage a wide range of disorders, such as urinary tract infections, kidney disease and diabetes. A urinalysis involves checking the appearance, concentration and content of urine. For example, a urinary tract infection can make urine look cloudy instead of clear.

Urine Examination

9.1 Urine test for sugar (glucose)

Urine does not normally contain glucose. The kidneys filter blood, keeping substances the body needs, while getting rid of waste products. Kidneys constantly reabsorb glucose so that it doesn't enter urine. However, if the glucose level goes above a certain level, the kidneys can't reabsorb all of the glucose. This means that some glucose will 'spill' through the kidneys into the urine.

A simple urine dipstick test can detect glucose in a sample of urine. In a dipstick test a doctor or nurse uses a special chemical strip which he/she dips into a sample of your urine. Colour changes on the strip show whether there is glucose in the urine sample. If glucose is in the urine, patient is likely to have diabetes mellitus
.It is important to recognize that the preanalytical handling of blood samples intended for glucose measurement can influence the laboratory results. When left unprocessed, glycolysis occurs in the cellular component of a blood sample and may consume 5%–7% of the sample's glucose content per hour.



The enzyme glucose oxidase reacts with glucose, water, and oxygen to form gluconic acid and hydrogen peroxide. The hydrogen peroxide can then be used to oxidize a chromogen or the consumption of oxygen measured to estimate the amount of glucose present. Glucose oxidase is specific for β-D-glucose, so cross reaction with other sugars is not a problem. In aqueous solution, approximately 66% of D-glucose is in the β state and 34% exists as α- D-glucose. The rate of interconversion is pH and temperature dependent. Some methods add a glucomutarostase to the reagents to speed up the conversion to the beta anomere, but this does not seem to alter the clinical results. The measurement of generated hydrogen peroxide is not as specific as the first glucose oxidase reaction. Numerous reducing substances can potentially inhibit the oxidation of the chromogen. Although uric acid and creatinine, even in uremic patients, seem to have little effect on the results, ascorbic acid will yield spuriously low blood glucose measurements. The high concentration of uric acid found in urine will affect the result and so **glucose oxidase** methods are not directly applicable to urine samples. The measurement of oxygen consumption using an oxygen-specific electrode avoids the problem of interfering reducing agents. In general, the glucose oxidase method is relatively inexpensive and specific.

9.2 GLOMERULAR FILTERATE AND URINE ANALYSIS

9.2.1 Urine production:

Urine is produced and excreted by the kidneys. Each kidney contains over 1 million functional units called nephrons. The filtration part of each nephron consists of a cup-shaped structure called the Bowman's capsule which surrounds a mass of blood capillaries called the glomerulus. Each Bowman's capsule leads into a complex nephron tubule (renal tubule). The pressure in the glomerulus is higher than that in the capsule and this results in water, glucose, electrolytes, amino acids and the waste products of metabolism (urea, creatinine, uric acid) passing from the blood into the capsule. The water and substances filtered from the blood as it passes through the kidneys is known as the glomerular filtrate. About 2 ml of glomerular filtrate is normally produced per second.

9.2.2 Glomerular filtration rate (GFR).

A major measure of kidney function is the **glomerular filtration rate** (**GFR**). The glomerular filtration rate is the flow rate of filtered fluid through the kidney.

9.2.3 Creatinine clearance rate (**CrCl**) is the volume of blood plasma that is cleared or creatinine per unit time and is a useful measure for calculating the GFR. To calculate creatinine clearance, the creatinine level in the urine is multiplied by the total volume of urine produced over 24 hours. This is then divided by the amount of creatinine in the blood. The final value is converted to milliliters of blood per minute (mL/min).

Creatinine Clearance is measured by the formula = $U_x V / P$

Where

U= urinary concentration of creatinine

V= urinary volume

P= plasma concentration of creatinine

The normal range of GFR, adjusted for body surface area, is 100–130 average 125 mL/min/1.73m^2 in men and 90–120 mL/min/1.73m^2 in women younger than the age of 40. In children, GFR measured clearance is 110 mL/min/1.73 m^2 until 2 years of age in both sexes, and then it progressively decreases. After age 40, GFR decreases progressively with age, by 0.4–1.2 mL/min per year.

9.2.4 Estimated Glomerular Filteration Rate, eGFR

eGFR is estimated GFR and is based on a patient's serum creatinine level, age, sex and race. This is usually calculated by the laboratory analyzing the blood sample and reported along with the serum creatinine result. A number of recognized and validated formulae have been used for this purpose including the MDRD and CKD-EPI equations. The correction for body surface area "per 1.73m^2" which is important for certain patient groups, e.g. amputees, extremes of body habitus. It is best to follow the locally calculated eGFR if possible although can be calculated using an eGFR calculator.

Creatinine, a waste product that comes from muscle activity, is a key indicator of kidney function. When kidneys are working well they remove creatinine from the blood; but as kidney function slows, blood levels of creatinine rise. The level of creatinine in the blood is a useful guide to kidney function or the glomerular filtration rate (GFR). The terms kidney function and GFR should be considered as interchangeable. Formulas which combine the serum creatinine level with other information about a patient such as their age, sex and race, can provide a more accurate measure of kidney function which is termed the estimated GFR or eGFR. The eGFR is usually calculated and reported by the laboratory measuring the creatinine level. If not, it can be calculated using an online eGFR calculator.

Acute kidney injury (AKI), also known as acute renal failure (ARF), is a sudden episode of kidney failure or kidney damage that happens within a few hours or a few days. AKI causes a build-up of waste products in blood and makes it hard for kidneys to keep the right balance of fluid in body. AKI can also affect other organs such as the brain, heart, and lungs. Acute kidney injury is common in patients who are in the hospital, in intensive care units, and especially in older adults.

A patient is said to have chronic kidney disease (CKD) if they have abnormalities of kidney function or structure present for more than 3 months. The definition of CKD includes all individuals with markers of kidney damage or those with an eGFR of less than 60 ml/min/1.73m^2 on at least 2 occasions 90 days apart with or without markers of kidney damage. Markers of kidney disease include: albuminuria i.e. Albumin to creatinine Ratio (ACR) > 3 mg / mmol, hematuria, electrolyte abnormalities due to tubular disorders, renal

histological abnormalities, structural abnormalities detected by imaging (e.g. polycystic kidneys, reflux nephropathy) or a history of kidney transplantation.

CKD is classified based on the eGFR and the level of proteinuria. Patients are classified into 5 stages, based on the eGFR, and A1-A3 based on the ACR (albumin : creatinine ratio) as detailed below:

- **Stage 1** with normal or high GFR (GFR > 90 mL/min)
- **Stage 2** Mild CKD (GFR = 60-89 mL/min)
- **Stage 3A** Moderate CKD (GFR = 45-59 mL/min)

  Stage 3B Moderate CKD (GFR = 30-44 mL/min)
- **Stage 4** Severe CKD (GFR = 15-29 mL/min)
- **Stage 5** End Stage CKD (GFR <15 mL/min)

Albumin:creatinine ratio (ACR) is the preferred test for detection of small amounts of albumin in the urine. Proteinuria and microalbuminuria are both signs of renal involvement in the disease process and are risk factors for cardiovascular (CVD) morbidity and mortality. Measurement forms part of the diagnosis, staging and monitoring of chronic kidney disease (CKD). ACR is also the recommended method for people with diabetes. ACR ≥3.0 mg/mmol for both men and women is clinically significant. Excess amounts of protein in urine are a marker of kidney damage and increase kidney disease and CVD risk.

<3 mg/mmol: normal to mildly increased
30 mg/mmol: moderately increased, relative to young adult level. Regard aconfirmed ACR of 3 mg/mmol or more as clinically important proteinuria.
>30.0 mg/ mmol: severely increased, including nephrotic syndrome (urine ACR usually >220 mg/ mmol)

9.2.5 Volume of Urine

Polyuria The volume of urine excreted is usually between 1–2 liters per 24 hours. An increase in the volume of urine is called **polyuria.** It occurs in diabetes mellitus due to an increase in the osmolality of the filtrate preventing the normal reabsorption of water (osmotic diuresis). Polyuria also occurs when the secretion of the antidiuretic hormone ADH is reduced, e.g. in diabetes insipidus.

Oliguria A decrease in the volume of urine excreted is called oliguria**.** It occurs when the renal blood flow and/or, glomerular filtration rate is reduced.

Severe dehydration

Cardiac failure

Acute glomerulonephritis (inflammation of the kidney glomeruli)

Early stages of acute tubular necrosis.

If severe oliguria progresses to a complete cessation of urine flow, this is called **anuria** and is usually due to severe damage to the renal tubules.

9.2.6 Appearance

Normal fresh urine is clear and pale to dark yellow. The yellow colour is due to the pigments urochrome, urobilin, and porphyrins. When normal urine has been allowed to stand for some time, a white phosphate deposit may form if the urine is alkaline (dissolved by adding a drop of acetic acid) or a pink uric acid deposit may form if the urine is highly acidic or concentrated (disappears on warming).

The appearance of urine may be altered in many conditions including:

● Urinary tract infections UTI, in which the urine appears cloudy because it contains pus cells and bacteria.

● Urinary schistosomiasis in which the urine often appears red and cloudy because it contains blood (haematuria).

● Malaria haemoglobinuria (blackwater fever) and other conditions causing intravascular haemolysis in which the urine appears brown and cloudy because it contains free haemoglobin (haemoglobinuria).

● Jaundice in which the urine may appear yellow brown or green-brown because it contains bile pigments or increased amounts of urobilin.

9.2.7 Composition and pH,

The composition of urine is greatly dependent on diet and the metabolic activities of the body's cells. In health, urine contains about 95% of water, electrolytes and the waste products of metabolism including urea, uric acid, and creatinine. Urine also contains surplus acids and alkalis (in buffered form) to maintain the acid-base balance in the body. The normal fresh urine is slightly acidic, pH around 6.0.

Abnormal chemical constituents in urine include:

9.2.8 Proteins, found in urinary schistosomiasis, urinary tract infections, nephrotic syndrome, pyelonephritis and glomerulonephritis, renal tuberculosis, pregnant women and sometimes in healthy young individuals.

9.2.9 Glucose and **Ketones** found in the urine of diabetic patients and occasionally in some healthy individuals.

9.2.10 Bilirubin is found in persons with hepatocellular jaundice or obstructive jaundice.

9.2.11 Urobilinogen in increased amounts, is found in the urine of those with conditions causing hemolysis.

9.2.12 Nitrite found in the urine of patients with urinary tract infection caused by nitrate-reducing bacteria.

9.2.13 Blood in urine is found in infections, acute glomerulonephritis, sickle cell disease, infective endocarditis, calculi (stones) in the urinary tract, malignancy of the urinary tract, and hemorrhagic conditions. Free hemoglobin in urine can be found in malarial hemoglobinuria.

9.2.14 specific gravity,The normal specific gravity of urine varies from 1.002–1.025 depending on the state of hydration of the person and the time of day. It is highest at the beginning of the day. Normal mass density is mainly proportional to the urea and sodium concentrations in the urine.

