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FACULTY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOMEDICAL LABORATORY SCIENCES

CLINICAL CHEMISTRY I (CCH 1512)

MODULE HANDOUT 2018/2019

Year I, Semester II

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COURSE CONTENTS OUTLINE

- Introduction to clinical chemistry: history, concepts and relationship to other medical sciences, uses in clinical medicine
- Definitions and explanations of clinical chemistry terminologies; screening, monitoring, diagnosis, prognosis
- Description of different body fluids and their normal constituents: Whole blood, Plasma , Serum, Urine, Cerebral spinal fluid (C.S.F), Saliva and Sweat
- Anticoagulants,, use of anticoagulants in clinical chemistry;
- The patho-physiological processes of urine constituents, normal and abnormal constituents of urine
- Urine constituents (composition): Urine volume, appearance, odour, PH, Sugar, Protein, reducing substances, bile pigments, Bile salts, Ketone bodies.
- The principles for qualitative , semi-quantitative and quantitative determinations of various parameters in urine
- Units of measurement in (SI units) clinical chemistry and international standards
- Introduction to reference values, Quality assurance and quality control procedures
- Interpretation of results in relation to normal parameter results
- Precision and accurate measurements in clinical chemistry

CHAPTER I

1. INTRODUCTION

Clinical biochemistry is the area of clinical pathology that is generally concerned with analysis of bodily fluids. The discipline originated in the late 19th century with the use of simple chemical tests for various components of blood and urine.

Clinical biochemistry, chemical pathology and clinical chemistry ' are all names for this subject.,

Clinical biochemistry is a branch of laboratory medicine in which chemical and biochemical methods are applied to the study of disease.

All biochemical tests come under chemical pathology. These are performed on any kind of body fluid, but mostly on serum or plasma. Serum is the yellow watery part of blood that is left after blood has been allowed to clot and all blood cells have been removed. This is most easily done by centrifugation, which packs the denser blood cells and platelets to the bottom of the centrifuge tube, leaving the liquid serum fraction resting above the packed cells.

This initial step before analysis has recently been included in instruments that operate on the "integrated system" principle. Plasma is in essence the same as serum, but is obtained by centrifuging the blood without clotting. Plasma is obtained by centrifugation before clotting occurs. The type of test required dictates what type of sample is used.

A large medical laboratory will accept samples for up to about 700 different kinds of tests. Even the largest of laboratories rarely do all these tests themselves, and some must be referred to other labs.

This large array of tests can be categorised into sub-specialities of:

- General or routine chemistry – commonly ordered blood chemistries (e.g., liver and kidney function tests).
- Special chemistry - elaborate techniques such as electrophoresis, and manual testing methods.
- Clinical endocrinology – the study of hormones, and diagnosis of endocrine disorders.
- Toxicology – the study of drugs of abuse and other chemicals.
- Therapeutic Drug Monitoring – measurement of therapeutic medication levels to optimize dosage.
- Urinalysis – chemical analysis of urine for a wide array of diseases, along with other fluids such as CSF and effusions

SCOPES OF CLINICAL BIOCHEMISTRY

Clinical biochemistry is a valuable subject in medical laboratory, without which there would have been no such advancement in the field

- The importance of clinical biochemistry can be seen from the fact that it is used in many daily activities.

- It is used in clinical diagnosis, manufacture of various biological products, treatment of diseases, in nutrition, in research industries etc….

Fundamentals of clinical biochemistry tests

Clinical biochemistry mainly deals with the biochemical aspects that are involved in several clinical conditions.

- The results of qualitative and quantitative analysis of body fluids assists the clinicians in the diagnosis ,treatment and prevention of the disease and drug monitoring, tissue and organ transplantation ,forensic investigations etc…

Sample used:

- Blood
- Urine
- Pleural fluids
- cerebrospinal fluids(CSF)
- Peritoneal fluids
- Synovial fluids.

OVERVIEW OF MEDICAL LABORATORY

- Our body is one of the most important metabolic processing machine .It maintains its balance with organic and inorganic chemicals present in the body.
- Balanced condition in the body is continuously disturbed by the external factors like microorganisms, environmental factors etc. A person considered to be normal that means his physical, chemical, biological and mental conditions are normal.
- Imbalance in body‘s metabolism of an individual becomes miserable. He/she becomes a patient. In order to bring the patient back to normal life, the cause of illness must be known.
- Generally cause of illness is assessed by physician through physical examination and recommended drug based on the sign and symptoms.
- Some of diseases are not responded with such therapy; in such condition physician seek assistance in the form of laboratory report of chemical and biology investigation from medical laboratory.

- Medical laboratory assist physician for the diagnosis of infection or body‘s imbalanced condition through various physical ,chemical ,biological analysis of body fluid, tissue etc

There are three categories of laboratory:

- Primary
- Secondary
- tertiary laboratories

PRIMARY LABORATORY

- In rural setups, for instance, a primary laboratory may provide only the basic investigations.
- These investigations are simply to perform and do not involve expensive machinery usage. Such laboratories are attached to the physician room nowadays.
- Such laboratories may perform the following simple investigations:

  • Hemograms (hemoglobin estimation ,total and differential counts, erythrocyte sedimentation rate, and packed cell volume etc)

  • Routine and microscopic studies of urine and stool

Secondary laboratory

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

• Routine examination of all body fluids(eg: semen,csf,blood,etc…)

• Routine bacteriological studies (stains,cultures,antibiograms.)

• Routine immunoserological tests(eg:widal,ELISA,…)

• Routine biochemistry investigation and organ profile test .e.g lipid ,cardiac, liver ,renal profiles.

TERTIARY LABORATORY

- These laboratories should be able to do and perform all kinds of investigations.
- Besides doing all investigations that are conducted in secondary laboratories they also carry out the following:

  • Complete biochemical essay

  • Complete immunology based essay for hormones, cancer markers, hepatitis marker

  • All microbiological processes (e.g cultures aerobic and anaerobic,fungal etcs…)

  These laboratory are totally automated, they also undertake all histopathology and cytopathology processing and report

Medical laboratory organization

- Very specific and goal oriented organizational structure is mandatory for the clinical laboratory service. Pathologist is the in charge of medical laboratory, who should possess medical degree and specialist in one area .He will be assisted by the laboratory supervisor, who could have research aptitude. Medical laboratory technicians are work horse who assists

laboratory supervisor. They have higher secondary qualification with diploma in medical laboratory technology. He performs test, records results in a laboratory book and send results to the laboratory supervisor. Laboratory supervisor transcribe the report in a specified format and submit to the pathologist. The final report will be signed by the pathologist and submitted to thephysician.

MEDICAL LABORATORY STAFF

The following is the hierarchy of the clinical laboratory staff from highest authority to lowest

- Pathologist
- Laboratory supervisor
- Laboratory technician
- Specimen collection & processing
- Haematology
- Immuno Heamatology
- Microbiology
- Clinical pathology
- Serology
- Blood banking
- Cytology
- Histopathology

CODE OF CONDUCTS FOR MEDICAL LAB TECHNICIANS

- Lab technician should follow certain ethics and abide by the code of conduct to discharge his duties perfectly
- • Punctuality: Lab workers should maintain punctuality in attending the duty. In case of emergency calls, maintenance of punctuality is quite essential as it saves the life of the patient.
- • Promptness(an action or feeling): Lab workers should be prompt in his work. Postponement of the work delays diagnosis and thus treatment.
- Accurancy: Lab workers should maintain accuracy and correctness in reporting. Guessing and assumption should not be done in reporting. Exhaustive methods are followed for accuracy in analysis.
- • Confidentiality. Clinical revealing‘s should be maintained confidential with the doctor. They should not be disclosed to the patient, patient‘s relations or others.
- • Courtesy. Courteous, kind and sympathetic approach should be followed towards the patient and patient attendants. Technician should be loyal and obedient to the superior‘s
- superintendent, deputy superintendent, pathologist, biochemist and micro biologist, blood bank medical officer and medical officer to discharge the duties correctly.

MAIN TEST PERFORMED IN CLINICAL PATHOLOGY

- **SUGAR**

- **RFT(Renal function Test):**
  - Creatinine
  - Blood Urea
  - Uric acid

- **LFT(Liver function test)**
  - SGOT(serum glutamic oxaloacetic transaminase)

OR AST(aspartate aminotransferase)

  - SGPT(serum glutamic pyruvate transaminase)

Or ALT(alanine aminotransferase)

  - BILIRUBIN

- **ELECTROLYTES**
  - Calcium
  - Potassium
  - Sodium
  - Chloride

Etc..

- **Lipid Profile**

- Cholesterol
- HDL(high density lipoprotein)
- LDL(Low density lipoprotein)
- Triglycerides

- **Cardiac Profile**

- CK(creatinine kinase)
- CPK(Creatinine phosphokinase)
- etc

CHAPTER 2

CLINICAL CHEMISTRY TERMINOLOGIES

- Biochemical tests are involved, to varying degrees in every branch of clinical medicine.
- The results of biochemical tests may be of use in diagnosis and in monitoring of treatment.
- Biochemical tests may also be of value in screening for disease or in assessing the prognosis once a diagnosis has been made.
- The biochemistry laboratory is often involved in research into a biochemical basis of disease and clinical trials of new drugs.

Screening tests

- Screening tests are laboratory tests that help to identify people with increased risk for a condition or a disease before they have symptoms or even realize they may be at risk so that preventive measures can be taken.
- Screening test help detect disease in its earliest and most treatable stages. They are most valuable when they are used to screen for diseases that are both serious and treatable, so that there is a benefit to detecting the disease before symptoms begin.

The goal of screening is to reduce mortality and morbidity (or avoiding expensive or toxic treatments).Screening is a form of secondary prevention

- ❑ The premise of screening is based on concept that early treatment will stop or retard progression of disease
- ❑ Screening therefore has both diagnostic and therapeutic components
- ❑ Screening involves the examination of asymptomatic people who are then classified as either

o Unlike to have disease

o Likely to have disease and therefore require further diagnostic evaluation.

Examples:

- Cholesterol testing
- Screening tests in pregnancy (you will be offered some screening tests to find any health problems that could affect you or your baby, such as infectious diseases…..

There are two fundamentally different types of screening:

1.Mass or population –based screening

Is the application of screening tests to large, unselected populations.

e.g. .Mammography screening (Screening mammograms simply look for signs of cancer. These procedures are x-ray exams) for breast cancer in woman <40yrs of age.

2.Case finding

Is the use of screening by clinicians to identify disease in patients who present for other unrelated problems .

E.g. Blood pressure measurement

Characteristics of screening tests

A. **Sensitivity** (also called the true positive rate , i.e. the percentage of sick people who are correctly identified as having the condition)

B. **Specificity** (also called the true negative rate, i.e., the percentage of healthy people who are correctly identified as not having the condition)

C. **Yield**: the amount of previously unrecognized disease that is diagnosed and brought to treatment as a result of screening.Yield is affected by sensitivity of screening test(a lower sensitivity means that a smaller fraction of disease individuals are

detected at any screening ,a higher prevalence will increase the yield,).

MONITORING TESTS

(Observe or and check the progress of a condition over a period of time)

In medicine, monitoring is the observation of a disease condition one or several medical parameters over time.

It can be performed by continuously measuring certain parameters by using a medical monitor and /or by repeatedly performing medicals tests

Examples of monitoring tests:

oblood glucose monitoring with a glucose meter in a people with diabetes mellitus

o continuously measuring vital signs by a bed side monitor

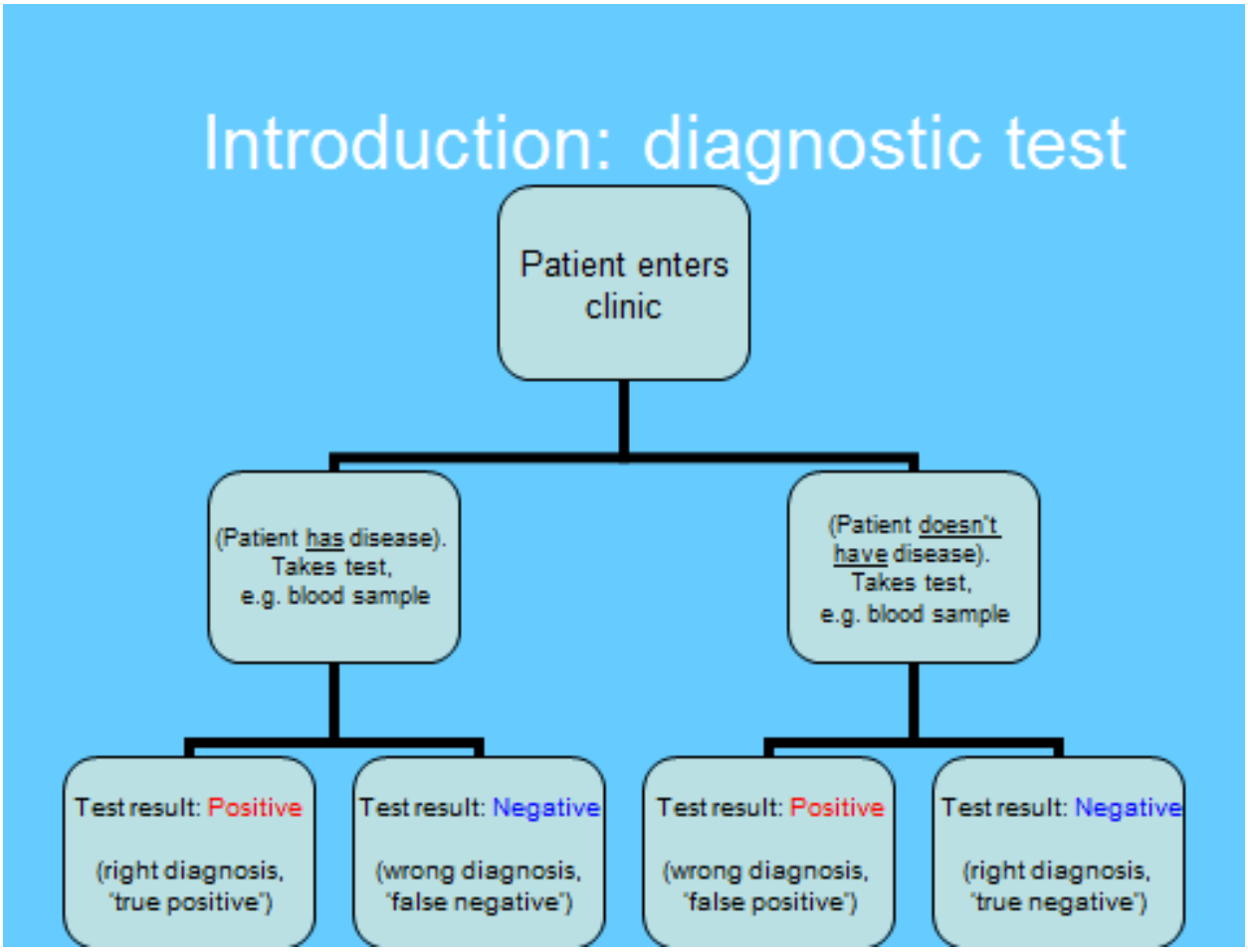
oonce a specific cancer is diagnosed ,many tests like blood cell counts, blood chemistries, CT Scan, are used during and after treatment to monitor how well therapies are working .

oMonitoring tests test also may be used to check for any sign of recurrence(come back).

DIAGNOSTIC TEST

A diagnostic test is a procedure performed to confirm, or determine the presence of disease in an individual suspected of having the disease, usually following the report of symptoms, or based on the results of other medical tests.

Medical diagnosis is a process of determining which disease or condition explain a person‘s symptoms and signs.



Diagnostic tests examples:

- ✓ Utilizing nuclear medicine techniques to examine a patient having lymphoma
- ✓ Measuring the blood sugar in a person suspected of having diabetes mellitus, after period of increased urination.
- ✓ Taking a complete blood count of an individual experiencing a high fever ,to check for bacterial infection.
- ✓ Monitoring electrocardiogram readings on a patient suffering chest pain,to diagnose or determine any heart irregularities.

METHODS OF DIAGNOSIS

- ✓ a.Observations made by the physician
- ✓ b.Question asked in the taking of a medical history of an individual
- ✓ c.Tests performed in physical examination
- ✓ d.Radiologic tests for example X-RAYS
- ✓ e.In vivo diagnostics which test in body ,such as monometry
- ✓ f.In vitro diagnosis ,which test a sample of tissue ,or bodily fluids ,such as
- ✓ o microbial culture which determines the presence or absence of microbes in a sample from the body,
- ✓ o Genetic testing
- ✓ o Blood glucose

- ✓ o Calcium
- ✓ o Liver function tests
- ✓ o Electrolytes in the blood ,such as sodium, potassium…
- ✓ In vitro tests can be classified according to the location of the sample being tested including:
- ✓ o Blood test
- ✓ o Urine tests, including naked eyes (gross)exam of the urine
- ✓ o Stool tests, including naked eye
- ✓ o Sputum

Prognostic tests

- ✓ • In medical field ,diagnosis relates to identifying and understanding the nature of a disease or disorder ,while prognosis is a prediction (the proportions of positive and negative results) of the probable outcome of a disease or disorder
- ✓ • Prognosis is a medical term for predicting the likely outcome of one's current standing. We can call also prognosis the Doctor's judgment of the likely or expected development of a disease or of the chances of getting better.
- ✓ An opinion, based on medical experience, of the likely course of a medical condition.
- ✓ For example, prognosis after the operation was for a full recovery

- ✓ Medical studies have demonstrated that most doctors are overly optimistic when they are giving prognostic information, that is, they tend to overstate how long a patient might live. For patients who are critically ill, particularly those in an intensive care unit, there are numerical prognostic scoring systems that are more accurate.

CHAPTER 3

BODY FLUIDS AND ITS NORMAL CONSTITUENT

Definition

- ✓ Body fluid, bodily fluids or biofluids are liquids originating from inside the bodies of living people. They include fluids that are excreted or secreted from the body.
- ✓ The dominating content of body fluids is body water. In humans approximately 60-65% of body water is contained within the cells (in intracellular fluid) with the other 35-40% of body water contained outside the cells (in extracellular fluid).
- ✓ This fluid component outside the cells includes the fluid between the cells (interstitial fluid), lymph and blood. There are approximately 6 to 10 liters of lymph in the body, compared to 3.5 to 5 liters of blood.

WHOLE BLOOD

- ✓ A venous, arterial or capillary blood sample in which the concentrations and properties of cellular and
- ✓ extra-cellular constituents remain relatively unaltered when compared with their in-vivo state.
- ✓ Whole blood contains red cells, white cells, and platelets .

The composition of whole blood for more information:

- Plasma: 55% of whole blood;
- Buffy Coat: Leukocytes and platelets (<1% of whole blood);
- Erythrocytes: 45% of whole blood

BLOOD SERUM

- The undiluted, extracellular portion of blood after adequate coagulation is complete.
- In blood, the serum is the component that is neither a blood cell (serum does not contain white or red blood cells) nor a clotting factor; it is the blood plasma not including the fibrinogens. Serum includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms).
- The study of serum is serology, which may also include proteomics(Proteomics is the large-scale study of proteins). Serum is used in numerous diagnostic tests.
- Blood is centrifuged to remove cellular components. Anti-coagulated blood yields plasma containing fibrinogen(a protein that is essential for blood clot formation)and clotting factors. Coagulated blood (clotted blood) yields serum without fibrinogen.
- Serum is an essential factor for the self-renewal of embryonic stem cells(Embryonic stem cells are stem cells derived from the undifferentiated inner mass cells of a human

- embryo. Embryonic stem cells are pluripotent, meaning they are able to grow into all derivatives).
- Blood serum is blood plasma without clotting factors; in other words, "pure" blood.Plasmapheresis is a medical therapy that involves blood plasma extraction, treatment, and reintegration.
- If you're sick, your plasma can contain antibodies that attack the immune system. A machine can be used to remove the affected plasma and replace it with good plasma or a plasma substitute. This is also known as plasma exchange. The process is similar to kidney dialysis
- The serum of convalescent patients successfully recovering (or already recovered) from an infectious disease can be used as a biopharmaceutical in the treatment of other people with that disease, because the antibodies generated by the successful recovery are potent fighters of the pathogen. Such convalescent serum (antiserum) is a form of immunotherapy

BLOOD PLASMA

- The virtually cell-free supernatant of blood containing anticoagulant obtained after centrifugation. Blood plasma is a straw coloured liquid component of blood that normally holds the blood cells in whole blood in suspension; this makes plasma the extracellular matrix of blood cells. It makes up about 55% of the body's total blood volume. It is the intravascular fluid part of extracellular fluid (all body fluid outside of cells)..
- It is mostly water (up to 95% by volume), and contains dissolved proteins (6–8%) (i.e. albumins, globulins, and fibrinogen), glucose, electrolytes (Na+, Ca2+, Mg2+,

HCO_3(bicarbonate), Cl^-, etc.), hormones, carbon dioxide (plasma being the main medium for excretory product transportation) and oxygen. Plasma also serves as the protein reserve of the human body. It plays a vital role in an intravascular osmotic effect that keeps electrolytes in balanced form and protects the body from infection and other blood disorders.

- Blood plasma is prepared by spinning a tube of fresh blood containing an anticoagulant in a centrifuge until the blood cells fall to the bottom of the tube. The blood plasma is then poured or drawn off. Blood plasma has a density of approximately 1025 kg/m3, or 1.025 g/ml.

COLLECTION OF BLOOD

- Collection of blood from a subject is necessary for
- 1. Hematological study
- 2. Biochemical analysis
- 3. Bacteriological culture
- 4. Immunological study
- 5. Blood transfusion services

SITES FOR BLOOD COLLECTION

- Blood is usually collected from veins, capillaries and arteries. Venous blood is most commonly collected from media cubital vein. Blood also collected from veins of arm, dorsum of hands.
- Capillary blood is used where small quantity of blood is required
  e.g., blood grouping.

- This is collected from the fingertips or lobule of ear. In infants, the thumb is used to collect the capillary blood.

Preservation and storage of blood sample

- Plasma or serum should be separated at least within 2 hours after blood collection. lt is advisable to analyse blood, plasma or serum, immediately after the specimen collection. This however, may not be always possible. In such case, the samples (usually plasma/ serum) can be stored at 4 degreeC until analysed. For enzyme analysis, the sample are preserved at -20degreeC.

SALIVA

- Saliva is a watery substance formed in the mouths of animals, secreted by the salivary glands. Human saliva comprises 99.5% water, plus electrolytes, mucus, white blood cells, epithelial cells (which can be used to extract DNA), glycoproteins, enzymes (such as amylase and lipase), antimicrobial agents such as secretory IgA and lysozyme.The enzymes found in saliva are essential in beginning the process of digestion of dietary starches and fats.
- These enzymes also play a role in breaking down food particles entrapped within dental crevices, thus protecting teeth from bacterial decay. Further more, saliva serves a lubricative function, wetting food and permitting the initiation of swallowing, and protecting the mucosal surfaces of the oral cavity from desiccation.

- Various animal species have special uses for saliva that go beyond pre-digestion. Some spider use their gummy saliva to build nests.

URINE

- Urine is an aqueous solution of greater than 95% water. Other constituents include urea, chloride, sodium, potassium, creatinine and other dissolved ions, and inorganic and organic compounds. Urea is a non-toxic molecule made of toxic ammonia and carbon dioxide.

COLLECTION OF URINE

- Urine is collected for chemical, cytological and microbiological investigations. Various metabolic disorders, renal dysfunction and urinary tract infection can be diagnosed.
- A random urine sample can be collected in a container which is used for the quantitative estimation of glucose, proteins, Ph, specific gravity, bile pigments and for the presence of blood,pus or crystals.
- Often the first morning voided specimens are recommended because it gives the urine concentration most accurately.
- Otherwise the time of collection of the sample is not important.

METHODS FOR COLLECTION OF URINE

- Midstream specimen – for all examinations

- Three separate specimens – 3 samples were collected in a 3 separate jars without stopping the flow and labeled accordingly.
- Catheter specimens – this is done for unconscious patients or bed ridden patients. At present urobag contains the urine can be collected for examination.