In renal failure, the ability of the kidneys to concentrate and dilute urine is reduced. Normal concentrating power can be assumed if the **specific gravity** of a urine sample is 1.018 or over. Unusually high **specific gravity** measurements may be found when the urine contains glucose, protein etc.

9.2.15 Proteinuria:

Most plasma proteins are too large to pass through the glomeruli of the kidney. The small amount of protein which does filter through is normally reabsorbed back into the blood by the kidney tubules. Only trace amounts of protein (less than 50 mg per 24 h) can therefore be found in normal urine. These amounts are insufficient for detection by routine laboratory tests. When more than trace amounts of protein are found in urine this is termed proteinuria. The condition is often referred to as albuminuria because when there is glomerular damage most of the protein which passes through the glomerular filter is albumin because this protein molecule is smaller than most of the globulins.

The following methods are used to test for proteinuria: Protein reagent strip tests detect mainly albumin. The test area is saturated with the dye tetrabromophenol blue or a tetrabromophenolphthalein ethyl ester and buffered to an acid pH. In the presence of protein there is a change in the colour from light yellow to green-blue. The urine specimen must be fresh. The strips are very sensitive, detecting as little as 0.1g/l of albumin. The reaction is unaffected by turbidity in the urine.

Proteins such as globulin, mucoprotein, haemoglobin, and Bence Jones protein give only weak reactions.

False positive results may be obtained when the urine is contaminated with disinfectants (quaternary ammonium compounds or chlorhexidine). Strongly alkaline urine can give false positives results and also if the urine is contaminated with vaginal or urethral secretions. **Sulphosalicylic acid test** is based on the precipitation of protein, particularly albumin, by sulphosalicylic acid.

Causes

- Glomerular or tubular urinary disease.
- acute glomerulonephritis
- HIV associated renal disease and treatment with nephrotoxic antiretroviral drugs.
- Pyogenic or tuberculous pyelonephritis.
- Severe lower urinary tract infection.
- Nephrotic syndrome which is a condition characterized by heavy proteinuria and oedema. The oedema is caused by a reduction in the colloid osmotic pressure due to a fall in the level of plasma albumin brought about when proteinuria rises to 5 or 10 g/l per day.
- Eclampsia
- Urinary schistosomiasis which is accompanied by both proteinuria and haematuria.
- Severe febrile illnesses including malaria.
- Occasionally in diabetes. Diabetic nephropathy sometimes causes a nephrotic syndrome.
- Hypertension.

Whenever proteinuria is found, the urine should be examined for bacteria, pus cells, red cells,and casts.

9.2.16 Bence Jones protein in urine

Bence Jones protein is an abnormal low molecular weight globulin. It may be found in the urine of patients with multiple myeloma which is a cancerous disease of the plasma cells, mainly affecting bone.

Hematological investigations are also required, including a bone marrow examination.

9.3 Glycosuria (glucose in urine):

Almost all the glucose which passes from the blood into the glomerular filtrate is normally reabsorbed back into the circulation by the kidney tubules. Usually less than 0.8 mmol/l is excreted in the urine. The term glycosuria (glucosuria) refers to the presence of more than the usual amount of glucose in the urine.

9.3.1 Renal threshold for glucose

The highest level that the blood glucose reaches before glycosuria occurs is referred to as the renal threshold for glucose.

In health it is about 9–10 mmol/l (160–180 mg/dl) which represents the normal maximum reabsorptive capacity of the kidney tubules. The threshold is lower in persons suffering from renal insufficiency

9.3.2 Glucose reagent strip tests; **Benedict's test** is recommended in preference to Benedict's test. Glucose is oxidized to gluconolactone by the enzyme glucose oxidase with the release of hydrogen peroxide. A dye is then oxidized by the hydrogen peroxide and by the peroxidase enzyme to convert the dye from a reduced colourless state to a coloured oxidized state. There are differences in the stability and sensitivity of the different strips and the degree of interference from substances other than glucose which may be present in the urine. The manufacturers' literature must therefore be read carefully. Strips measure glucose semi quantitatively in urine are recommended.

A number of urinary or contaminating substances may give false negative or false positive glucose strip test results.

9.3.3 INTERFERENCE

The most commonly occurring of these interfering substances are as follows:

Substances which can be oxidized by the hydrogen peroxide in preference to the chromogen. This can lead to a loss in sensitivity of the strip. Such substances include ascorbic acid. Acetoacetate in urine as can be found in specimens from diabetic patients and can give misleading results. Catalase, when present in high concentration in the urine (severe E. coli infections) can destroy hydrogen peroxide and so cause a false negative results. Disinfectants such as bleach which oxidize the chromogen directly and therefore cause a false positive reaction.

9.3.4 Benedict's test

When boiled in alkaline copper sulphate, glucose and other sugars, reduce cupric (copper II) ions to red-brown cuprous (coppe I) oxide, the degree of reduction corresponding to the concentration of reducing substance present.

9.3.5 Clinitest Clinitest is a modification of the Benedict's dry reagent test method in tablet form. Each tablet contains copper sulphate, sodium carbonate, sodium hydroxide, and citric acid. As the diluted urine acts upon the tablet, the citric acid is neutralized by the sodium carbonate and sodium hydroxide with the production of intense heat and the release of carbon dioxide. The heat produced brings the mixture to the boil and the copper ions are reduced by the glucose, the degree of reduction corresponding to the concentration of reducing substance present. Substances which reduce copper ions in the Clinitest are similar to those for Benedict's test. Clinitest is unable to detect less than 13.8 mmol/l (250 mg /dl) of glucose in urine whereas the strip tests are more sensitive, detecting as little as 5.5 mmol/l (100 mg/dl) of glucose.

Glucose will not appear in the urine until the blood glucose is well over 10 mmol/l (180 mg%). With diabetes mellitus the renal threshold for glucose is often raised, especially in elderly diabetic patients, those with cardiac failure, or those in diabetic coma with shock.

A reduced rate of reabsorption of glucose by the kidney tubules occurs in serious tubular damage or an inherited defect of tubular absorption. Glucose appears in the urine when the blood glucose level is below 10 mmol/l (180 mg /dl). This is often referred to as renal glycosuria.

9.4 TESTING URINE FOR KETONES

Ketonuria: Acetoacetate, beta-hydroxybutyrate and acetone are collectively referred to as ketones. The excretion of more than a trace of these substances in the urine is called ketonuria.

9.4.1 Formation of ketones: The metabolism of glucose normally provides the body with its energy requirements. If the intake of glucose is insufficient as in starvation, or glucose metabolism is defective due to a lack of insulin as occurs in untreated or uncontrolled diabetes, the body obtains its energy by breaking down fats. It is this increase in fat metabolism which leads to buildup of ketones in the body (ketosis). Ketones are toxic to the brain and can contribute to the coma found in diabetic ketoacidosis. Metabolic acidosis and ketonuria is always present.

9.4.2 Urine ketone tests detect acetoacetate and acetone. Betahydroxybutyrate is not detected.

The following methods are used to test for Ketonuria.

- Ketone reagent strip tests
- Nitroprusside tube or tile test

Acetoacetate and acetone react with sodium nitroprusside in an alkaline medium to give a violet dye complex. The strips are more sensitive to acetoacetate than acetone. The urine must be tested soon after it is passed before the acetoacetate is carboxylated toacetone.

9.4.3 Nitroprusside tube or tile test

The nitroprusside tube or tile test is a sensitive technique for detecting ketones in urine. Fresh urine is reacted with a dry reagent containing sodium nitroprusside, ammonium sulphate and sodium carbonate. In an alkaline medium, sodium nitroprusside reacts with acetoacetate and acetone to give a mauve-purple colour.

9.4.4 ketonuria Causes

Uncontrolled diabetes

Starvation when fat metabolism is increased.

Eating a diet that is very low in carbohydrates.

Severe dehydration following prolonged vomiting or diarrhea.

Glycogen storage disease.

9.5 TESTING URINE FOR BILIRUBIN

Bilirubin is not normally present in the urine. Urine containing 8.4 mol/l (0.5mg/dl) or more, bilirubin appears in urine and has a characteristic yellow-brown colour (hepatocellular jaundice) or a yellow-green appearance (obstructive jaundice)

The following methods are used to test for bilirubin in urine:

9.5.1 Specimen: Freshly passed urine is required. It should be protected from daylight and fluorescent light because bilirubin is rapidly oxidized by ultraviolet light to biliverdin which is not detected by the reagents used in the tube or strip tests.

9.5.2 Fouchet's test, is sensitive, easy to perform, inexpensive and stable when compared with the strip tests.

9.5.3 Principle, Barium chloride is used to precipitate the sulphates in the urine. Any bilirubin present becomes attached to the precipitated barium sulphate. When Fouchet's reagent is added to the precipitate, the ferric chloride oxidizes the bilirubin to green-blue biliverdin.

9.5.4 False reactions

Sensitivity of urine bilirubin strip tests is reduced by nitrite which may be present with some bacterial

urinary infections. Rifampicin and phenazopyridine may mask a small reaction.

False positive reactions may be produced by drugs that colour the urine red or turn red in an acid environment, e.g. chlorpromazine metabolites.

9.5.5 Causes

Bilirubin can be found in the urine whenever there is an increase of conjugated bilirubin in the blood

Obstructive and hepatocellular jaundice (blood contains both conjugated and unconjugated bilirubin).

Bilirubin is not found in the urine in haemolytic jaundice or in other conditions in which the excess bilirubin in the blood is of the unconjugated type.

Viral hepatitis, bilirubinuria together with raised aminotransferase levels is found before a patient becomes clinically jaundiced.

9.6 UROBILINOGEN IN URINE,

normally small amounts of urobilinogen are found in the urine which is reabsorbed from the intestine. Urine is often tested for increases in urobilinogen when investigating haemolysis or liver disorders in which liver function is impaired. The following methods are used to test for increased urobilinogen in urine:

9.6.1 Urobilinogen strip tests

Specimen: Freshly passed urine is required because urobilinogen which is colourless is rapidly oxidized to the orange pigment urobilin which is not detected by Ehrlich's test or by urobilinogen. If a delay in testing is unavoidable a technique must be used which detects urobilin in urine such as the Schlesinger test.

Urobilinogen reacts with p-dimethylaminobenzaldehyde dye to form a red product. The intensity of colour produced corresponds to the concentration of urobilinogen present. Bilirubin interferes with the reaction and therefore if present it must first be removed by reacting the urine with barium chloride.

A false negative reaction may occur if the urine contains nitrite as in some bacterial urinary infections. The nitrite will oxidize the urobilinogen to urobilin which is not detected by Ehrlich's reagent. A negative reaction may also occur if a patient is receiving intensive antimicrobial therapy. The antimicrobials will reduce the number of bacteria in the intestine and so prevent urobilinogen being formed. Substances which react with Ehrlich's reagent include the porphobilinogen, p-aminosalicylic acid and sulphonamides.

9.7 HAEMOGLOBIN IN URINE (Hemoglobinuria):

The presence of free haemoglobin in urine is called haemoglobinuria. It occurs with severe intravascular haemolysis when the amount of haemoglobin being released into the plasma is more than can be taken up by haptoglobin (the plasma protein that binds free haemoglobin to prevent it being lost from the body). The renal threshold for free haemoglobin is 1.0–1.4 g/l. The following methods can be used to test for haemoglobinuria:

Reagent strip tests

Guaiac test

Specimen:

Urine containing haemoglobin appears brown or brown-grey in colour and is usually cloudy. It should be tested as soon as possible after it has been passed.

9.7.1 Urine haemoglobin strip tests

Principle; Hemoglobin and myoglobin catalyze the oxidation of a colour indicator by an organic peroxide to give a blue-green complex. A uniform green colouring of the test area indicates the presence of free haemoglobin, or haemolyzed erythrocytes or myoglobin in the urine. A green spotting of the area indicates the presence of intact red cells in the urine. Differentiation between haemoglobinuria and haematuria is not

possible when the urine contains visible amounts of blood. The strips are more sensitive to free haemoglobin than intact red cells.

Strip tests are capable of detecting 150–620 g/l free haemoglobin or up to 5–20 intact red blood cells per microlitre in urines. The test is less sensitive to urine with a high relative mass density. Several multiple strip tests also contain areas for detecting free haemoglobin and intact red cells.

9.8 Nitrite and leukocytes in urine:

The detection of nitrite in urine is a useful test in the investigation of urinary tract infections caused by nitrate-reducing bacteria. Most pathogenic bacteria reduce the nitrate normally found in urine to nitrite. The presence of leukocytes in urine indicates inflammation of the urinary tract.

9.8.1 specimen for testing is urine that has remained in the bladder for 4–8 hours such as the first passed urine of the morning.

9.8.2 Principle. Sulphanilamide reacts with nitrite in an acid buffer medium to form a diazonium compound. The diazonium produces a red azo dye, changing the colour of the test area from white to pink. The test detects nitrite in a concentration as low as 11 mol/l. When first morning urine is tested, the nitrite reaction is positive in about 90% of all urinary tract infections. When testing urine collected at other times, up to 70% of infections will be detected where there is significant bacteriuria.

9.8.3 The leukocyte reaction detects the presence of esterases that occur in granulocytic leukocytes. Esterase enzymes cleave an indoxyl ester which reacts with a diazonium salt to produce a violet colour. Both intact and lyzed leukocytes are detected. The reaction is not affected by bacteria or red cells in the urine. The detection limit is 10–25 leukocytes/µl.

Nitrite and leukocyte testing areas can also be found on strip tests. A negative reaction will be obtained if an infection is caused by bacteria that do not reduce nitrite.

False negatives can occur if-

An infection is caused by bacteria that do not reduce nitrite.

Urine has been in the bladder too short a time.

Urine is not fresh and the nitrite has decomposed.

Urine contains insufficient nitrate.

High concentrations of ascorbic acid in the urine.

Sensitivity is reduced when the urine has a high specific gravity.

False positive results are rare.

9.8.4 False leukocyte reactions

Medication with antibiotics containing imipenem, meropenem or clavulanic acid may cause false positive reactions.

9.9 Specific gravity of urine

The relative mass density (d), formerly known as specific gravity (SG), of any liquid is its density compared with distilled water which has a density of 1.000. By measuring the mass density of urine, information can be obtained regarding the concentrating and diluting ability of the kidneys. The following methods are commonly used to test the mass density of urine:

- Urinometer
- Refractometer
- Reagent strip tests

9.9.1 Specimen:

First urine passed at the beginning of the day with the patient having taken no fluid for 10 hours.

9.9.2 Urinometer method, a specially calibrated hydrometer. The lower the concentration of solutes the further the urinometer will sink in the urine. A new urinometer must be checked for accuracy by being floated in distilled water. The reading should be 1.000 at the temperature specified on the urinometer. If the reading is not 1.000, subtract the difference in value from each urine reading. For example, if the density of the distilled water is 1.002, subtract 0.002 from each urine reading.

9.9.3 Refractometer method A refractometer measures refractive index. The measurement is based on the number of dissolved particles in the urine. The higher the concentration of particles, the greater the increase in refractive index.

9.9.4 Urine specific gravity strip tests

The specific gravity reagent test responds to the concentration of ions in the urine. It contains certain pretreated electrolytes, the pKa of which changes depending on the ionic concentration of the urine. The indicator bromothymol blue is used to detect the change. Colors range from deep blue green when the urine is of low ionic concentration, through green to yellow-green when the specimen is of high ionic concentration. The strip specific gravity test does not indicate the amount of non-ionic urinary constituents present such as urea, creatinine, or glucose. The specific gravity scale covers the range 1.000 to 1.030. Increased accuracy can be obtained by adding 0.005 to the reading if the pH of the urine is 7.0 or more.

9.9.5 Low values

A consistently low urine mass density usually indicates poor tubular reabsorption. Excessive fluid intake. In general, the greater the volume of urine excreted, the lower is its density.

9.9.6 High values

A high urine mass density may be the result of heavy perspiration, dehydration, or to the presence of substances not normally found in urine such as glucose or protein.

Sample Question, describe the specific gravity and the causes of high protein content in urine?

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Chapter 10

INFERTILITY

Objectives,

The ultimate objective of studying infertility is to improve clinical practice and optimize the chances of people with fertility problems achieving parenthood.

10.1 INFERTILITY

A semen analysis, also called a sperm count, measures the quantity and quality of semen and sperm. Semen is the thick, white fluid released during sexual orgasm. This release is called ejaculation. Semen contains sperm, the cells made in the male reproductive system that include the genetic material needed to make a baby. If female spouse hasn't been able to get pregnant, a problem with semen or sperm may be one of the reasons. Problems such as a low sperm count or sperm that don't move properly can cause infertility, which is the inability to conceive a baby after a year of trying. A semen analysis can help figure out if a problem with semen or sperm is likely to be causing infertility.

A vasectomy is a surgery on the male reproductive system that's done for birth control. To prevent pregnancy, a vasectomy cuts the tubes that carry sperm out of testes so that the sperm can't get into semen. A semen analysis is usually done 8 to 16 weeks after a vasectomy to make sure semen has no sperm. Partners have been trying to have a baby for at least 12 months without success. Semen analysis, also known as a sperm count test, analyzes the health and viability of a man’s sperm. Semen is the fluid containing sperm (plus other sugar and protein substances) that’s released during ejaculation. A semen analysis measures three major factors of sperm health:

- the number of sperm
- the shape of the sperm
- the movement of the sperm, also known as “sperm motility”

Doctors will often conduct two or three separate sperm analyses to get a good idea of sperm’s health. According to the American Association for Clinical Chemistry (AACC), the tests should be conducted at least seven days apart and over the course of two to three months. Sperm counts can vary on a daily basis. Taking an average of the sperm samples can give the most conclusive result.

10.2 Why undergo semen analysis?

10.2.1 Test for male infertility A semen analysis is often recommended when couples are having problems getting pregnant. The test will help determine if a man is

infertile. The analysis will also help determine if low sperm count or sperm dysfunction is the reason behind infertility.

10.2.2 Test for vasectomy success Men who have had a vasectomy undergo semen analysis to make sure no sperm are in their semen. In a vasectomy, the tubes that send sperm from the testicles to the penis are cut and sealed as a permanent form of birth control. After a vasectomy, doctors often recommend that men take a sperm analysis once a month for three months to ensure that sperm is no longer present in their semen.

It's very important to follow these instructions for accurate results. To get the best sample:

- Avoid ejaculation for 24 to 72 hours before the test.
- Avoid alcohol, caffeine, and drugs such as cocaine and marijuana two to five days before the test.
- Stop taking any herbal medications.
- Avoid any hormone medications.

Two main factors are crucial to having a good testing sample. First, the semen must be kept at body temperature. If it gets too warm or too cold, the results will be inaccurate. Second, the semen must be delivered to the testing facility within 30 to 60 minutes of leaving the body.

10.2.3 Test interference, Some factors can negatively affect the test, including:

- semen coming into contact with spermicide
- taking the test when patient is ill or stressed
- lab technician error
- contamination of the sample

Use of cimitidine, some herbs, alcohol, caffeine or tobacco can reduce sperm count

Home semen tests are available. However, they only test for sperm count. They don't analyze sperm motility or shape. Results for at-home tests are usually available within 10 minutes. A normal sperm count above 20 million sperm per milliliter of semen from a home test doesn't necessarily mean that a man is fertile, since it doesn't consider all the possible causes of male infertility.

When investigating infertility, the basic analysis of semen usually includes:

- Measurement of volume
- Measurement of pH
- Examination of a wet preparation to estimate the percentage of motile spermatozoa and viable forms to look for cells and bacteria.
- Sperm count Examination of a stained preparation to estimate the percentage of spermatozoa with normal morphology.

10.3 SAMPLE COLLECTION,

Sample should be collected in a clean, dry, leak-proof container at home following 3–7 days of sexual abstinence. When a condom is used to collect the fluid, powder should be washed which coats the rubber. It must be dried completely before use. Coitus interruptus not be used because the first portion of the ejaculate may be lost. Also the acid pH of vaginal fluid can affect sperm motility and the semen may become contaminated with cells and bacteria.

Ask the person to write his name, date and time of collection, period of abstinence and to deliver the specimen to the laboratory within 1 hour after ejaculation. Fluid should be kept near to body temperature. This is best achieved by placing the container inside a plastic bag and transporting it in a pocket in the person's clothing.

10.4 LABORATORY EXAMINATION OF SEMEN

Specimen should be handled with care because it may contain infectious pathogens, e.g. HIV, hepatitis viruses, herpes viruses.

10.4.1 Measure the volume Normal semen is thick and viscous when ejaculated. It becomes liquefied usually within 60 minutes due to a fibrinolysin in the fluid. When liquefied, measure the volume of fluid in millilitres using a small graduated cylinder. Normal specimens is usually 2 ml or more.

10.4.2 Measure the pH, A narrow range pH paper is used, e.g. pH 6.4–8.0 and a drop of liquefied semen is spread on the paper. After 30 seconds pH is recorded. pH of normal semen should be 7.2 or more within 1 hour of ejaculation. When the pH is over 7.8 this may be due to infection. When the pH is below 7.0 and the semen is found to contain no sperm, this may indicate dysgenesis of the vas deferens, seminal vesicles or epididymis.

10.4.3 Estimate the percentage of motile and viable spermatozoa Motility, A drop (10–15µl) of liquefied semen is placed on a slide and cover with a 20 20 mm or 22 22 mm cover glass. Close the condenser iris sufficiently to give good contrast. Ensure the spermatozoa are evenly distributed (if not, re-mix the semen and examine a new preparation). Using the 40 objective, examine several fields to assess motility, i.e. whether excellent (rapid and progressive) or weak (slow and non-progressive). Count a total of 100 spermatozoa, and note out of the hundred how many are motile. Record the percentage that are motile and nonmotile.