Collection of urine sample:

- The patients were given a clean, preferably sterile container of appropriate size (50ml).
- The container must be free of detergents, which may give false results.
- The container should be prelabeled with the identification data of the patient.
- For quantitative examination, the whole volume of the urine excreted during 24 hours must be carefully collected in a 2 L container.
- The urethra is swabbed and cleaned before collection of the sample.
- The patients should be instructed not to touch the inside or rim of the container
- The patients hands should be cleaned
- The patient is asked to void the first portion of the urine.
- The midstream sample of the urine should be collected in a clean sterile container.
- The container should be capped without holding its top.

Collection of urine from infants

- ✓ A condom fixed to the penis or around the babies genitalia.

- ✓ A plastic bag with an adhesive mouth to be fixed around genitalia and left for 3 to 4 hours
- ✓ A colostomy bag also used.

Preservation and storage of urine sample

- ✓ For the collection of 24 hrs urine samples, preservative have to be used or else urine undergoes changes due to bacterial action. Hydrochloric acid, toluene,light petroleum, thymol, formalin etc., are among the common
- ✓ Preservatives used.

CSF(Cerebral spinal Fluid)

- ✓ Cerebrospinal fluid (CSF) is a clear, colorless body fluid found in the brain and spine. It is produced in the choroid plexuses of the ventricles of the brain. It acts as a cushion or buffer for the brain's cortex, providing basic mechanical and immunological protection to the brain inside the skull. The CSF also serves as vital function in cerebral autoregulation of cerebral blood flow
- ✓ The CSF is created from blood plasma and is largely similar to it, except that CSF is nearly protein-free compared with plasma and has some modified electrolyte levels. CSF contains approximately 0.3% plasma proteins, or approximately 15 to 40 mg/dL, depending on sampling site,and it is produced at a rate of 500 ml/day. Since the subarachnoid space around the brain and spinal cord can contain only 135 to 150 ml, large amounts are drained primarily into the blood through arachnoid granulations in the superior sagittal sinus. This continuous flow into the venous system dilutes the concentration of larger, lipid-insoluble molecules penetrating the brain and CSF.

COLLECTION OF CEREBRAL SPINAL FLUID (CSF)

- ✓ CSF is a fluid of the nervous system. lt is formed by a process of selective dialysis of plasma by the choroid plexuses of the ventricles of the brain.
- ✓ The total volume CSF is 100-200 ml.

Collection of CSF

- ✓ CSF is collected by puncturing the interspace between the 3rd and the 5th lumbar vertebrae under aseptic conditions and local anesthesia.
- ✓ Cerebrospinal fluid (CSF) collection is a test to look at the fluid that surrounds the brain and spinal cord.
- ✓ CSF acts as a cushion, protecting the brain and spine from injury.
- ✓ The fluid is normally clear.
- ✓ It has the same consistency as water.
- ✓ The test is also used to measure pressure in the spinal fluid.

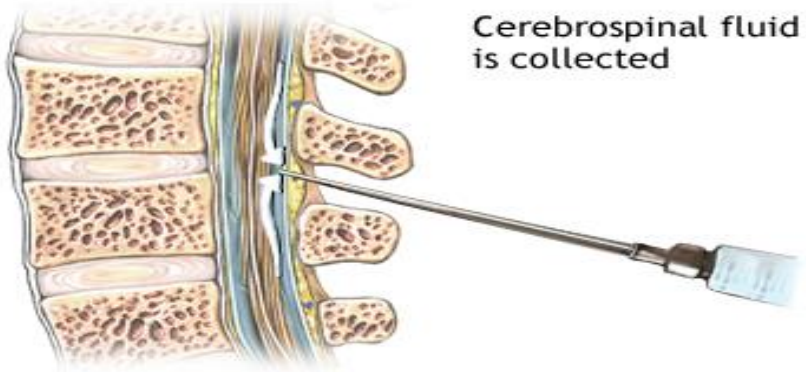
How the Test is Performed

- ✓ There are different ways to get a sample of CSF. Lumbar puncture (spinal tap) is the most common method.

To have the test

- ✓ •You will lie on your side with your knees pulled up toward the chest, and chin tucked downward. Sometimes the test is done sitting up, but bent forward.

- ✓ •After the back is cleaned, the health care provider will inject a local numbing medicine (anesthetic) into the lower spine.
- ✓ A spinal needle will be inserted.
- ✓ •Once the needle is in position, the CSF pressure is measured and a sample of 1 to 10 mL of CSF is collected.
- ✓ •The needle is removed, the area is cleaned, and a bandage is placed over the needle site. You may be asked to remain lying down for a short time after the test.
- ✓ Occasionally, special x-rays are used to help guide the needle into position.
- ✓ This is called fluoroscopy.



Rarely, other methods of CSF collection may be used.

o **Cisternal puncture** uses a needle placed below the occipital bone (back of the skull). It can be dangerous because it is so close to the brain stem. It is always done with fluoroscopy.

o **Ventricular puncture** may be recommended in people with possible brain herniation. This is a very rarely used

method. It is most often done in the operating room. A hole is drilled in the skull, and a needle is inserted directly into one of the brain's ventricle.

Fluoroscopy is a study of moving body structures--similar to an X-ray "movie." A continuous X-ray beam is passed through the body part being examined. The beam is transmitted to a TV-like monitor so that the body part and its motion can be seen in detail.

SWEAT

Sweat is mostly water. Dissolved in the water are trace amounts of minerals, lactic acid, and urea. Although the mineral content varies, some measured concentrations are: sodium (0.9 gram/liter), potassium (0.2 g/l), calcium (0.015 g/l), and magnesium (0.0013 g/l).
Also many other trace elements are excreted in sweat, again an indication of their concentration is (although measurements can vary fifteenfold) zinc (0.4 milligrams/liter), copper (0.3–

0.8 mg/l), iron (1 mg/l), chromium (0.1 mg/l), nickel (0.05 mg/l), and lead (0.05 mg/l). Probably many other less-abundant trace minerals leave the body through sweating with correspondingly lower concentrations.
In humans, sweat is hypoosmotic relative to plasma (i.e. less concentrated). Sweat typically is found at moderately acidic to neutral pH levels, typically between 4.5 and 7.0.

ERRORS IN CLINICAL CHEMISTRY WHICH CAN AFFECT LABORATORY SAMPLE and RESULTS

Three phases of laboratory testing:

Pre-analytical, analytical and post-analytical

1. **Pre-analytical**—specimen collection, transport and processing

2. **Analytical**—testing

3. **Post-analytical**—testing results transmission, interpretation, follow-up, retesting.

PRE-ANALYTICAL ERRORS

• Errors at any stage of the collection, testing and reporting process can potentially lead to a serious patient misdiagnosis.
• Errors during the collection process are not inevitable but can be prevented with a diligent application of quality control, continuing education and effective collection systems.
• 32 - 75% of all test errors occur in the pre-analytical phase

The Pre-Analytical process

1. Patient Identification
2. Sampling Technique(example phlebotomy)
3. Test Collection Procedures
4. Specimen Transport
5. Specimen Processing

Collection of sample:

- Locate Patient
- Prepare Patient
- Draw Sample
- Label
- Dispose of supplies

PATIENT IDENTIFICATION

- ❑ When identifying the patient, have them provide their full name, address, identification number and/or date of birth.
- ❑ In Hospital patients should be wearing an identification band with the above information, which the phlebotomist should confirm before the venipuncture.
- ❑ It is important to identify a patient accurately so that blood is collected from the correct person.
- ❑ Drowing blood from the wrong person or labeling the correct patient‘s sample with a different patient‘s label can certainly contribute to laboratory error. (Mislabeling)

Factor affecting lab result

Patient variables that affect test results

- ❑ • Age
- ❑ • Gender
- ❑ • Diet & Nutrition status
- ❑ • Genetic variation
- ❑ • Obesity

- ❑ • Posture
- ❑ • Hemolysis
- ❑ • Special habits
- ❑ • Drugs

Phlebotomy: is a highly complex skill requiring expert knowledge, and critical judgment

- ❑ • Venipuncture is a frequent medical procedure.
- ❑ • Phlebotomy errors may cause harm to patients or result in needle stick injury to the phlebotomist
- ❑ Posture:
- ❑ • The patient should be comfortably seated or supine(lying face upwards) for 20 minutes before sampling. Not standing
- ❑ • The patient arm should be extended in a straight line from the shoulder to the wrist.

Collection site

- ❑ • The median cubital vein is the preferred site.
- ❑ • Veins on the hand

Phlebotomy Technique Errors

Tourniquet Application

- Tourniquet tied too close to the venipuncture site can cause hematoma
- Veins may not become prominent if tourniquet is tied too high (more than 3 to 4 inches above venipuncture site)
- Tourniquet left on longer than one minute can result in hemo concentration , affecting some test results
- Tourniquet should be released as soon as needle is in the lumen of the vein and blood flow established

TEST COLLECTION ERRORS

- • Blood collected insufficient to amount of additive in tube.
- (EDTA, citrate, lithium heparin , oxalate, flouride)
- • Traumatic venipuncture
- • Blood collected from area with hematoma
- • Vigorous shaking of tubes after collection
- • Air bubbles in the sample
- Proper Tube Mixing:
- All tubes with additives need to be inverted to mix the additive evenly with the blood. Improper mixing of the tube after venipuncture could contribute to sample clotting

SPECIMEN TRANSPORT

Transport errors:

Temperature

- Specimens must be transported at the appropriate temperature for the required test
- • On ice
- • Warmed -- (37° C)

- ❑ • Avoid temperature extremes if transported via vehicle from other collection site

Light

Some samples need to be protected from light, for example, bilirubin

Blood Specimen Transport

Transport of blood specimens in the proper manner after collection ensures the quality of the sample

Timing

Some specimens must be transported immediately after collection.

- Specimens for serum or plasma chemistry testing should be centrifuged and separated within two hours

ANALYTICAL FACTORS THAT CAN AFFECT LABORATORY TESTS

- Instrument calibration and maintenance
- Standards and procedure control
- Test procedure logistics (reagents, pipetting, timing etc)
- Interfering conditions or substances

POST-ANALYTICAL FACTORS

- Primarily in reporting and charting of the results, including transcription and clerical errors. Use of computers as well as proper patient identifiers, laboratory request form, labels and specimen containers can greatly aid in minimizing these errors.

CHAPTER 4

ANTICOAGULANTS AND USE OF ANTICOAGULANTS

- Once the blood is drawn out, within 10 minutes it clots, if we don‘t want it to clot, it should be mixed with anticoagulants in a

proper proportion. Anticoagulants are the chemicals which prevent blood coagulation. Except heparin most anticoagulants coagulate by removing ca ++(factor iv). Heparin acts by destroying thrombin, thromboplastin .

The important anticoagulants used are :

– EDTA
– CITRATE
- ACID CITRATE DEXTROSE (ACD)
- TRISODIUM CITRATE
- CITRATE PHOSPHATE DEXTROSE
- CITRATE PHOSPHATE DEXTROSE ADENINE

– HEPARIN
-HIRUDIN
– SODIUM FLUORIDE
– DOUBLE OXALATE

1. EDTA

- It's the most commonly used anticoagulant in routine practice
- The potassium and sodium salts of EDTA are powerful anticoagulants.

- **Mechanism of action:**

➢ It acts by chelating the calcium molecules in the blood. (is a type of bonding of ions and molecules to metal ions)

1.2mg od EDTA is required for each ml of blood to get the desired results.

EDTA is used for mainly blood counts

COMPOSITION OF EDTA

- Ethylenediamine tetra acetic acid.
- Water-1litre.
- NEUTRAL EDTA
- Ph:7.0
- EDTA,dipotassium salt 44.5g and disodium salt 41.0g
- NaOH:75ml

ADVANTAGES

✓ It's the anticoagulants of choice in routine hematological work.
✓ Best for platelet counts.

DISADVANTAGES:

✓ RBC morphology is hampered if the concentration is more than the required.
✓ Not good for coagulation studies.

2. CITRATE

❑ ACID CITRATE DEXTROSE (ACD)

Composition:Sodium citrate - 1.32 gm.
Citric acid - 0.48 gm.
Dextrose - 1.40 gm.
Distilled water- 100 ml.

Above mixture is used the 4 ml blood for 1 ml ACD mixture. It precipitate the calcium ions. It is used in the Blood Bank for collecting blood for transfussion. It is also used for enzyme study, red cells preservation and for hemolytic process. It is not suitable for Haemoglobin % and blood cells count because it is used as liquid that dilute the cellular elements.