10.4.4 Normal motility: Over 50% of spermatozoa are motile within 60 minutes of ejaculation. The spermatozoa remain motile for several hours. When more than 60% of spermatozoa are nonmotile, examine an eosin preparation to assess whether the spermatozoa are viable or non-viable.

10.4.5 Presence of cells in semen: Report when more than a few leucocytes (pus cells) or red cells are present. When pus cells are seen, examine a Gram stained smear for bacteria.

10.4.6 Viability, One drop of semenis mixed well with 1 drop of 0.5% eosin solution*on a slide. *Dissolve 0.1 g of eosin in 20 ml of fresh physiological saline. After 2 minutes examine the preparation microscopically. Use the 10 objective to focus the specimen and the 40 objective to count the percentage of viable and non-viable spermatozoa. Viable spermatozoa remain unstained, non-viable spermatozoa are staineds red. Normal viability: 75% or more of spermatozoa should be viable which are unstained. A large proportion of non-motile but viable spermatozoa may indicate a structural defect in the flagellum.

10.4.7 Perform a sperm count, Use a graduated tube or small cylinder, dilute the semen 1 in 20: Fill the tube to the 1 ml mark with well-mixed liquefied semen. Add sodium bicarbonate-formalin diluting fluid to the 20 ml mark, and mix well. Using a Pasteur pipette, fill an Improved Neubauer chamber with diluted semen. Wait 3 to 5 minutes for the spermatozoa to settle. By using the 10 objective with the condenser iris closed sufficiently to give good contrast, count the number of spermatozoa in an area of 2 sq mm, i.e. 2 large squares. Calculate the number of spermatozoa in 1 ml of fluid by multiplying the number counted by 100 000. Normal count is 15 million to 200 million/ ml of semen.

Sample Question; Define infertility and describe various steps in performing the semen analysis.

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Chapter 11

CSF Examination

Objectives,

The objective of this chapter is to teach students CSF to measure different substances in cerebrospinal fluid. It may include tests to diagnose: Infectious diseases of the brain and spinal cord, including meningitis and encephalitis and some other diseases. CSF tests for infections look at white blood cells, bacteria, and other substances in the cerebrospinal fluid.

11.1 CEREBROSPINAL FLUID (CSF) is the liquid that surrounds the brain and spinal cord. The brain and spinal cord are covered by the meninges that consist of three layers: the dura mater, arachnoid, and pia mater. Cerebrospinal fluid (CSF) is contained in the cavity that surrounds the brain in the skull and the spinal column. CSF flows between the arachnoid and the pia mater in an area referred to as the subarachnoid space. It nourishes the tissues of the central nervous system and helps to protect the brain and spinal cord from injury. Choroid plexuses present in ventricles of the brain secrete it continuously at a rate of 500 ml/day. From here it circulates in the sub-arachnoid space of both brain and spinal cord and is absorbed into the blood of dural venous sinuses by arachnoid villi.

The three functions of the CSF are

- physical support and protection,
- provision of a controlled chemical environment to supply nutrients to the tissues and removal of wastes,
- intracerebral and extracerebral transport.

The CSF is cushion for the brain. The denser brain floats in the less dense fluid, allowing movement within the skull. The significance is demonstrated by the result of a blow to the head. The initial shock is transferred to the entire brain, instead of inflicting damage to one area. The other function is the maintenance of a constant gross chemical matrix for the CNS. Serum components may vary greatly, but constituent levels of CSF are maintained within narrow limits.

Specimens for CSF analysis are obtained by lumbar puncture, usually at the interspace of vertebrae L 3, 4 or lower by using aseptic technique. The fluid obtained is usually separated into three numbered aliquots:

- for chemistry and serology,
- for microbiology,
- for hematology.

It should be analyzed immediately and any remaining sample should be preserved because of its limited availability. The order of the tubes reflects the presumed order for minimization of interference from less than optimal collection technique, with tube 3 presumably least contaminated by cells of intervening tissue

11.2 CEREBROSPINAL FLUID (CSF) EXAMINATION

CSF is composed of substances present in plasma but its composition differs, as it is not formed by simple filtration. The entry of many substances into CSF is controlled by the so-called Blood Brain Barrier, which allows free entry of some substances into CSF but inhibits the entry of others. This barrier is however, deranged in inflammation. Therefore, changes in composition of CSF may occur not only in diseases of the brain and spinal cord but also in metabolic diseases like diabetes mellitus, infections etc. It maintains the volume of the brain inside the cranial cavity and provides nutrition. The normal volume of the CSF is 100-150 ml.

11.2.1 NORMAL CSF

Normal CSF is a colorless, clear, watery fluid and no coagulum is formed when it is allowed to stand undisturbed in a refrigerator. It contains only 1-5 cells/mm and these are lymphocytes.

The chemical composition of CSF is as follows:

Proteins: Normal value of protein in CSF is 0.2-0.45 g/L. Higher the level of collection of CSF, lower the protein. In neonates, protein concentration may be as high as 1.7 g/L.

Glucose: It is 50-80 mg/dl and the value is usually 2/3 of the blood glucose level at any time. In diabetes or continuous intravenous glucose infusion, the value may be high. It is better that a sample for blood glucose also be collected simultaneously to make the interpretation easier. Chlorides: 118-127 mmol/L. The estimation of chlorides is of some value in tuberculous meningitis and heat stroke.

	Normal	Bacterial	Viral	Fungal/TB
Pressure (cmH20)	5-20	> 30	Normal or mildly increased	
Appearance	Normal	Turbid	Clear	Fibrin web
Protein (g/L)	0.18-0.45	> 1	< 1	0.1-0.5
Glucose (mmol/L)	2.5-3.5	<2.2	Normal	1.6-2.5
Gram stain	Normal	60-90% Positive	Normal	
Glucose - CSF:Serum Ratio	0.6	< 0.4	> 0.6	< 0.4
WCC	< 3	> 500	< 1000	100-500
Other		90% PMN	Monocytes 10% have >90% PMN 30% have >50% PMN	Monocytes

11.2.2 SAMPLE COLLECTION AND STORAGE

CSF is normally collected from the sub-arachnoid space of the spinal cord at lumbar level by puncture with a long needle. A physician in the ward, under strict aseptic conditions, performs the procedure. The specimen is to be collected in 2-4 ml quantities in 3-4 sterile capped bottles that are serially numbered and must be sent to the laboratory immediately. In case the CSF is to be cultured for M. tuberculosis, then at least a 5 ml sample is needed.

CSF should be tested as soon as it arrives in the laboratory. The CSF in the first bottle is sometimes contaminated with blood and should be kept aside. Fluid from the second bottle is used for routine tests while the fluid from the third bottle is used for bacterial culture etc. If tuberculous meningitis is suspected, the 4th bottle is kept undisturbed in a refrigerator to see **whether a** pellicle or coagulum forms. Otherwise CSF must never be refrigerated (if for bacterial culture as it kills H.influenzae) and should be kept at 37°C.

11.3 ROUTINE EXAMINATION

11.3.1 Appearance

If blood is visible it should be noted whether it is present in all bottles equally or it is present in the first bottle and then disappears. The amount of blood should also be noted. If there is gross contamination of CSF with blood in all of the bottles then the chemical values will not be true. If no blood is seen, then note the color. A yellowish color (Xanthochromia) is commonly seen in sub-arachnoid hemorrhage persisting for several weeks, in the neonatal period, brain tissue destruction and sometimes in long-standing jaundice. Pseudomonal meningitis may be associated with bright green CSF. Note the translucency or turbidity. If the number of WBCs is high in the CSF, then the fluid becomes turbid. In such cases a cell count can be omitted with the main emphasis on gram stain and culture. Finally, check if there is clot or pellicle formation in the CSF. It indicates increased fibrinogen in the CSF, which is a sign of inflammation.

11.3.2 Cell Counts

The CSF may contain WBCs in varying quantities in certain diseases. The cell count should be carried out as soon as possible after collection of the specimen, since the cells are rapidly lysed. Table below depicts the WBC counts in different CSF samples.

Table: WBC count in various conditions

Conditions	WBC /mm² count	Predominant cell type
Normal adult CSF	0-25	Lymphocytes
Normal neonatal CSF	<30	Neutrophils
Tuberculous meningitis	100-500	Neutrophils
Viral meningitis	10-500	Lymphocytes

If CSF is clear then the cells can be counted by charging a Neubauer counting chamber with well-mixed, un centrifuged, undiluted fluid. Cells in all of the nine WBC squares should be counted. If the count is expected to be high then CSF has to be diluted for cell counting. Diluting fluid for CSF is prepared by dissolving 200 mg crystal violet in 100 ml of 10% acetic acid. The method for counting and calculation is the same as for counting WBCs in peripheral blood. In the case of gross contamination of CSF with blood, blood-derived leucocytes will be present in the CSF, therefore, the count is to be corrected. For this purpose perform an RBC and WBC count in both the CSF and the peripheral blood.

Corrected WBC CSF = Measured WBC CSF – (WBC blood × RBC CSF/RBC blood)

Having less than 100 WBCs per mm^3 is more common in patients with viral meningitis. Elevated WBC counts also may occur after a seizure, in intracerebral hemorrhage, with malignancy, and in a variety of inflammatory conditions. Table 2 lists common CSF findings in various types of meningitis.

	Gross	Proteins mg/dl	Glucose mg/dl	Cells /cmm
Normal	Clear	15-40	50-80	0-5 Lymphocytes
Bacterial	Cloudy	60-1000	0-45	1000-50000 Mostly neutrophils
Aseptic (Viral)	Clear	Normal or increased	Normal	100-1000 Mostly lymphocytes
TB	Clear/Clot	Moderate rise (45-300)	Normal or decreased	10-1000

11.3.3 Microscopic Examinations

If the CSF does not contain numerous cells (<200x10/L), centrifuge 2-4 ml CSF in a conical test tube, preferably, at a slow speed for 5-10 min. Save most of the supernatant in a clean test tube for chemical analysis. Re-suspend the sediment in a drop of remaining CSF. Prepare at least three smears on glass slides and dry these in the air. Stain one smear with Leishman Stain (for the type of WBC), one with Gram Method (for bacteria) and the third with the Ziehl-Neelsen method of staining (for acid-fast bacilli).

11.3.4 ESTIMATION OF PROTEINS

An increase in protein is the commonest abnormality of CSF. Protein should always be estimated quantitatively. The easiest is the turbidimetric method using a proteinometer. A Proteinometer is a set of standard tubes showing the turbidity of known amounts of proteins in CSF.

Sulfosalicylic Acid Test

Take 3 ml of 3% sulfosalicylic acid in a tube and add I ml of supernatant clear CSF in it. The cloudiness of the test is compared with that of a standard tube.

11.3.5 Biuret Method

Biuret reagent is made up of Copper sulfate ($CuSO_4$), sodium tartrate (NaK), and sodium hydroxide (NaOH), Trichloracetic acid.

Principle: CSF proteins can be estimated colorimetrically by using the Biuret methods.