❑ TRISODIUM CITRATE

It is used for coagulation studies.

Mechanism of action:

It also works on the principle of calcium chelation(is a type of bonding of ions and molecules to metal ions)
•9 volumes of blood are added to 1 volume of sodium citrate for coagution studies.
•For ESR estimation,4 volumes of blood are added to 1 volume of sodium citrate solution and well mixed.
Trisodium citrate has the chemical formula of $Na_3C_6H_5O_7$. It is sometimes referred to simply as sodium citrate.It converts the ionised calcium into non-ionised form.
It is used in a concentration of 2 - 3 mg for 1 ml of blood, it is used to perform coagulation test, blood transfusion and ESR estimation.

❑ CITRATE PHOSPHATE DEXTROSE(CPD)

Composition:

- Trisodium citrate,dehydrate(102mmol/l)-30g.

- Sodium dihydrogen phosphate,monohydrate(1.08mmol/l)-0.15g.
- Dextrose(11mmol/l)-2g
- pH:6.9

CITRATE PHOSPHATE DEXTROSE ADENINE

COMPOSITION:

- Trisodium citrate,dihydrate(89mmol/l)-26.30g.
- Citric acid ,monohydrate (17mmol/l)-3.27g.
- Sodium dihydrogen phosphate,monohydrate(16mmol/l)-2.22g.
- Dextrose(177mmol/l)-31.8g.
- Adenine (2.04mmol/l)-0.275g
- Water:1 litre
- pH:5.6-5.8
- CPD,ACD,CPDA & the related solutions are used in blood banking and blood transfusion purposes.

3.HEPARIN

- Its used for chemistry,blood analysis and emergency tests.
- This anticoagulant is used when plasma is required urgently for certain emergency estimation of blood gases and electrolytes . It is used in concentration of 1 - 2 mg per ml of blood.
- It's the best anticoagulant for osmotic fragility tests(Osmotic fragility is a blood test to detect whether red blood cells are more likely to break down) and immunophenotyping(

Immunophenotyping is the analysis of heterogeneous populations of cells for the purpose of identifying the presence and proportions of the various populations of interest.

- The lithium or sodium salt of heparin at a concentration of 10-20IU/ml blood is used generally.
- • It is not suitable for blood counts as it induces platelet and leucocytes clumping and gives faint blue color

4. HIRUDIN

- Hirudin is a naturally occurring peptide in the salivary glands and medicinal leeches that has a blood anticoagulant property.
- Hirudin is an antithrombin extracted from leeches or prepared by a genetic engineering process.
- Hirudin inhibits thrombin by forming hirudin-thrombin complex. Hirudin is used at a
- concentration of 10 mg/L

5.SODIUM FLUORIDE

- Sodium fluoride is an inhibitor. It is used for determination of blood sugar. It preserves blood for 24 hours at room temprature and 4 to 6 day in refrigerator
  - It prevents glycolysis
  - It is the ideal additive used for blood sugar estimation.

6.DOUBLE OXALATE

- It is a mixture of ammonium oxalate and potassium oxalate. It is used in concentration of 2 mg for the 1 ml of blood. Oxalates acts by removing calcium ions from the plasma as in the insoluble oxalate. It is used for determination of Haemoglobin %, ESR estimation, RBCs count and leucocytes count.

  It contains:

  - Ammonium oxalate
  - Potassium oxalate
  - This combination minimizes the morphological changes that occur in the erythrocytes.
  - Routine hematological examinations and ESR determination.

CHAPTER 5

PATHO-PHYSIOLOGICAL PROCESSES OF URINE CONSTITUENTS

• Urine is an excretory product of the body.
• It is formed in the kidney
• Urine examination helps in the diagnosis of various renal as well as systemic diseases.

URINE SAMPLE PRESERVATION

For determination of

urea, ammonia, nitrogen, and calcium – **hydrochloric acid** is used.

For determination of
sodium,potassium,chloride,bicarbonate,calcium,phosphorus,urea,ammonia,amino acids, creatinine ,proteins, reducing substances, and ketone bodies –**THYMOL** is used .

For determination of
ascorbic acid : **acetic acid** is used

❑ Normal constituents of urine

Normal urine is composed by:
1) Water :90-95%
2) Solids:5-10%
Solids are of two types:

- organic(urea, uric acid, and creatinine)
- Inorganic (sodium, potassium, calcium, and phosphate).
3) Others trace elements
Ammonium chloride, oxalates, and minerals

❑ Abnormal constituents of urine

• Proteins

Presence of protein in urine is proteinuria. Appearance of albumin in urine is albuminuria; albumin is seen in pregnancy and kidney diseases like nephritic syndrome.

• Glucose

Presence of reducing sugar in urine is glycosuria, it is seen in diabetes mellitus and renal glycosuria

Ketone bodies

(Acetone, aceto acetic acid, and beta –hydroxybutyric acid are called ketone bodies) they are present in the urine during long fasting and uncontrolled diabetes mellitus.

- **Bilirubin**

It appear in urine in case of jaundice

- **Blood**

In disease of kidney like nephritis, RBC appear in urine .hemoglobin appear in urine in conditions with excessive breakdown of RBC.

- **Creatinine**

creatinine appears in the urine whenever there is muscle wasting as in myopathies (a disease of muscle tissue) and starvation.

A routine urinalysis consists of three parts which are the following:

1.Physical examination
2.Microscopic examination

3.Chemical examination

1.Physical examination of urine

Introduction

A routine urinalysis consists of three parts: physical, chemical and microscopic examination. The physical examination is the first part of urinalysis performed and includes observing urine color and transparency and measuring specific gravity. It is the quickest and simplest part of routine analysis and can be performed while the specimen is being prepared for other procedures.
Physical characteristics of urine

The physical characteristics of urine are

- colour
- transparency
- specific gravity
- odor

Colour

The normal color of urine ranges from pale yellow to amber. Variation in colour can be caused by diet, medications, physical activity and disease state. Colour can therefore be a clue to certain disease or conditions.

- **Yellow urine:**

The pigment that produces the normal color of urine is urochrome. As the urine concentration varies. Dilute urine samples are pale while more concentrated samples are darker yellow and amber.

- **Red urine:**

The abnormal color seen frequently in urine is red brown. Cloudy urine can be due to hematuria, the presence of blood cells. Red blood cells, hemoglobin and myoglobin form red brown color acidic urine. Porphyrins can cause the urine to be red or wine- red.

- **Brown or black urine**

Hemoglobin will become brown in acidic urine that has been standing. The presence of melanin, a dark pigment will also cause the urine to become dark. This can occur in patients with advanced melanoma, a tumor of melanin producing cells

- **Yellow brown or green brown urine**

Bilirubin or bile pigment causes urine to be dark yellow brown or green brown. Urine specimens containing these substances can be present in the urine of patients with hepatitis

Transparency (clarity)

Fresh urine is usually clear immediately after voiding. As the urine reaches room temperature, or after refrigeration, it can become turbid or cloudy. Depending on the pH of the urine,

this cloudiness can be due to amorphous urates or phosphates. The transparency of urine can give clues to possible problems. Clear urine usually has normal microscopic results; any abnormalities in a clear specimen is usually detected in the chemical examination. The cause of a cloudy or turbid urine specimen usually becomes evident during microscopic examination.
Turbidity or cloudiness in a freshly voided urine sample can be an indication of disease. Four common causes of turbid urine are white and red blood cells, epithelial cells and bacteria. Red blood cells in urine give it a cloudy, red appearance. Mucus in the urine also causes urine to appear milky.

Odor

Normal, recent voided urine has a characteristic aromatic and unpleasant odor. Changes in urine odor can be due to disease, diet, or the presence of microorganisms. Although urine odor is not usually reported on the urinalysis form, it is noticeable property that can alert the technologist of possible abnormalities or improper handling of urine sample.
The odor of urine from patients with uncontrollable diabetes is described as fruity. This is due to the presence of ketones, a product of fat metabolism. Other metabolic diseases can also cause unusual urine odor. Phenylketonuria (PKU) an inherited condition in which the amino acid phenylalanine is not metabolized causes urine to have a mousy or musty odor. All newborns are tested for PKU because mental retardation will result if the condition is allowed to grow untreated. Maple syrup urine disease is a rare metabolic condition in which the urine has a maple syrup odor. It is evident in the early weeks of life of infected infants

If urine is allowed to remain un-refrigerated for a few hours, any bacteria present can break down urea to form ammonia; the resulting urine odor is similar to ammonia. A recent voided sample of urine with a foul, pungent odor suggests urinary tract infection.
Food such as garlic and asparagus can also produce an abnormal urine odor. Although urine odor may be stinking in certain instances, odor alone is not reliable enough characteristic to use in making a diagnosis.

Specific gravity

The specific gravity of solution is the ratio of the weight of the solution compared to the weight of an equal volume of distilled water at the same temperature.
The range of specific gravity for normal urine is 1.005 to 1.030 with most samples falling between 1.010 to 1.025. Specific gravity is the highest in the morning specimen. It estimates the concentration of solutes such as urea, phosphates, chloride, proteins and sugars in the urine. It is an indicator of renal tubular function and it is used to assess the ability of the kidney to reabsorb essential chemicals and water from the globular filtrate
Darker urine samples are usually more concentrated than pale urine samples. Patients who are dehydrated may have highly concentrated urine with high specific gravity.

Measuring specific gravity

Specific gravity can be measured using a urinometer, refractometer, or reagent strip. Urinometer method requires 20-50ml of urine depending on the size of the urinometer. The refractometer method requires only a drop of urine.

Urinometer method

Well mixed urine is poured into a special glass cylinder and the urinometer, a weighted float with a calibrated stem is placed in the urine with a slight spinning motion. The S.G is read at the urine's meniscus on the float stem. The urinometer will float high in concentrated sample and will sink lower in a dilute sample. A disadvantage of this method is the large urine volume required and the need to disinfect cylinder and urinometer.

HIGH SPECIFIC GRAVITY

Excessive sweating
Glycosuria
Acute nephritis
Albuminuria

All causes of oliguria

LOW SPECIFIC GRAVITY (LESS THAN 1.010)

Excessive water intake
Chronic nephritis
Diabetes insipidus
All causes of polyuria

LOW AND FIXED SPECIFIC GRAVITY (1.010 to 1.012)

Chronic nephritis (end stage kidney)
Arterosclerosis kidney.

URINARY VOLUME

The average 24 hrs urinary output in the adult is around 1200 to 1500ml and the night urine should not be more than 400ml
A volume more than 2000ml is termed **as polyuria**
Oliguria implies excretion of urine less than 500ml .
Anuria is a complete cessation .
Nocturia is excretion by an adult of urine more than 500ml with a specific gravity of less than 1.018 at night (characteristic of chronic glomerular nephritis)

POLYURIA

Causes:

- Neurotic polydipsia
- Diabetes mellitus/insipidus
- Diuretics(substance that promotes diuresis:increased production of urine)

- Intravenous saline/ glucose
- Chronic renal failure
- Addison's disease ,decrease of adrenocortical hormones.

OLIGURIA

Causes:

- dehydration
- vomiting
- diarrhea
- excessive sweating
- renal ischemia
- acute renal tubular necrosis
- acute glomerulonephritis
- obstruction to urinary outflow

2.Microscopic examination of urine

2.1.Introduction

- Microscopic examination of urine sediment is the second part of the routine urinalysis. The examination can reveal infection, disease, or trauma in the urinary tract. In addition, certain findings, such as the presence of abnormal crystals, can suggest a metabolic disorder.

2.2.Components of urine sediment

- Urine sediment is the solid that settle at the bottom of the urine specimen when urine is allowed to stand undisturbed after centrifugation. Most of the supernatant, the liquid lying

over the sediment is then removed and the remaining sediment is microscopically examined.

- The components that can be seen in urine sediment include blood cells, epithelial cells, crystals, casts and microorganisms. Urine sediment can be observed unstained or using stains like KOVA or Sedi-stain for component identification.

a.Cells

- Blood cells and epithelial cells can be found in normal urine but are present in low numbers. An Increase in a particular cell type can indicate the presence of certain pathological conditions. Cells in urine sediment are identified, quantified , and reported as part of the microscopic examination of urine.

Blood cells

Blood cells can be microscopically seen in urine with a 40X objective. Red blood cells look like pale, light refractive disks viewed under high power. The presence of high numbers of red blood cells in urine is called hematuria and is an abnormal condition indicating of urinary system disease or trauma. White blood cells- A few white blood cells can be present in normal urine. Neutrophils are the most predominant type. They are slightly larger than red blood cells, have a granular appearance and have a visible nucleus. They are increased in urinary tract infections.

Epithelial cells

They are larger than white cells and are slightly flatter with a distinct nucleus and large cytoplasm. The presence of large numbers of epithelial cells indicate possible chronic or acute renal disease particularly affecting the renal tubules.

b.Microorganisms

They should not be present in normal urine and their presence indicates infection.

- **Bacteria:** they appear as rod shaped or cocci shaped. Cocci often resemble amorphous material.

- **Yeast cells:** mostly, Candida albicans is seen. It appears as budding or in chains. They can be confused to red cells and the best way to distinguish them is by adding a drop of dilute acetic acid which will destroy red cells but will not affect yeast cells.

Protozoa: these are free living or parasites eukaryotes. The most common is trichomonas vaginalis that can be identified due to its twitching movement

•Spermatozoa

They can be seen with their oval head and a single long flagellum. They can be motile or non-motile

•Casts

Kidneys tubules usually secrete small amount of mucoprotein. In condition of slow urine, acid pH, increased solutes, the protein accumulates and begins to gel forming casts.
Casts are cylindrical with round or flat ends and are classified according to the substance observed in them.

C.crystals

- Abnormal crystals

They can occur in urine of patients with certain metabolic diseases or after administration of low solubility drugs like sulfa drugs. They are cystine, tyrosine, leucine, cholesterol, hippuric acid. They are not common but when present, they are usually in acidic urine.

- **Normal crystals in acidic urine**

- **<u>Amorphorous urates</u>**

They are called amorphous because they have no specific shape. When amorphorous urates are present, the centrifuged sediment may appear pink but microscopically the sediment will appear as fine yellowish granules.

- **<u>Calcium oxalate</u>**

Calcium oxalate forms colorless, refractive octahedral crystals. Microscopically they look like envelopes having an X intersecting the crystal and can vary in size. They are usually seen after ingesting large doses of vitamin C.

Normal crystals in neutral or alkaline urine

- **Amorphous phosphates**

  They appear as white precipitate in the sediment of centrifuged urine having neutral to alkaline pH. Microscopically, they appear as colorless, amorphorous granular particles. Amorphorous phosphates are soluble in 10% acetic acid

- **Triple phosphate**

  Ammonium magnesium phosphate crystals commonly known as triple phosphates are six sided, colorless, highly refractile prisms. They can be present in alkaline and neutral urine

- **Calcium phosphate**

  These occur in neutral or alkaline urine. They are large, flat, thin plates that can appear granular and can be mistaken for squamous epithelial cells

- **Calcium carbonate**

  Calcium carbonate forms small, colorless, dumbbell shaped or leaf shaped crystals in alkaline urine.

3.CHEMICAL EXAMINATION OF URINE

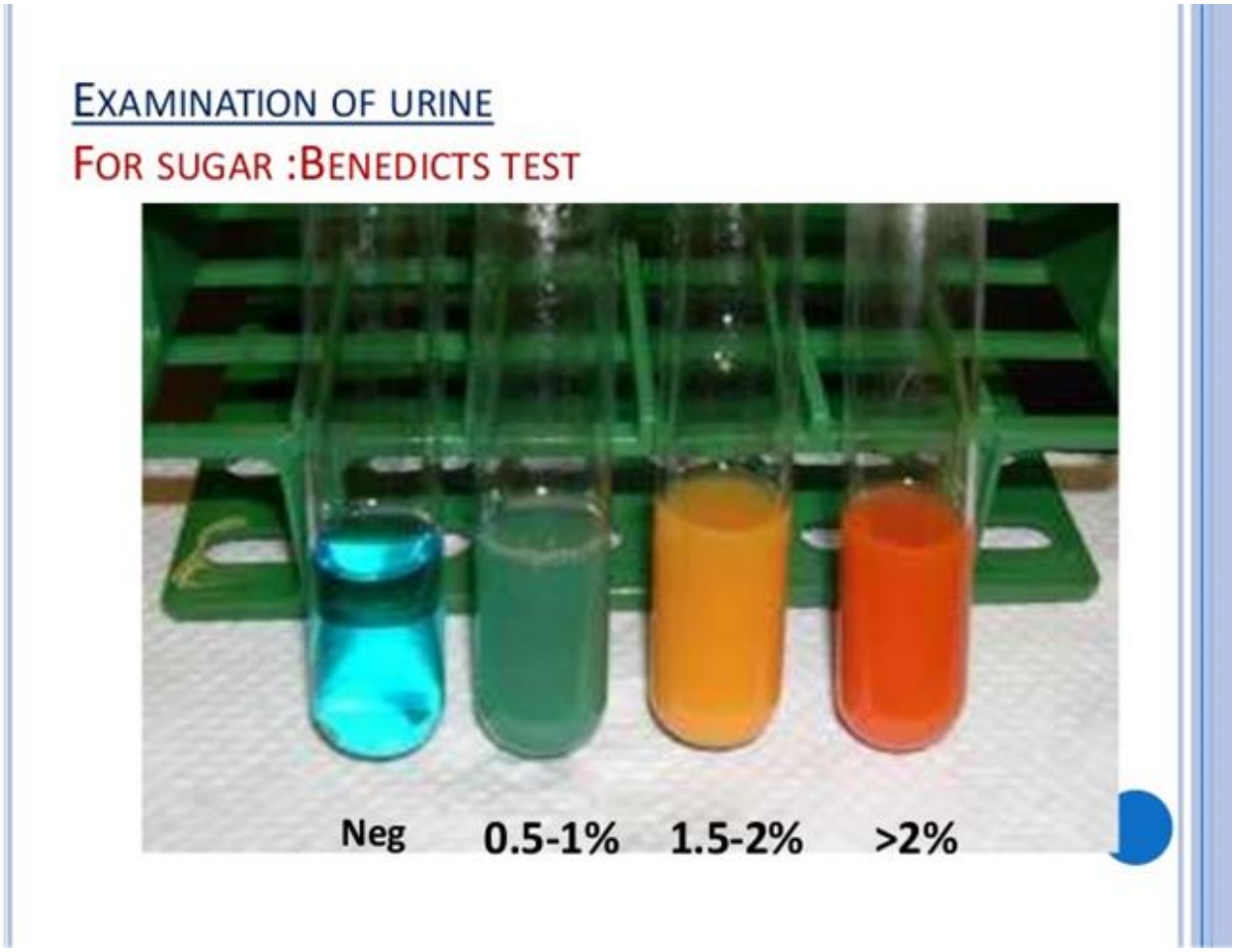
❖ Benedict test

Principle

When benedict's qualitative reagent 5ml is heated with eight drops of urine about 0.5ml glucose present in urine reduces cupric ions present in reagent to cuprous ions.Alkaline medium is provided to the reaction by sodium carbonate present in the reagent .The original color of Benedict's reagent is blue.It changes to green ,yellow ,orange or red,according to the concentration of glucose present in urine.

Procedure:

1.Pipette 5ml of benedict's reagent in a test tube .
2.By using Pasteur pipette ,add eight drops of Urine.
3.Heat carefully on the flame of a gas burner or spirit lamp or place in boiling water for 5-10 mins.
4.cool under tap water or by placing in a beaker containing tap water.
5.Then observe for color change.



❖ **GLUCOSE OXIDASE METHOD(strip)**

Glucose oxidase reacts with glucose to yield gluconic acid and hydrogen peroxide. hydrogen peroxide and orthotolidine yield a blue color .

This is a specific test, the reagents may be impregnated on a paper strips .and dipping them in urine provide the results in lesser time as compared to benedict's method (sensitivity=0,1%)

TEST FOR PROTEINS

Qualitative estimation of protein in urine

- Heat and acetic acid test
- Paper strips method
- Sulphosalicylic acid test

HEAT AND ACETIC ACID TEST

Procedure:

- Take a test tube 2/3rd full with urine
- Boil upper portion of urine for 2minutes
- Lower portion is not heated so that it can be used as a control for comparing
- Now turbidity can arise because of phosphates ,carbonates or protein.
- Add a few drops of 10% acetic acid
- Persistence or development of turbidity implies proteinuria.

INTERPRETATION

- Negative : no cloudiness
- \+ : definite cloudiness ,but no granularity (is the extent to which a material or system is composed of distinguishable pieces or grains)and flocculation Flocculation (occurs as a result of a chemical reaction between the clay particles and another substance, usually salt water.)

- ++ : granular cloudiness ,but no flocculation seen from above ,the cloud is dance but not opaque protein content is about 0.1%
- +++: dense opaque cloud, clearly flocculated about 0.2 to 0.3% protein

- ++++: very thick precipitation ,almost a solid. Protein concentration >0.5%

- **PAPER STRIP METHOD**

  - Paper strips impregnated with bromphenol blue and salicylate buffer are dipped in urine.
  - Presence of protein is indicated by change of color from light yellow to blue.

- **Sulphosalicylic acid test (SSA)**

  Principle

  SSA is an anionic precipitant which neutralizes the cationic of the protein resulting in precipitation due to denaturation of the protein structure. The turbidity formed is proportional to the amount of protein present in the urine sample.

Procedure:

- 0.5 ml of 20-25% of SSA is added to 5ml of clear urine or 1ml urine to 3ml 3% SSA.

- Heat it until the turbidity arise.
  - In the presence of protein a white precipitate appears, the turbidity being proportional to the amount of protein present.
  - Absence of cloudiness means absence of protein.

False positive can be caused by high levels of uric acid in urine, but the turbidity due to uric acid will disappear on warming.

Bilirubin

- **Fouchets test**

Principle

Barium chloride reacts with sulphate radicals in urine to form a precipitate of barium sulphate. Any bile pigments present will adhere to the precipitate. Addition of FeCl3(Ferric chloride) will oxidize the bilirubin (yellow) to biliverdin (green).

Procedure:

1. Place 5ml 10% barium chloride in a test tube and mix with 10 ml of Urine.
2. Mix well and filter
3. Add one drop of Fouchet's reagent.
4. In the presence of bilirubin a green colour will form
5. Report results.

Ictotest

The Ictotest is a specific test for bilirubin and is four times as sensitive as the reagent strip method.

PROCEDURE:

- The test uses a tablet and absorbent mat.
- A few drops of urine are placed on the mat .
- The tablet is placed on the moist surface
- Water is dropped on the tablet.
- If biliribin is present, a purple colour will develop on the mat within 60 seconds.

KETONE BODIES

The three ketone bodies that can be detected in urine are:

- ❑ Acetone
- ❑ Acetoacetic acid
- ❑ B-hydroxybutyric acid

Ketone bodies are products of incomplete fat metabolism and their presence is indicative of acidosis.