Biuret solution is a blue-colored reagent used in biochemistry to test for the presence of proteins in a sample. The reagent contains copper ions that react with peptide bonds in proteins, resulting in the formation of a complex that absorbs light at a wavelength of around 540 nm, which causes the blue solution to change color to violet or pink. The intensity of the color change is directly proportional to the concentration of proteins present in the sample. Biuret solution is commonly used in laboratory experiments to quantitatively determine protein concentration in a given solution and is also used in clinical diagnosis to detect the presence of proteins in various bodily fluids.

The mechanism copper (II) binds to protein peptide nitrogen atoms. This assay was used for protein determination in entire tissue samples without being affected by amino acids. Ammonium sulfate ($(NH_4)_2SO_4$) precipitation purifies proteins, but buffers like ammonia interfere with biuret assay. Copper (II) ions react with peptide nitrogens to displace peptide hydrogens (as long as the environment is sufficiently alkaline).

11.3.6 Dye-Binding Method

There are certain dyes that bind with protein to give colour complexes. These have been used for measuring small amounts of protein in body fluid such as CSF e.g. Pyrogallol Red Method.

Sample Question, Describe the importance of CSF in diagnosing various diseases. Write note on biuret and dye binding methods of protein analysis.

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Chapter 12

gastric Functions tests

Objectives

By performing these tests students will learn to identify the cause of ulcers, to detect duodenal regurgitation, to evaluate the cause of malabsorption, to assess the adequacy of anti-ulcer medications, and to evaluate secretion of gastrin.

12.1 GASTRIC FUNCTION TESTS

The gastrointestinal tract consists of an oral cavity, oesophagus, stomach, and small & large intestines. Important glands like the pancreas, salivary glands, gall bladder, gastric and intestinal glands secrete enzymes, hormones and juices, which help in the digestion of food. The main functions of the gastrointestinal tract are digestion, partial storage, absorption of ingested food and the excretion of waste material.

12.2 GASTRIC FUNCTION

The stomach secretes pepsin, hydrochloric acid and intrinsic factor. The total volume of gastric secretions is 2800 ml. It digests proteins by converting large protein molecules into small polypeptides. The main disorder of gastric functioning is hypersecretion, which causes duodenal ulcers. A less common disorder is achlorhydria, in which gastric acid secretion is reduced.

12.3 GASTRIC FUNCTION TESTS

Since the discovery of Helicobacter Pylori and its role in the pathogenesis of peptic ulcer disease and acid secretion, the conventional gastric function tests involving the measurement of acid secretion have largely been replaced by endoscopic examinations and biopsies. However, following is a list of commonly used gastric function tests:

1. Tests for H. pylori
2. Gastric Acid Measurement
    a) Basal Acid output
    b) Peak and maximum acid output following pentagastrin stimulation
    c) Determination of Free Hydrochloric acid in Gastric juice
3. Measurement of Plasma Gastrin
4. Secretin stimulation test
5. Endoscopy
6. Barium Meal Examination

12.3.1 TESTS FOR H. PYLORI

A wide range of invasive and Noninvasive tests for H. pylori infection have been described. These are; Invasive tests - On Gastric Biopsy specimens;

1. Histology Giemsa/Silver staining Immunohistochemical staining
2. Direct urease test
3. Culture
4. PCR of specific DNA sequences

Noninvasive tests - Breath, Blood, Faeces

1. Breath tests; rise in breath CO_2 or CO^{13} after ingestion of C^{12} labelled urea

2. Serum: Detection of specific IgG antibody

3. Fecal test: Detection of specific antigen

Urea breath test:

It is a rapid diagnostic procedure used to identify infections by H. pylori implicated in gastritis, gastric ulcer, and peptic ulcer disease .It is based upon the ability of H. pylori to convert urea to ammonia and carbon dioxide.

Procedure:

a) A baseline breath sample is taken at the outset.
b) Patient swallows urea labelled with the isotope i.e., C^{13} or C^{12}.
c) After 10-30 minutes, detection of isotope- labelled carbon dioxide in exhaled breath indicates that the urea was split.

Acid reducing medication and antibiotic treatment can produce false negative results. Therefore, these need to he stopped 14 and 28 days prior to the test respectively.

Stool Antigen Test (SAT):

Indications:

Diagnosis of active infection in patients with dyspeptic symptoms

Monitoring effectiveness of antibiotic.

The direct antigen test is superior to the serology test because it can differentiate between active and latent H. pylori infection.

Serological tests only detect exposure. SAT has >95% sensitivity and specificity for diagnosing H. pylori infection and correlation to endoscopy of 95.5%. The test is FDA-approved for monitoring during the course of treatment. The test requires 10 ml. fresh stool collected in a clean, leak- proof plastic container with no preservatives, delivered to the laboratory within 2 h of collection. Patients DO NOT have to fast, have blood samples drawn or ingest anything before the stool sample is collected.

Interpretation: A positive stool antien test a week after initiating therapy indicates a "treatment failure" which can be due to lack of compliance, resistance or lack of efficacy of a treatment regimen. A negative result would indicate that the therapy has eliminated or reduced infection below level of detection, although eradication (at this point) cannot be confirmed. A negative stool antigen test at a minimum of 4 weeks after completion of treatment indicates successful eradication.

Dyspeptic symptom-relieving compounds, such as bismuth, H2 blockers or PPI's interfere with the test results, therefore these medications need to be discontinued for 2 weeks before conducting the test.

12.3.2 GASTRIC ACID MEASUREMENT

1. The patient should have had an overnight fast.

2. A gastric tube is passed into the stomach under fluoroscopy so that the tip lies in the antrum of the stomach

3. Aspirate and discard the baseline gastric juice since this represents overnight gastric acid collection.

4. Basal acid output (BAO), the normal minute amount of acid being produced) is obtained by aspiration of gastric acid for an hour.

5. Collect basal venous blood sample (5 ml) in a bottle containing heparin, pre-cooled on ice, for plasma Gastrin levels.

6. For the stimulation test, administer a stimulant, e.g. pentagastrin 6 µg/kg body weight intramuscularly. Aspirate the stomach every 15 minutes for one hour (stimulated secretions).

7. Determine volume, pH and acid content separately for pre and post stimulation samples of gastric aspirate.

8. Maximal acid output (MAO) is defined as the total acid secretion for the period of 60 min after administration of a gastric stimulant.

Peak Acid Output (PAO) is the highest rate of gastric acid secretion that can be achieved after injection of gastric stimulant.

Calculate the acid output for four 15-minute post-stimulation specimens. Select the two specimens with the highest acid output. Take the mean of the two values and this gives peak acid output.

12.3.3 ESTIMATION OF FREE HYDROCHLORIC ACID IN GASTRIC JUICE:

Principle: A known amount of gastric juice residue is titrated with 0.1 mol/L sodium hydroxide to a pH of 3.5 using a pH meter or Toepfer's Reagent as indicator.

Reagents:

1. Sodium hydroxide 0.1 mol/L. Dissolve 4g sodium hydroxide and make up to 1 L with distilled water.

Procedure:

In the analysis of gastric juice, the amount of "hydrochloric acid," of "total acid," and sometimes of "free acid— organic and inorganic" which it contains are determined. In

these determinations a sample of the gastric juice is titrated with alkali to three different hydrogen ion concentrations, shown by the end-points with the indicators dimethylamino-azobenzene (Töpfer's reagent), phenolphthalein and alizarin, respectively. For titrating the "free acidity, organic and inorganic, Add 3 drops of alizarin sulphonate solution to the contents of vessel B (a beaker or porcelain dish containing 10 c.c. of strained gastric juice) and titrate with N/10 sodium hydroxide until a *violet* color is produced. In this titration the red color must be entirely replaced by a *distinct violet color*."

12.3.4 GASTRIN

Gastrin is a peptide hormone consisting of 34 amino acids (G-34) produced and stored mainly by the endocrine cells (G cells) of antral mucosa and to a lesser extent by G cells of proximal duodenum and delta cells of pancreatic islets. It stimulates secretion of gastric acid, and pepsin, increases gastrointestinal motility and growth of gastric mucosa.

Increased vagal discharge, gastric distension and amino acid peptides in the stomach and calcium in the blood stimulate its secretion. It is inhibited by gastric acidity and gastrointestinal hormones e.g., secretin. Antral pH of 5-7 causes maximal secretion of Gastrin, whereas a pH of 2.5 reduces the secretion by 80%, maximal suppression occurs at pH 1.0.

Indications;

Investigation of achlorhydria or pernicious anemia.

Investigation of Zollinger Ellison syndrome.

Diagnosis of Gastrinoma (Basal and secretin stimulated gastrin measurement).

Monitoring of recurrence of Gastrinoma Measurement of Plasma Gastrin.

Gastrin is unstable in plasma or serum, mainly because of the activity of proteolytic enzymes. Collection of blood samples into tubes containing heparin as anticoagulant and aprotonin prevents proteolytic activity. Samples should be mixed by inversion and transported immediately on ice to the laboratory where plasma is separated by refrigerated centrifuge. If there is delay in transportation the sample has to be frozen at -20°C within 15 minutes of venipuncture. Medicines that interfere with gastric acid secretion (particularly proton pumo inhibitors) increase the levels of Gastrin, these need to be discontinued for a week before measurement of gastrin.

Reference Range and Interpretation:

Isolated serum gastrin levels can only be interpreted in fasting patients: non fasting specimens are uninterpretable;

Normal	up to 100 ng/L
Duodenal ulcer	100-200 ng
Zollinger-Ellison Syndrome	>200 ng/L

Hypergastrinemia may be observed in following situations

- Improperly prepared patients (c.g. after gastroscopy, non-fasting, or diabetic patients taking insulin)
- Patients with pheochromocytoma
- Patients with cancer of the colon, pancreas, breast or lung.
- Renal failure prolongs the serum half life of gastrin and is associated with increased serum gastrin levels.

12.3.5 SECRETIN STIMULATION TESTS

Although secretin normally inhibits secretion of Gastrin it paradoxically stimulates the release of this hormone by gastrinoma cells, and therefore most patients with these tumors have a dramatic rise in plasma gastrin in response to a secretin infusion.

Indications:

To differentiate patients with gastrinomas from those with other causes of hypergastrinemia.

Patient suspected to have Zollinger Ellison Syndrome (ZES) who has a nondiagnostic fasting plasma gastrin concentration.

Procedure:

After an overnight fast of 12 h duration, two plasma samples are taken for determination of fasting gastrin level.

2 U/kg body weight of secretin is administered intravenously over one minute.

Post injection plasma gastrin samples are taken 2, 5, 10, and 15minutes later.

An increase in serum gastrin > 200 pg/mL is diagnostic of Gatrinoma.

Sample Question, Describe the process of gastric acid measurement?

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Chapter 13

HYPERGLYCEMIA

Objectives

The objective of this chapter is to teach students about treatment goals of hyperglycemia involving eliminating the symptoms related to hyperglycemia and reducing long-term complications. Glycemic control in patients with type 1 diabetes is achieved by a variable insulin regimen along with proper nutrition.