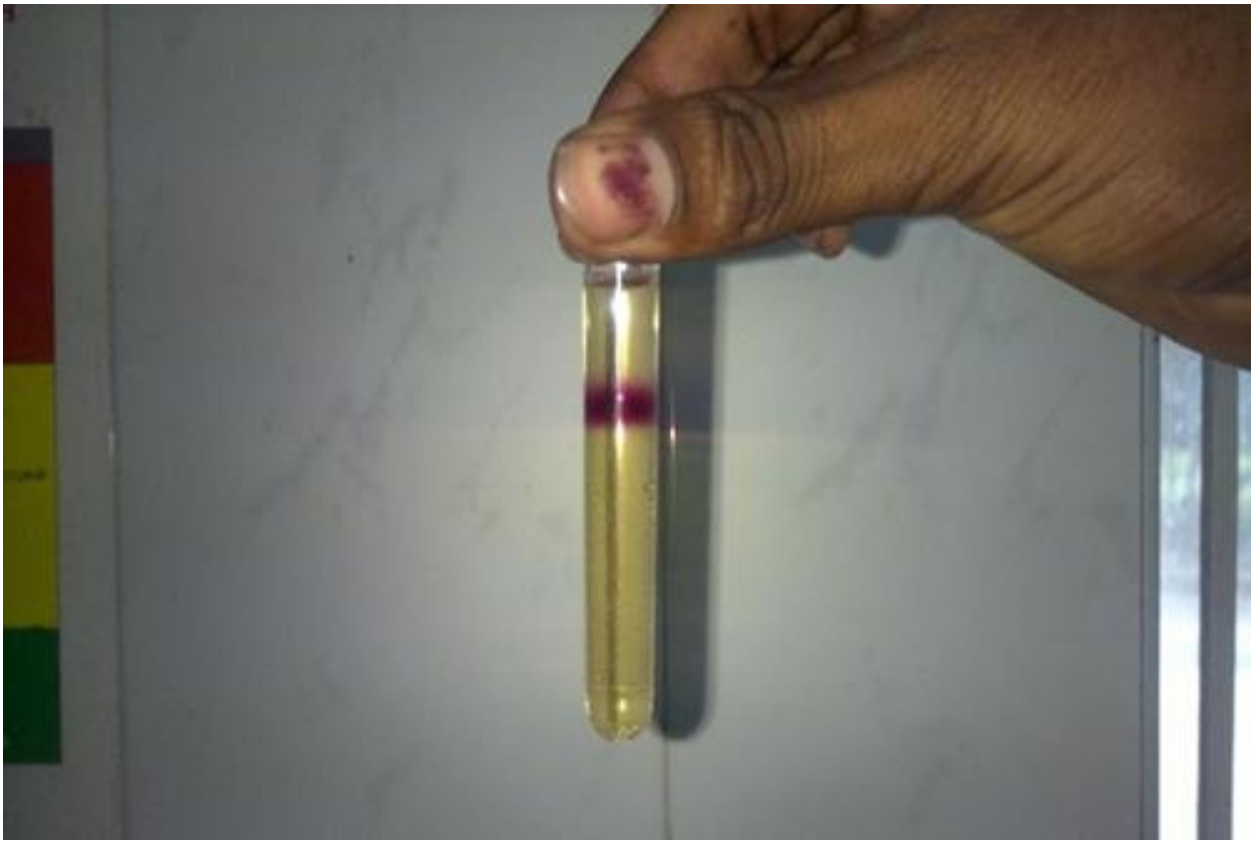
TEST FOR KETONE BODIES

- ❑ • Rothera's test
- ❑ • Legal's test
- ❑ • Paper strip
- ❑ • Gerhard's test

ROTHERA'S TEST

PROCEDURE:

- Saturate 5ml of urine with ammonium sulfate
- Add a few Crystals of sodium nitroprusside
- Shake it well.
- . Add a liquor ammonia from the side of the test tube
- Formation of a purple ring at the junction indicate a positive test.



❑ LEGAL'S TEST

PROCEDURE

- Take 10ml of urine in a test tube and add a few crystals of sodium nitroprusside .
- Acidify with glacial acetic acid(2-3drops) .
- Invert to mix.
- Overlay with strong liquor ammonia
- let stand for 5minutes.
- A violet ring indicate a positive test.
- The degree of positivity depends upon the speed of the reaction.

❑ PAPER STRIP

These contain sodium nitroprusside ,amino acetic acid and disodium phosphate .

A positive test is indicated by development of a purple color.

❑ Gerhard's test

Principle

Ferric chloride reacts with aceto-acetic acid to give a characteristic purple colour.

Procedure:

- Into a test tube place 5ml of urine
- Then add drop by drop 10% ferric chloride.
- observe the development of a purple colour

CAUSE OF KETONURIA

In case of Diabetes:
Whenever glycosuria is more than 2+
Always test for ketone bodies also.
ketonuria indicates ketoacidosis and if unchecked may go to coma.

BILE SALTS

Bile salts when present decrease the surface tension of urine.
How to check it in urine?
When sulphur powder is added on the surface of urine sulphur particles sink to the bottom of the test tube .
In normal urine sample sulphur particles float on the surface of the urine.

PROCEDURE

- Take about 10 ml of urine in a test tube
- Sprinkle a little dry sulphur powder on the surface of the urine
- Observe the sulphur particles

INTERPRETATION

- If sulphur particles sink to the bottom: bile salts present
- If sulphur particles remain floating: bile salts absent
- Dipstick tests are available.

BILE PIGMENTS

Definition:

- Colored compound breakdown products of the blood pigment haemoglobin that are excreted in bile pigment bilirubin which is orange or yellow and its oxideed from biliverdin which is green
  Bile pigments (always use a fresh specimen)
  Normal level of bile pigments in urine is **<0.02mg %**

TESTS FOR BILE PIGMENTS

- Foam test
- Iodine ring test
- Harrison test
- Diazo test

- **Harrison test**

PROCEDURE:

- To 5 ml of urine ,add 5ml of 10% barium chloride in a test tube .
- Shake well the tube and then filter it off
- Let the filter paper dry.

- when dry add 1-2 drops of fouchet's reagent to the dried precipitate .
- A green color indicates bilirubinuria

❑ Iodine ring Test

A sensitive reliable test .

PROCEDURE:

Layer a solution of 10% alcoholic iodine on urine in a test tube
A green ring indicates presence of bile.

BLOOD IN URINE (HEMATURIA)

Hematuria can be gross
Urine appears reddish due to blood
It can also be microscopic
When it is not visible to the naked eye
Here various tests are performed for confirmation:

- Guaic test
- Benzidine test
- Paper strips

❑ GUAIC TEST

PROCEDURE:

- In one test tube mix 2ml of 10%acetic acid
, 5ml of ether and 5ml of urine.

- In a second test tube place 5ml of 95% alcohol ,2ml fresh hydrogen peroxide and a pinch of powdered guaic .
- Now pour the guaic solution slowly down the side of the 1st tube .
- Blood in urine causes blue color to appear at the zone of contact between the guaic and ether.

❑ BENZIDINE TEST

PROCEDURE:

- Saturate 2ml of glacial acetic acid with benzidine and pour off the clear supernatant fluid.
- Add 1ml of fresh hydrogen peroxide and 2ml of urine.
- Development of blue color indicates a positive test
- If the blue color develops before the addition of urine ,the glassware is contaminated.

❑ PAPER STRIP

Blood reacts with the peroxide –orthotolidine reagent to produce a blue color.

CAUSES

- Bleeding diathesis (generally result in excessive bleeding and a lack of clotting.)
- Local disorders of kidney and genitourinary tract etc…

RENAL CAUSES

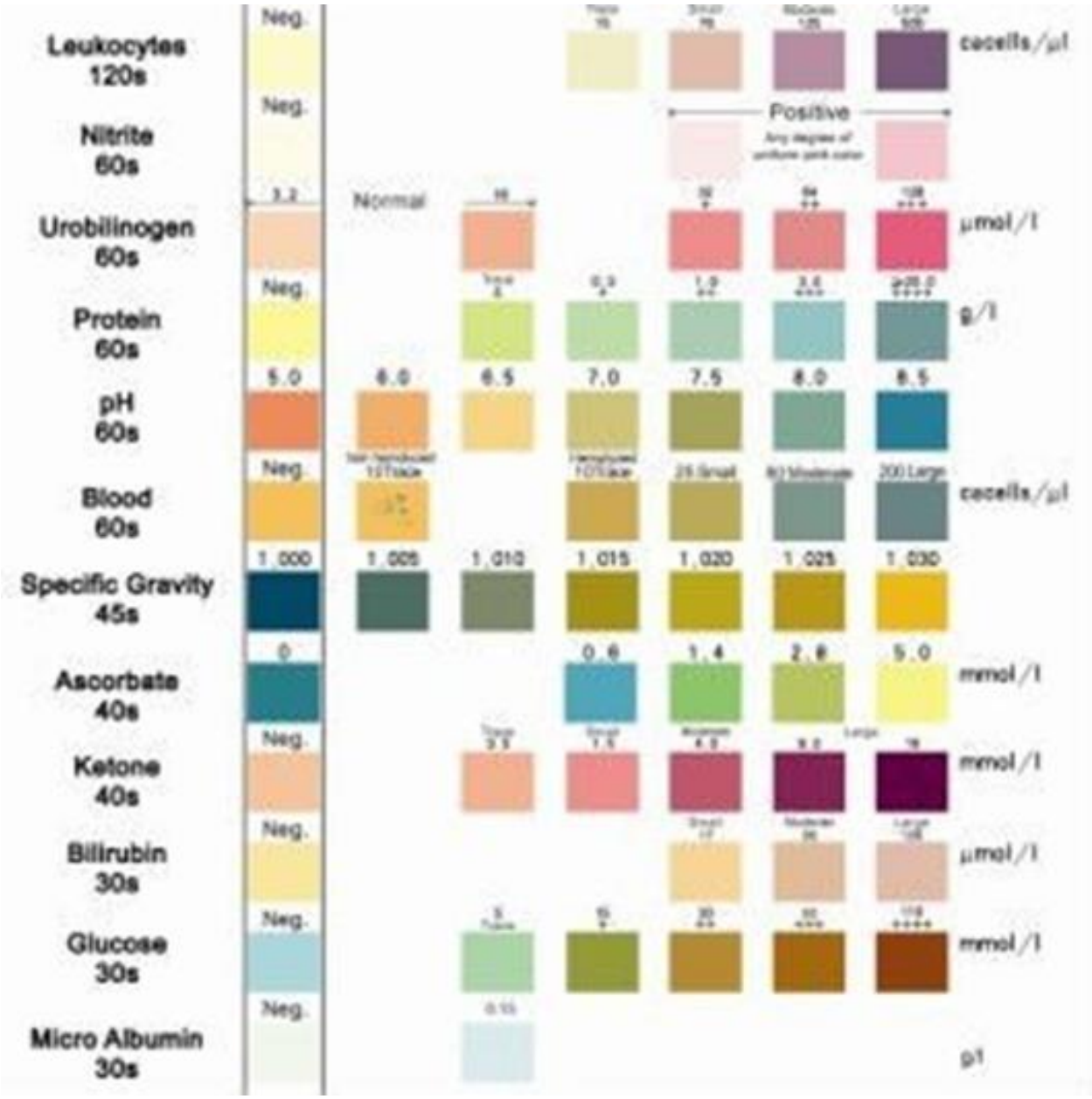
- • Renal disease which diminishes the glomerular filtration rate leads to urea retention (a relatively increased concentration in the blood, is the result of increased resistance to the excretion of urea through the kidneys)
  and increase in plasma concentration until the higher concentration in the glomerular filtrate compensated for its diminished volume in slowly progressive, chronic failure.
- • In acute renal failure with anuria , the rate of increase in plasma concentration is much more rapid.
- Glomerular nephritis or Bright's disease.
- Obstruction to the flow of urine after it leaves the kidney leads to back pressure on the renal pelvis and diminished glomerular filtration of urea.
- • If prolonged secondary renal damage occurs, renal uremia is added
- Urinary calculi, uretheral structure and malignant tumors involving both ureters.

MATERIALS/EQUIPMENT REQUIRED FOR DIPSTICK TESTING

- Reagent/test strips – in-date and stored correctly
- Watch
- Urine sample in suitable container
- Gloves
- Good lighting
- Access to hand washing and drying
- Suitable room for testing
- Suitable waste disposal .

MANUAL TEST PROCEDURE

- Wear gloves.
- Ensure the sample is in the correct container.
- Check the appearance of the sample and record results.
- Ensure the strips have been stored properly & are in-date.
- Remove the cap, take out strip and replace the cap on the bottle.
- Using the appropriate reagent strip completely immerse all reagent areas into the sample.
- Dip briefly and remove immediately to avoid dissolving out the reagents.
- While removing the strip, run the edge against the rim of the urine container to remove excess urine.
- Hold the strip in a horizontal position to prevent possible mixing of chemicals from the adjacent areas.
- After the appropriate time, compare test areas closely with the corresponding colour chart on the bottle label at the specified time. Hold the strip close to the colour blocks and match carefully.
- Always record results.



SOURCES OF ERROR

- ❑ Incorrect dipping of reagent strip.
- ❑ Incomplete wetting of strip.
- ❑ Incorrect storage of strips – always check manufacturers instructions.
- ❑ Sample error – sample must be allowed to return to room temperature, non sterile containers; sample needs to be fresh for best results.

- Contamination of the reagent pad by handling or non sterile container.