13.1 HYPERGLYCEMIA

High blood sugar, also called hyperglycemia, affects people who have diabetes. Several factors can play a role in hyperglycemia in people with diabetes. They include food and physical activity, illness, and medications not related to diabetes. Skipping doses or not taking enough insulin or other medication to lower blood sugar also can lead to hyperglycemia.

Hyperglycemia is an increase in plasma glucose levels. In healthy patients, during a hyperglycemia state, insulin is secreted by the cells of the pancreatic islets of Langerhans. Insulin enhances membrane permeability to cells in the liver, muscle, and adipose tissue. It also alters the glucose metabolic pathways. Hyperglycemia, or increased plasma glucose levels, is caused by an imbalance of hormones and the destruction of the cells of pancreatic Islets of Langerhans. Hyperglycemia usually doesn't cause symptoms until blood sugar (glucose) levels are high, above 180 to 200 milligrams per deciliter (mg/dL), or 10 to 11.1 millimoles per liter (mmol/L).

Recognizing early symptoms of hyperglycemia can help identify and treat it right away. Watch for:

- Frequent urination
- Increased thirst
- Feeling weak or unusually tired
- Increased hunger

Diabetes Mellitus

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from

defects in insulin secretion, insulin action, or both. National Diabetes Data Group developed a

classification and diagnosis scheme for diabetes mellitus.

This scheme included dividing diabetes into two

broad categories: type 1, insulin-dependent diabetes mellitus (IDDM);

type 2, non–insulin-dependent diabetes mellitus (NIDDM).

I . Type 1 diabetes

II. Type 2 diabetes

III . Other specifc types of diabetes

IV . Gestational diabetes mellitus (GDM)

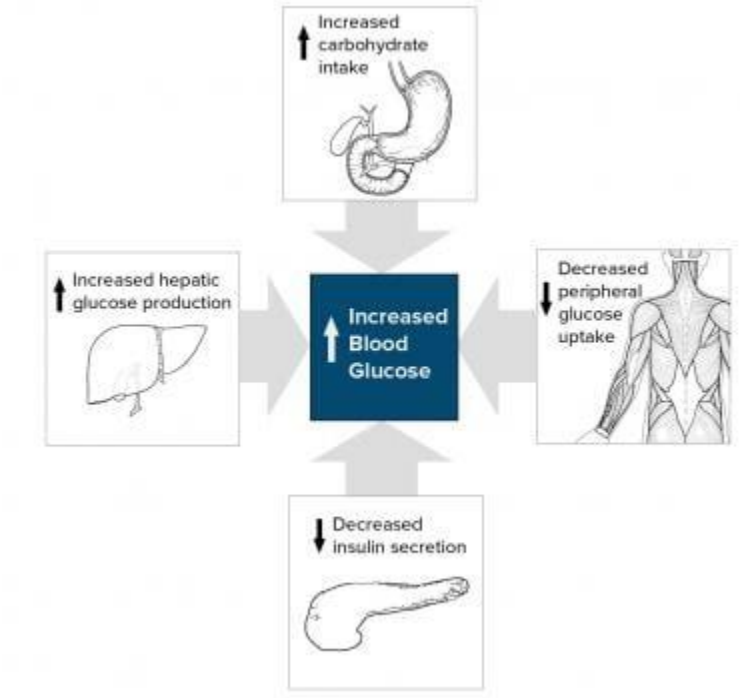
13.2 TYPE 1 DIABETES

Type 1 diabetes mellitus (T1D) is an autoimmune disease that leads to the destruction of insulin-producing pancreatic beta cells. Individuals with T1D require life-long insulin replacement with multiple daily insulin injections. Without insulin, diabetic ketoacidosis (DKA) develops and is life-threatening. In addition to insulin therapy, a continuous glucose monitoring is recommended. Self-management education and support should include training on monitoring, insulin administration, ketone testing when indicated, nutrition including carbohydrate estimates, physical activity, ways of avoiding and treating hypoglycemia, and use of sick day rules. Psychosocial issues also need to be recognized and addressed. This activity reviews the evaluation and management of T1D.

In type 1 diabetes, pancreas doesn't make insulin or makes very little insulin. Insulin helps blood sugar enter the cells in the body for use as energy. Without insulin, blood sugar can't get into cells and builds up in the bloodstream. High blood sugar is damaging to the body and causes many of the symptoms and complications of diabetes. Type 1 diabetes was once called insulin-dependent or juvenile diabetes, but it can develop at any age. Type 1 diabetes is less common than type 2, about 5-10% of people with diabetes have type 1.

13.3 TYPE 2 DIABETES

Type 2 diabetes mellitus consists of an array of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion and excessive or inappropriate glucagon secretion.



Diagnostic criteria by the American Diabetes Association (ADA) include the following

- A fasting plasma glucose (FPG) level of 126 mg/dL (7.0 mmol/L) or higher, *or*
- A fasting plasma glucose (FPG) level of 126 mg/dL (7.0 mmol/L) or higher, *or*
- A random plasma glucose of 200 mg/dL (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis
- Whether a hemoglobin A1c (HbA1c) level of 6.5% or higher should be a primary diagnostic criterion or an optional criterion remains a point of controversy.

DIABETES MELLITUS CLASSIFICATION	**PATHOGENESIS**
Type 1	- **β** -Cell destruction -Absolute insulin deficiency - Autoantibodies • Islet cell autoantibodies • Insulin autoantibodies • Glutamic acid decarboxylase autoantibodies • Tyrosine phosphatase IA-2 and IA-2B autoantibodies
Type 2	Insulin resistance with an insulin secretory defect Relative insulin deficiency Other Associated with secondary conditions
Other	genetic syndromes • Genetic defects of β-cell function • Pancreatic disease • Endocrine disease • Drug or chemical induced • Insulin receptor abnormalities
Gestational	Glucose intolerance during pregnancy Due to metabolic and hormonal changes

13.4 OGTT Oral Glucose Tolerance Test

Indications:

OGTT is only indicated in the following conditions:

Diagnosis of Gestational Diabetes Mellitus (GDM)

An OGTT is performed when the patient's fasting glucose is equivocal (6.1 – 6.9 mmol/L), or during pregnancy to test for gestational diabetes.

An OGTT is not required if a patient's fasting glucose is unequivocally in the diabetic range.

13.4.1 Patient Preparation:

The OGTT should be administered in the morning after at least three days of unrestricted diet (greater than 150g of carbohydrate daily) and usual physical activity. The test should be preceded by an overnight fast of at least 12 hours, during which time only water may be drunk. Smoking is not permitted during the test. The presence of factors that may influence the interpretation of the results of the test must be recorded on the request form (e.g. medication, inactivity, infection, etc).

13.4.2 Test Protocol

1. Confirm that the patient has fasted for at least 12 hours (water only) and write this on the request form.
2. Perform a capillary blood glucose test OR take a venous blood sample for glucose according to the SOP/working instructions for the device. Record the result in the GTT diary and on the request form.
3. Adult patients should drink 75g of anhydrous glucose in 250 - 300 mL of water over the course of 5 minutes.
4. For children, the test load should be 1.75g of glucose per kg body weight up to a total of 75g of glucose.
5. Patient may drink water freely, but must not eat until the test is complete.
6. Take a further sample for glucose into a fluoride oxalate tube 2 hours from the start of glucose consumption. Label the tube clearly with the date and time.

 The patient should not leave the hospital until the test is complete.

 The patient should not leave the hospital until the test is complete.

Recommended Glucose Drink is 75 g anhydrous glucose in 250 – 300 mL cold water. Check carefully that the glucose is anhydrous - glucose obtained from pharmacies is often glucose.H2O, in which case 82.5g must be used.

3. Any drug that alters blood glucose levels should also be discontinued for three days prior to testing (e.g., salicylates, steroids, thiazide diuretics, anticonvulsants). If the patient is taking drugs which are known to lower the blood glucose, they should be discontinued at least on the day of the test.

Tea, coffee drinks, smoking is not allowed during the fast or at least in the morning before the OGTT and also during the OGTT.

No physical exercise is allowed during the test.

The patient should be seated quietly and relaxed for 30 minutes before taking the test.

TABLE -CATEGORIES OF ORAL GLUCOSE TOLERANCE

Normal 2-h PG glucose tolerance	<140 mg/dL (<7.8 mmol/L)
Impaired 2-h PG glucose tolerance	140–199 mg/dL (7.8–11.1 mmol/L)
Provisional 2-h PG diabetes diagnosis	≤200 mg/dL (≤11.1 mmol/L)

Sample Question; Describe the protocol and procedure of oral glucose tolerance test.

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Section2 Chapter 14

Serology

Objectives

Serological tests are important diagnostic tool in medical science. They measure the levels of different antibodies in the body. Serology teaching to students will help to determine illnesses, infections, and overall health.

14.1 Serology

Study of serum and other body fluids. The term usually refers to the diagnostic identification of antibodies in the serum. Such antibodies are typically formed in response to an infection (against a given microorganism), against foreign proteins (in response, for example, to a mismatched blood transfusion), or to one's own proteins (in instances of autoimmune disease). Serology has been used to confirm infections with bacteria, fungi, and viruses that are difficult to detect by other methods. The difficulty with serology is that some immunocompromised patients will not mount an adequate antibody response to infection, a significant increase in antibody titer may not be detected until weeks or months after the infection. Persistence of antibodies may make it difficult to differentiate between a recent and a past infection and cross-reactions may compromise the specificity of the antibody response.

The presence of antibodies against a pathogen in a person's blood indicates that they have been exposed to that pathogen. Most serologic tests measure one of two types of antibodies: immunoglobulin M (IgM) and immunoglobulin G (IgG). IgM is produced in high quantities shortly after a person is exposed to the pathogen, and production declines quickly thereafter. IgG is also produced on the first exposure, but not as quickly as IgM. On subsequent exposures, the antibodies produced are primarily IgG, and they remain in circulation for a prolonged period of time.

A positive result for IgM suggests that a person is currently or recently infected, while a positive result for IgG and negative result for IgM suggests that the person may have been infected or immunized in the past. Antibody testing for infectious diseases is often done in two phases: during the initial illness (acute phase) and after recovery (convalescent phase). A significantly higher amount of IgG in the convalescent specimen suggests infection as opposed to previous exposure. False negative results for antibody testing can occur in people who are immunosuppressed, as they produce lower amounts of antibodies, and in people who receive antimicrobial drugs early in the course of the infection.

Blood typing is typically performed using serologic methods. The antigens on a person's red blood cells, which determine their blood type, are identified using reagents that contain antibodies, called antisera. When the antibodies bind to red blood cells that express the corresponding antigen, they cause red blood cells to clump together which is called agglutination, and can be seen with naked eye. Other serologic methods include crossmatching and the direct and indirect antiglobulin tests. Crossmatching is performed before a blood transfusion to ensure that the donor blood is compatible and can be transfused. It involves adding the recipient's plasma to the donor blood cells and observing for agglutination reactions. The direct antiglobulin test is performed to detect if antibodies are bound to red blood cells inside the person's body, which is abnormal and can occur in conditions like autoimmune hemolytic anemia, hemolytic disease of the newborn and transfusion reactions. The indirect antiglobulin test is used to screen for antibodies that could cause transfusion reactions and identify certain blood group antigens.