- pH may be falsely elevated if the urine is stale.
- Some medication can affect some of the reagents on the strips (e.g. cephalosporins; L-dopa; high levels of salicylates; chlorhexadine; ferrous sulphate)
- Strips out of date.
- Vegetarians may have a urine pH >8.

CHAPTER VI

INTERNATIONAL SYSTEM OF UNITS (S.I. UNITS)

In an effort to standardize scientific measurements worldwide most countries have adopted the use of SI units, from the International System of units, the modern metric system of measurement. SI units are derived from the metric system and

are based on seven fundamental units, from which commonly used units are see table below. These seven units have internationally agreed-upon values and were selected because they make possible more precise, reproducible measurements.

SI base units

The SI is founded on seven SI base units for seven base quantities assumed to be mutually independent, as given in Table below

Base quantity	Name	Symbol
	SI base unit	
length	meter	m
mass	kilogram	kg
time	second	s
electric current	ampere	A
thermodynamic temperature	kelvin	K
amount of substance	mole	mol
luminous intensity	candela	cd

TERMINOLOGY OF THE METRIC SYSTEM AND SI UNITS

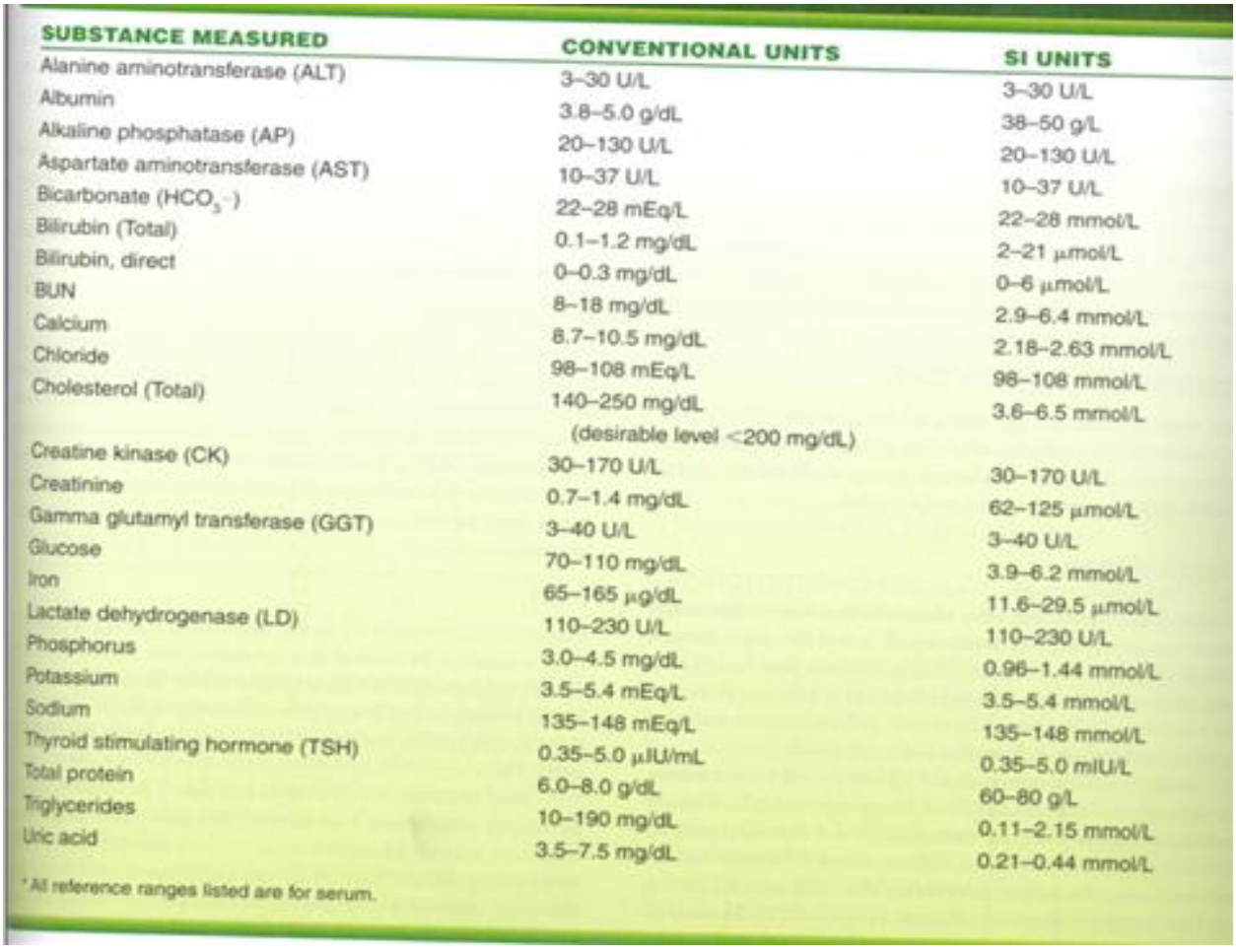
Clinical chemistry test results are usually reported in metric units or SI units. Commonly used units are milligrams (mg) or micrograms (µg) per deciliter (dL), millimoles per liter (mmol/L), or, in the case of enzymes, enzyme activity units per liter (U/L).

Factor	Name	Symbol	Factor	Name	Symbol
10^{24}	yotta	Y	10^{-1}	deci	d
10^{21}	zetta	Z	10^{-2}	centi	c
10^{18}	exa	E	10^{-3}	milli	m
10^{15}	peta	P	10^{-6}	micro	µ
10^{12}	tera	T	10^{-9}	nano	n
10^{9}	giga	G	10^{-12}	pico	p
10^{6}	mega	M	10^{-15}	femto	f
10^{3}	kilo	k	10^{-18}	atto	a
10^{2}	hecto	h	10^{-21}	zepto	z
10^{1}	deka	da	10^{-24}	yocto	y

NORMAL VALUES OR REFERENCE VALUES

Before a laboratory can determine whether a value is normal or abnormal, studies must be done to establish a range of normals for individual laboratories. Using the standard deviation formula, normals can be established through testing "normal" populations in the area where the laboratory is located. There is variation in populations due to age, sex, race, geography, cultural differences, and economic conditions. Traditionally normals have been established by testing healthy

persons. To establish normal ranges, the laboratory must always use statistical tools to set meaningful ranges.
For instance, when establishing the reference range for total protein, the laboratory has to test 100 random samples from healthy persons and calculate the mean value and the standard deviation of the set values. The reference range is then determined by adding (and subtracting) 2SD (±2SD) to the mean. Patient results are then compared to this reference range when results are reported to the physician. Many laboratories do not establish their own ranges, they often adopt normal values established by manufacturers of various test protocols. After normal ranges are set physicians are given information about these ranges for their use and interpretation of patient results.



SUBSTANCE MEASURED	CONVENTIONAL UNITS	SI UNITS
Alanine aminotransferase (ALT)	3–30 U/L	3–30 U/L
Albumin	3.8–5.0 g/dL	38–50 g/L
Alkaline phosphatase (AP)	20–130 U/L	20–130 U/L
Aspartate aminotransferase (AST)	10–37 U/L	10–37 U/L
Bicarbonate (HCO_3^-)	22–28 mEq/L	22–28 mmol/L
Bilirubin (Total)	0.1–1.2 mg/dL	2–21 μmol/L
Bilirubin, direct	0–0.3 mg/dL	0–6 μmol/L
BUN	8–18 mg/dL	2.9–6.4 mmol/L
Calcium	8.7–10.5 mg/dL	2.18–2.63 mmol/L
Chloride	98–108 mEq/L	98–108 mmol/L
Cholesterol (Total)	140–250 mg/dL (desirable level <200 mg/dL)	3.6–6.5 mmol/L
Creatine kinase (CK)	30–170 U/L	30–170 U/L
Creatinine	0.7–1.4 mg/dL	62–125 μmol/L
Gamma glutamyl transferase (GGT)	3–40 U/L	3–40 U/L
Glucose	70–110 mg/dL	3.9–6.2 mmol/L
Iron	65–165 μg/dL	11.6–29.5 μmol/L
Lactate dehydrogenase (LD)	110–230 U/L	110–230 U/L
Phosphorus	3.0–4.5 mg/dL	0.96–1.44 mmol/L
Potassium	3.5–5.4 mEq/L	3.5–5.4 mmol/L
Sodium	135–148 mEq/L	135–148 mmol/L
Thyroid stimulating hormone (TSH)	0.35–5.0 μIU/mL	0.35–5.0 mIU/L
Total protein	6.0–8.0 g/dL	60–80 g/L
Triglycerides	10–190 mg/dL	0.11–2.15 mmol/L
Uric acid	3.5–7.5 mg/dL	0.21–0.44 mmol/L

* All reference ranges listed are for serum.

CHAPTER VII

QUALITY ASSURANCE AND QUALITY CONTROL

DEFINITION

Laboratory Quality Control refers to the measures that must be included during each assay run to verify that the test is working properly.

Lab QC = Running controls and statistically analyzing the data before releasing patient results.

Quality control is a statistical system for measuring the reproducibility of degree of precision (exactness) in laboratory procedures. It is an excellent way of improving laboratory efficiency. The programmers ensure the physicians quality results and the patient's better results.

QUALITY CONTROL SAMPLES:

Quality control samples are "knowns." They have been made or manufactured with a composition similar to patient samples and have been analyzed for concentration before they are put into use so they have expected results.

Therefore, if Quality Control samples are analyzed and expected results are obtained, we can assume our system is operating correctly.

Quality control concepts

- Sensitivity
- Specificity
- Predictive value
- Precision
- accuracy
- Sensitivity:

  It is also called the true positive rate, the recall, or probability of detection in some fields) measures the proportion of cases with a positive screening test among all cases of pre-clinical disease.

  e.g : the percentage of sick people who are correctly identified as having the condition.

- **Specificity:**

  It is also called the true negative rate)measures the proportion of negatives that are correctly identified as such

  e.g :the percentage of healthy people who are correctly identified as not having the condition

- **Predictive value**

  Positive predictive value is the probability that subjects with a positive screening test truly have the disease.

  Negative predictive value is the probability that subjects with a negative screening test truly don't have the disease

- **Precision**

  This indicates how close test measurements are to each other, when the same test is run on the same sample repeatedly.

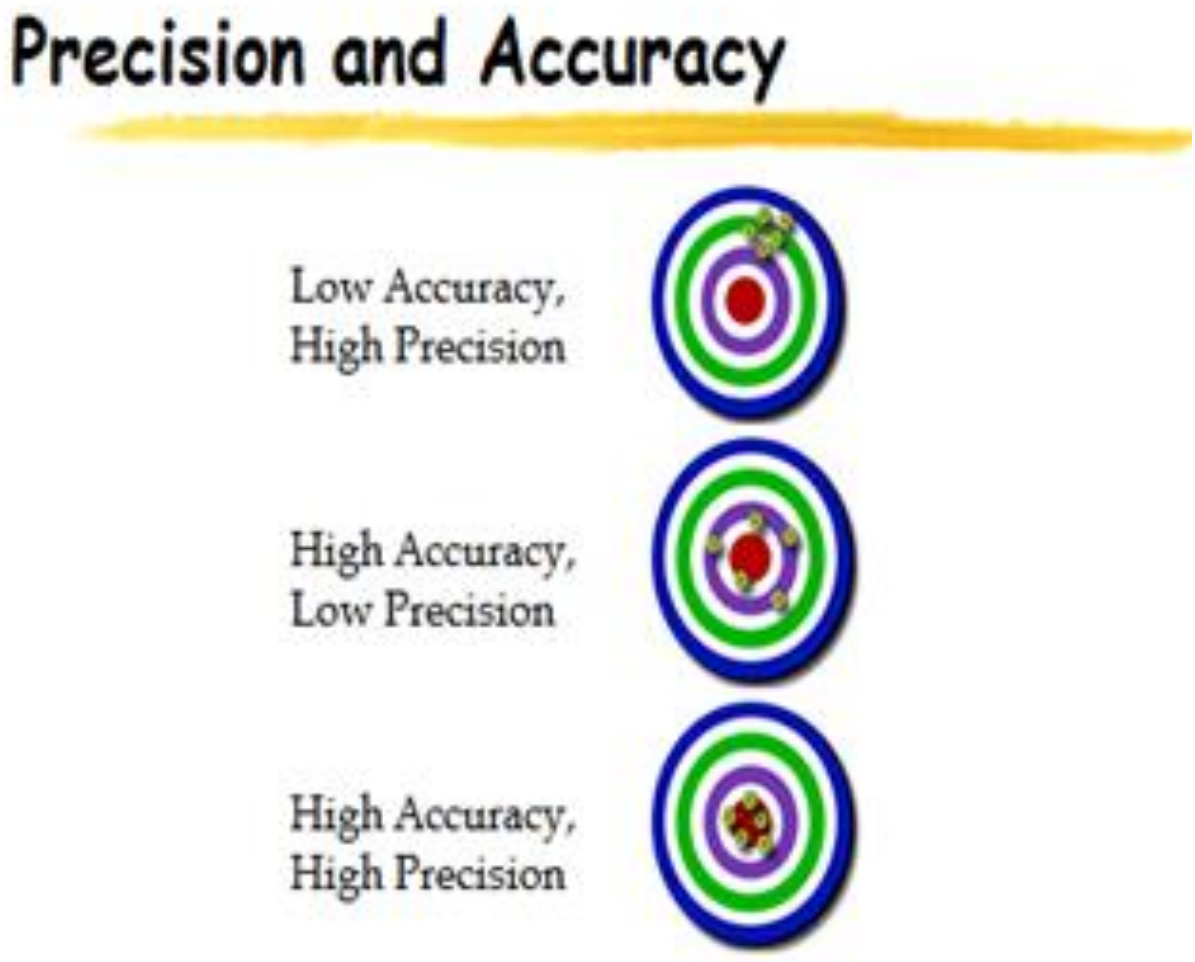
  Precision does not imply accuracy.

Precision is: The reproducibility or closeness of results to each other

- **Accuracy**

This indicates how close to the true value a measurement is. The close to the actual value, the more accurate. Briefly we can call accuracy the Closeness of the measured result to the true value

- Your control values may show precision but be inaccurate
- Instrument can repeatedly get the same value, but it's not the right value



Quality control in a clinical laboratory can be conducted by the following 2 methods:

1. Internal quality control
2. External quality control

1. Internal quality control

Internal quality control can be maintained by the following ways:

Use of standardized glassware, reagents and equipment
Employment of conscientious and well trained staff
Maintenance of

- Proper analytical skill from start to finish
- Required quality reagents
- Desired performance of this instrument

Selection of accurate and precise methods

There are 2 phases in internal quality control

a) Preventive phase
b) Retrospective method

a) Preventive phase:

In this phase, preventive precautions are taken at the following stages of specimen analysis:

- Collection of specimen
- Separation of serum
- Specimen analysis
- Photometric analysis and
- Calculations of test values etc.

b) Retrospective method:

- This phase includes the comparison between

    i) Optimal condition variance (OCV)
    ii) Routine condition variance (RCV)

OCV refers to the test results obtained under optimum conditions ie by using freshly prepared reagents and standardization A grade glass wares.

- RCV refers to the results obtained by using routine requirements (by using routine stored reagents and glassware in regular use).
- The difference between OCV and RCV should not be more than 3%.

2.External quality control

- The term external quality assessment, 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

Following are the various ways to observe external quality control:

- Use of a recognized sample
- Checking of the similarity of the reported values by sending the specimen to a recognized laboratory.
- Analysis of some specimen brought from recognized laboratories and comparative studies of the analyzed specimen.

- **RELIABILITY:**

Reliability explains about the quality of measurement. Reliability is the repeatability of measures. The term reliability means “repeatability” or “consistency”. A measure is considered reliable if it would give us the same over and over again.

- **REPRODUCIBILITY**

Reproducibility is the ability of an entire experiment or study to be duplicated, either by the same researcher or by someone else working independently. Reproducing an experiment is called replicating it. Reproducibility is one of the main principles of the scientific method. .

Experiments which cannot be reliably reproduced are generally not considered to provide useful scientific evidence. Results which prove to be highly reproducible are typically given more trust by scientists than those which are less reproducible.

STATISTICAL METHODS OF ANALYSIS OF RESULTS

Quality control programs use statistics, the branch of mathematics that deals with collection, classification, analysis and interpretation of numerical data.

A few common statistical measures used in quality control (QC) are mean, standard deviation, coefficient of variance and tolerance range.

MEAN

Mean is a measure of central tendency of the data set and denoted by X. It is calculated by summing up value of each observation, divided by the number of observations.

STANDARD DEVIATION

It is a commonly used measure of dispersion of the data and is measured by variability from the mean.

COEFFICIENT OF VARIANCE

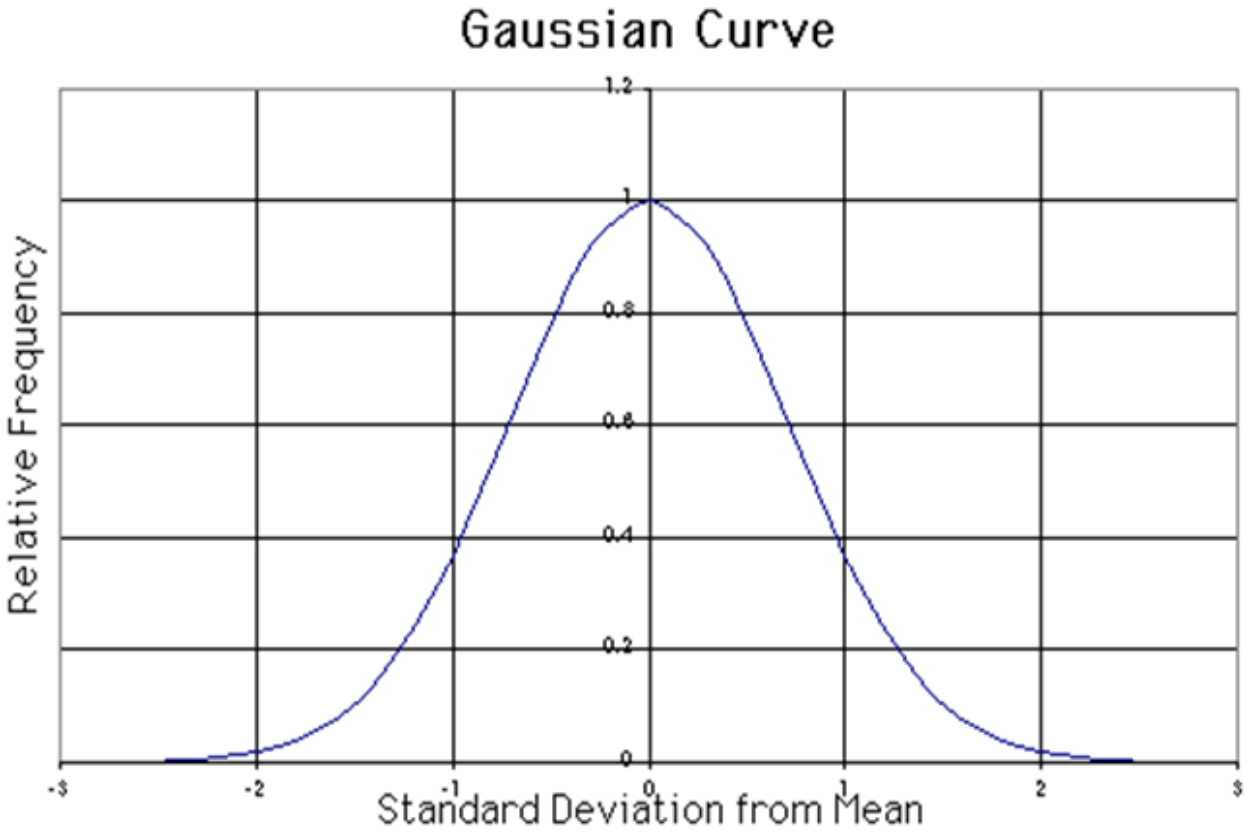
The standard deviation is expressed as a percentage of the mean value. This is called coefficient of variance (CV) or relative standard deviation (RSD)

TOLERANCE RANGE

It is the acceptance range of variation in quality control. This is equivalent to ± 2 SD.

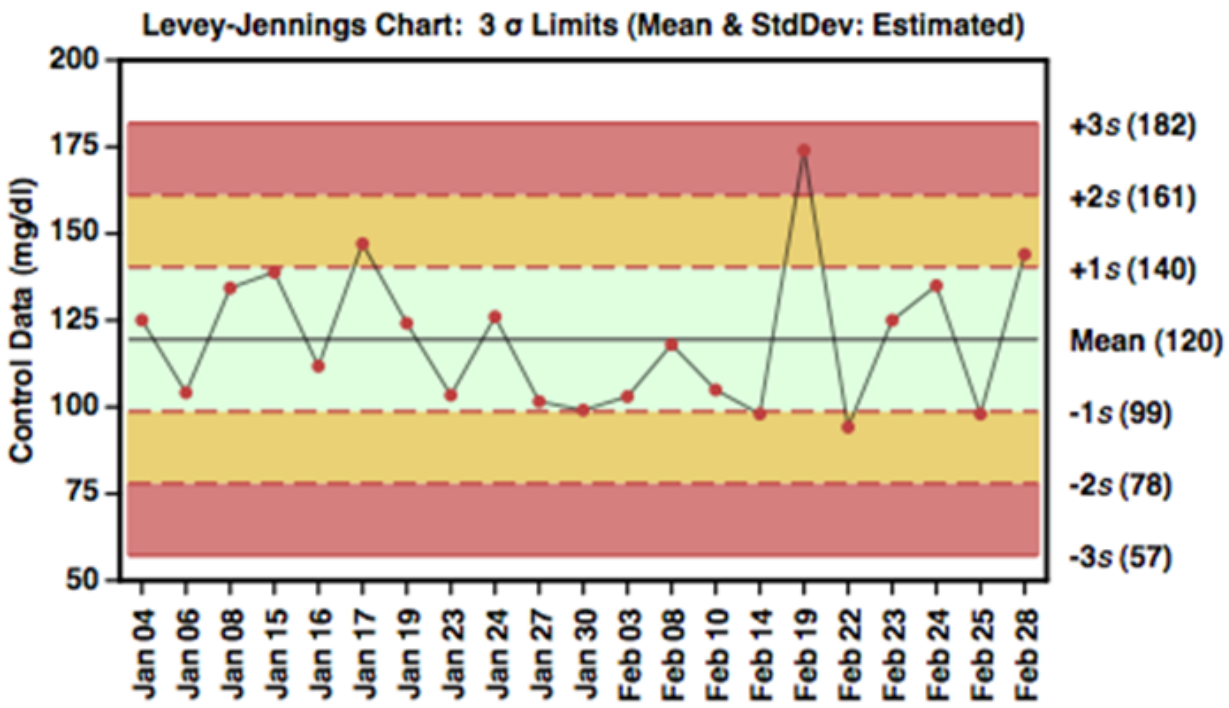
Gaussian curve

It is expected that the data for most situations in a laboratory will be form a bell-shaped curve also called normal distribution or Gaussian distribution.



LEVEY-JENNINGS CHART

It is a graph that quality control data is plotted on to give a visual indication whether a laboratory test is working well. The distance from the mean is measured in standard deviations (SD)


Levey-Jennings Chart: 3 σ Limits (Mean & StdDev: Estimated)
Control Data (mg/dl)
200
175
150
125
100
75
50
+3s (182)
+2s (161)
+1s (140)
Mean (120)
-1s (99)
-2s (78)
-3s (57)
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REFERENCES

- William J. Marshall, Stephen K Bangert, MártaLapsley, 2012, *Clinical Chemistry: With STUDENT CONSULT Access, 7e*, Seventh Ed. [ISBN: 978-0723437031]
- Anderson C. S. and Cockayne S. Clinical chemistry concepts and applications; McGraw-Hill (2003).
- ShaunnaA.andCockayne S. (2003). Clinical chemistry; concepts and applications. (9th edition)
- Karen Munson Ringsrud and Jean Jorgenson; Urinalysis and Body Fluids.
- Lorraine J. Doucette; Mathematics for the Clinical Laboratory
- David E. Golan. 2008. Principles of pharmacology The Pathophysiologic Basis of Drug Therapy. Lippincont Williams and Wilinks
- Tripathi, K.D. 2008. Essentials of medical pharmacology. Jp Medical Pub