Antibody serology tests check for the presence or level of specific antibodies in the blood. Antibodies are proteins that immune system makes to fight foreign substances. These substances are often pathogens, (disease-causing germs) such as viruses and bacteria. When you have an infection, body makes antibodies that are targeted to the pathogen. These antibodies may protect

from getting another infection or from getting severe symptoms. A vaccine can also provide protection by triggering immune system to make antibodies to pathogen.

14.2 Serological test, any of several laboratory procedures carried out on a sample of blood serum (the clear liquid that separates from the blood when it is allowed to clot) for the purpose of detecting antibodies or antibody-like substances that appear specifically in association with certain diseases. There are different types of serological tests, for example, flocculation tests, neutralization tests, hemagglutinin-inhibition tests, enzyme-linked immunosorbent assays (ELISAs), and chemiluminescence immunoassays.

Among flocculation tests, complement-fixation tests are the most common. These are based on the precipitation, or flocculation, that takes place when an antibody and specially prepared antigens (substances that provoke antibody production in the body) are mixed together.

Neutralization tests depend on the capacity of an antibody to neutralize the infectious properties of the infectious organisms. Hemagglutinin-inhibition tests are based on the ability of viruses to cause the red blood cells of certain animal species to agglutinate (clump together); this agglutination will be prevented by the antibody.

ELISAs make use of fluorescent, light (chemiluminescent), or colorimetric signal detection; the signals are produced by enzymatic reactions that occur during the detection and quantification of a specific antigen or antibody in a solution. Chemiluminescence immunoassays are based on the detection of light signals emitted through chemical reactions between enzymes or chemical probes that bind to antibodies.

Serological testing is particularly helpful in the diagnosis of certain bacterial, parasitic, and viral diseases, including Rocky Mountain spotted fever, influenza, measles, polio, yellow fever, and infectious mononucleosis. It is also useful in the detection of autoantibodies (harmful antibodies that attack components of the body) that are involved in autoimmune diseases, such as rheumatoid arthritis. As a practical mass-screening tool, serological testing has proved valuable in the detection of diseases such as syphilis, HIV/AIDS, and epidemic and pandemic infectious diseases (e.g., influenza and coronavirus disease).

14.3 Antigen,

A substance that is capable of initiating an immune response, specifically activating lymphocytes, which are the body's defense white blood cells. In general, two main divisions of antigens are recognized: foreign antigens (or heteroantigens) and autoantigens (or self-antigens). Foreign antigens originate from outside the body. Examples include parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals. Autoantigens, on the other hand, originate within the body. Normally, the body is able to distinguish self from nonself, but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. An antigen that induces an immune response—i.e., stimulates the lymphocytes to produce antibody or to attack the antigen directly—is called an immunogen.

On the surface of antigens are regions, called antigenic determinants, that fit and bind to receptor molecules of complementary structure on the surface of the lymphocytes. The binding of the lymphocytes' receptors to the antigens' surface molecules stimulates the lymphocytes to multiply and to initiate an immune response—including the production of antibody, the activation of cytotoxic cells, or both—against the antigen. The amount of antibody formed in response to stimulation depends on the kind and amount of antigen involved, the route of entry to the body, and individual characteristics of the host.

"An antigen is a molecule that initiates the production of an antibody and causes an immune response."

Antigens are large molecules of proteins, present on the surface of the pathogen- such as bacteria, fungi viruses, and other foreign particles. When these harmful agents enter the body, it induces an immune response in the body for the production of antibodies. The epitopes or antigenic determinants are the components of antigen. Every antigen has several epitopes. An antibody has at least two binding sites that can bind to specific epitopes on antigens.

14.3.1 Types of Antigens

1-Exogenous Antigens

Exogenous antigens are the external antigens that enter the body from outside, e.g. inhalation, injection, etc. These include food allergen, pollen, aerosols, etc. and are the most common type of antigens.

2-Endogenous Antigens

Endogenous antigens are generated inside the body due to viral or bacterial infections or cellular metabolism.

3-Autoantigens

Autoantigens are the 'self' proteins or nucleic acids that due to some genetic or environmental alterations get attacked by their own immune system causing autoimmune diseases.

4-Tumour Antigens

It is an antigenic substance present on the surface of tumour cells that induces an immune response in the host, e.g. MHC-I and MHC-II. Many tumours develop a mechanism to evade the immune system of the body.

5-Native Antigens

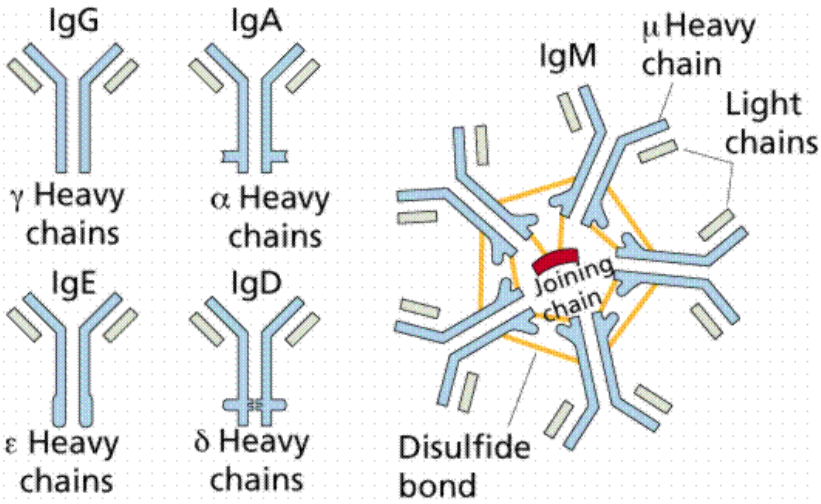
An antigen that is not yet processed by an antigen-presenting cell is known as native antigens.

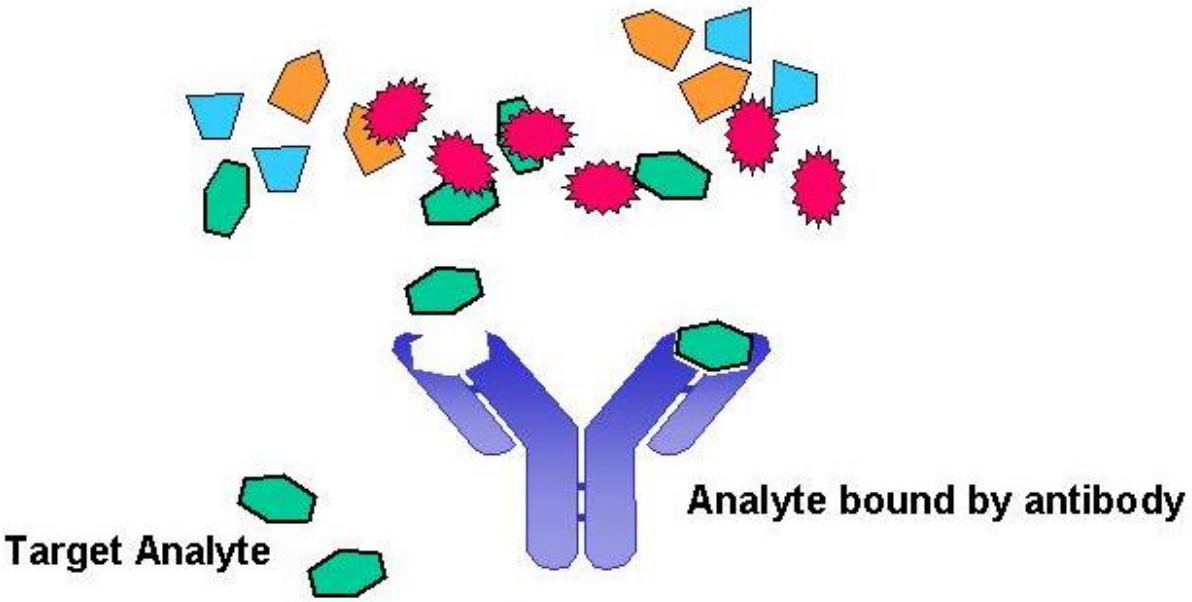
6-mmunogen

These may be proteins or polysaccharides and can generate an immune response on their own.

7-Hapten

These are non-protein, foreign substances that require a carrier molecule to induce an immune response.





14.4 Antibodies,

A protective protein produced by the immune system in response to the presence of a foreign substance, called an antigen. When an alien substance enters the body, the immune system is able to recognize it as foreign because molecules on the surface of the antigen differ from those found in the body. To eliminate it, the immune system calls on a number of mechanisms, including one of the most important—antibody production. Antibodies are produced by specialized white blood cells called B lymphocytes (or B cells). When an antigen binds to the B-cell surface, it stimulates the B cell to divide and mature into a group of identical cells called a clone. The mature B cells, called plasma cells, secrete millions of antibodies into the bloodstream and lymphatic system.

As antibodies circulate, they attack and neutralize antigens that are identical to the one that triggered the immune response. Antibodies attack antigens by binding to them. The binding of an antibody to a toxin, for example, can neutralize the poison simply by changing its chemical composition; such antibodies are called antitoxins. By attaching themselves to some invading microbes, other antibodies can render such microorganisms immobile or prevent them from penetrating body cells. In other cases the antibody-coated antigen is subject to a chemical chain reaction with complement, which is a series of proteins found in the blood. The complement reaction either can trigger the lysis (bursting) of the invading microbe or can attract microbe-killing scavenger cells that ingest, or phagocytose the invader. Once begun, antibody production continues for several days until all antigen molecules are removed. Antibodies remain in circulation for several months, providing extended immunity against that particular antigen.

The basic structure of these proteins consists of two pairs of polypeptide chains that form a flexible Y shape. The stem of the Y consists of one end of each of two identical heavy chains, while each

arm is composed of the remaining portion of a heavy chain plus a smaller protein called the light chain. The two light chains also are identical. Within particular classes of antibodies the stem and the bottom of the arms are fairly similar and thus are called the constant region. The tips of the arms, however, are highly variable in sequence. It is these tips that bind antigen. Thus each antibody has two identical antigen-binding sites, one at the end of each arm, and the antigen-binding sites vary greatly among antibodies.

14.5 B cells

provide one of the most important functions of immunity, which is to produce a tremendous number of protective proteins that make the body to remove all traces of that antigen. Collectively B cells recognize an almost limitless number of antigens; however, individually each B cell can bind to only one type of antigen. B cells distinguish antigens through proteins, called antigen receptors, found on their surfaces. An antigen receptor is basically an antibody protein that is not secreted but is anchored to the B-cell membrane.

"The production of antibodies is the main function of the **humoral immune system**".

14.6 T cells

type of leukocyte (white blood cell) that is an essential part of the immune system. T cells are one of two primary types of lymphocytes—B cells being the second type—that determine the specificity of immune response to antigens (foreign substances) in the body.

T cells originate in the bone marrow and mature in the thymus. In the thymus, T cells multiply and differentiate into

helper,

regulatory,

cytotoxic T cells

memory T cells

They are then sent to peripheral tissues or circulate in the blood or lymphatic system. Once stimulated by the appropriate antigen,

helper T cells secrete chemical messengers called cytokines, which stimulate the differentiation of B cells into plasma cells (antibody-producing cells).

Regulatory T cells act to control immune reactions, hence their name.

Cytotoxic T cells, which are activated by various cytokines, bind to and kill infected cells and cancer cells.

14.7 Complement system

a heat-labile component of normal plasma that augments the opsonization of bacteria by antibodies and allows antibodies to kill some bacteria. This activity was said to 'complement' the antibacterial activity of antibody, hence the name.Ccomplement can also be activated early in infection in the absence of antibodies. The C3 formed by early events of complement activation are bound covalently to the pathogen surface. Here they cleave C3 to generate large amounts of C3b, the main effector molecule of the complement system, and C3a, a peptide mediator of inflammation. The C3b molecules act as **opsonins**; they bind covalently to the pathogen and thereby target it for destruction by phagocytes equipped with receptors for C3b. C3b also binds the C3 convertase to form a **C5 convertase** that produces the most important small peptide mediator of inflammation, **C5a**, as well as a large active fragment, C5b, that initiates the 'late' events of complement activation.

14.8 TYPES OF ANTIGEN-ANTIBODY REACTIONS (Immunoassays)

PRINCIPLE: Immunoassays rely on the ability of an antibody to recognize and bind a specific macromolecule in what might be a complex mixture of macromolecules. In immunology the particular macromolecule bound by an antibody is referred to as an antigen and the area on an antigen to which the antibody binds is called an epitope. In some cases, an immunoassay may use an antigen to detect for the presence of antibodies, which recognize that antigen, in a solution. In other words, in some immunoassays, the analyte may be an antibody rather than an antigen. In addition to the binding of an antibody to its antigen, the other key feature of all immunoassays is a means to produce a measurable signal in response to the binding. Most, though not all, immunoassays involve chemically linking antibodies or antigens with some kind of detectable label. A large number of labels exist in modern immunoassays, and they allow for detection through different means. Many labels are detectable because they either emit radiation, produce a color change in a solution, fluoresce under light, or can be induced to emit light.

DIFFERENT TYPES : Competative immunoassays. Non-Competative immunoassays. Homogenous immunoassays. Heterogenous immunoassays.

Heterogeneous Assays . A conjugate useful in determining the amount of antigen or antibody in a liquid sample, said conjugate having a marker, an immunoreactive component (i.e. antigen or antibody) bound to the marker and an insolubilizing binding component which is also bound to the marker. The insolubilizing binding component portion of the conjugate will react with an insolubilizing receptor to form a solid product of conjugate and receptor unless the conjugate reacts with the corresponding antigen or antibody to be analyzed in which event the conjugate will not react with the insolubilizing receptor. . The conjugate will be added to a liquid sample containing an unknown amount of, for example, an antibody. A known amount of the corresponding antigen is also added which reacts with both the conjugate and antibody. After the reaction is complete, the liquid sample is contacted with the insolubilizing receptor. Since only the free conjugate reacts with the insolubilizing receptor the amount of antibody originally in the liquid sample can be determined by measuring the activity of the marker in the precipitate.

The antigen- antibody reactions used in diagnostic laboratories are based on various techniques which are broadly classified as conventional or old Techniques and newer techniques.

• Precipitation reaction

• Agglutination reaction

• Complement fixation test

• Neutralization test Newer techniques

• Enzyme linked immunosorbent assay (ELISA)

• lmmunofluorescence assay (IFA}

• Radioimmunoassay (RIA)

• Chemiluminescence-linked immunoassay (CLIA}

• immunohistochemistry

• Western blot

14.8.1 PRECIPITATION REACTION

When sufficient antigen and antibody molecules interact, they precipitate out of solution. When the reaction occurs in a liquid solution, the solid formed is called the precipitate. The chemical that causes the Solid to form is called precipitant.

14.8.2 AGGLUTINATION REACTION

When a particulate or its soluble antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated.

14.8.3 COMPLEMENT FIXATION TEST (CFT): It can be used to detect the presence of either specific antibody or specific antigen in a patient serum. It was widely used to diagnose infection, particularly with microbes that are not easily detected by culture methods and in rheumatic diseases. Procedure: The complement system react with antigen-antibody complexes and result in the formation of trans-membrane pores and therefore destruction of the cell.

The basic steps of a complement fixation test are as follows;

1- Serum of the patient is ontained
2- The serum is heated in such a way that all of the complement proteins But none of the antibodies within it are destroyed. A known amount of Standard complement proteins are added to serum.
3- The antigen of interest is added to the serum.
4- Sheep RBCs which have been pre-bound to Anti-sheep RBCs antibodies are added to the serum.

Result:

Haemolysis .....CFT Negative

No haemolysis.....CFT Positive

Neutralizaion Test: Its detects the presence of neutralizing antibody in patients serum. When the serum is mixed with a live viral suspension and poured onto cell line, specific serum antibody neutrilzes the surface antigen, making the virus unable to infect a cell line.

14.8.4 Enzyme Linked Immunosorbent Assay (ELISA), is a very sensitive immunochemical technique which is used to access the presence of specific protein (antigen or antibody) in the given sample and it's quantification. It employs an enzyme linked antigen or antibody as a marker for the detection of specific protein. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product.

Principle, ELISA is a plate-based assay technique. Along with the enzyme- labelling of antigens or antibodies, the technique involves following three principles in combination which make it one of the most specific and sensitive than other immunoassays to detect the biological molecule: An immune reaction i.e. antigen-antibody reaction. Enzymatic chemical reaction i.e. enzyme catalyzes the formation of colored (chromogenic) product from colorless substrate. Signal detection and quantification i.e. detection and measurement of color intensity of the colored products generated by the enzyme and added substrate.

Types of ELISA , allowing qualitative detection or quantitative measurement of either antigen or antibody.

1. Indirect ELISA

2. Sandwich ELISA

3.Competitive ELISA.

Sandwich ELISA is used to identify a specific sample antigen. The wells of microliter plate are coated with the antibodies. Non-specific binding sites are blocked using bovine serum albumin. The antigen containing sample is applied to the wells. A specific primary antibody is then added after washing. u This sandwiches the antigen. Enzyme linked secondary antibody is added that binds primary antibody. Unbound antibody-enzyme conjugates are washed off. The substrate for enzyme is introduced to quantify the antigens.

Competitive ELISA

This type of ELISA depends on the competitive reaction between the sample antigen and antigen bound to the wells of microliter plate with the primary antibody. First, the primary antibody is incubated with the sample. This results in the formation of Ag-Ab complex which are then added to the wells that have been coated with the same antigens. After an incubation, unbound antibodies are washed off. The more antigen in the sample, more primary antibody will bind to the sample

antigen. Therefor there will be smaller amount of primary antibody available to bind to the antigen coated on well. Secondary antibody conjugated to an enzyme is added, followed by a substrate to elicit a chromogenic signal. Concentration of color is inversely proportional to the amount of antigen present in the sample.

14.8.5 Immunofluorescence Assaay: In fluorescence, a photon of an appropriate energy excites the molecule from its ground state to a higher electronic state. When the molecule returns to the ground state, energy is released. The difference between the excitation wavelength and the emission wavelength is the stocks shift. A large stokes shift in nanometer means that there is a large difference between the excitation and emission wavelengths. In colorimetry, the light absorbed by a sample is related directly to the concentration of the absorbing molecule and is independent of the intensity. In fluorometry the intensity of the fluorescence emission is directly proportional to the intensity of the incident light. There are problems associated with fluorescence measurements.

Endogenous fluorophores , such as bilirubin and proteins, can increase the nonspecific background fluorescence and reduce the sensitivity of FIA. Light scattering by high concentrations of protein, lipid and other particles in serum will reduce the fluorescence signal. The inner filter effect of hemoglobin and albumin will absorb part of the excitation or emission beam affecting the results

14.8.6 RadioImmunoAssay

Radioimmunoassays (RIAs) use antibodies to detect and quantitate the amount of antigen (analyte) in a sample. These assays are typically very sensitive and specific. It is possible to detect as low as a few picograms of analyte by using antibodies of high affinity.

Principle of radioimmunoassay is competitive binding, where a radioactive antigen ("tracer") competes with a non-radioactive antigen for a fixed number of antibody or receptor binding sites. When unlabeled antigen from standards or samples and a fixed amount of tracer (labeled antigen) are allowed to react with a constant and limiting amount of antibody, decreasing amounts of tracer are bound to the antibody as the amount of unlabeled antigen is increased.

Separation of the antibody-antigen complexes from free antigen is achieved by precipitation of the antibody-bound tracer with either a secondary antibody solution directed against specific immunoglobulins of the primary antibody, or by use of polyethylene glycol. Both precipitators generally require the presence of carrier immunoglobulin. After centrifugation, the supernatant containing the unbound antigen is sepatated, and the pellet containing the antibody-antigen complex is counted in a scintillation counter. Results obtained for the standards are used to construct a standard curve from which the unknowns are calculated.

14.8.7 Chemiluminescence-linked immunoassay (CLIA)

The principles of are the same as an ELISA or fluoroimmunoassay, but the reporter is different. Luminescence is the release of light due to an electron being kicked up to a higher energy state and emitting a photon as it relaxes down. This is the same principle as fluorescence. The difference lies in the mechanism of kicking the electron up to a higher energy in the first place. In fluorescence

this is achieved with certain frequencies of light. In chemiluminescence this is achieved by a chemical reaction. These reactions require an emitter and a coreactant. A magneto-actuated chemiluminescence assay was developed to detect the presence of Zika virus in patient samples.

Immunohistochemistry uses the antibody, antigen to detect and localize specific antigens in cells and tissue, most commonly detected and examined with the light microscope. IHC is an essential technique in clinical diagnostics in anatomic pathology with antigen retrieval methods allowing to be performed conveniently on formalin fixed paraffin embedded and automated methods for high volume processing with reproducibility. IHC is frequently utilized to assist in the classification of neoplasms, determination of a metastatic tumor's site of origin and detection of tiny foci of tumor cells inconspicuous on routine hematoxylin and eosin staining. Furthermore, it is increasingly being used to provide predictive and prognostic information, such as in testing for *HER2* amplification in breast cancer.

14.8.8 Western blot

is used in research to separate and identify proteins. A mixture of proteins is separated based on molecular weight, and by type, through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein. The membrane is then incubated with labels antibodies specific to the protein of interest. The unbound antibody is washed off leaving only the bound antibody to the protein of interest.

The bound antibodies are then detected by developing the film. As the antibodies only bind to the protein of interest, only one band should be visible. The thickness of the band corresponds to the amount of protein present.

Sample Question, Name the various immunoassays, describe the ELIS?

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