CLINICAL BIOCHEMISTRY

Basic Concept of Clinical Biochemistry

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CONTENTS

Keywords

Quality control; Chemical units; Interpretation of results; Automation; Analyzers; Analysis of biological fluids**;** Renal function test.

Introduction

Clinical Biochemistry is one of the most rapidly advancing areas of laboratory and clinical medicine. The marked increase in the number and availability of laboratory diagnostic procedures has helped in the solution of clinical problems. Individual laboratory tests are rarely ordered and reported singly; usually combinations of lab tests are used. The physician should however be judicious in selecting the tests that really give a clue to the diagnosis of a disease. Some biochemical investigations however are done routinely for most patients e.g. qualitative tests with urine.

A trend is also emerging to conduct certain biochemical investigations, which could reveal predisposition to specific disease processes in healthy individuals. The physician can then suggest preventive measures to the person e.g. elevated levels of plasma cholesterol persisting for a long time contribute to the development of coronary artery disease and increased blood pressure. These individuals should be advised to avoid cholesterol-containing food so that the development of a serious disease could be retarded. However, one must be careful in interpreting these investigations and bear in mind the concept of normal variation from individual to individual.

Metabolic changes associated with specific disorders may give rise to a change in the biochemical profile of a particular body fluid, e.g. blood glucose in diabetes mellitus; glucose levels in the cerebrospinal fluid in bacterial meningitis (which are greatly reduced). Hence, specific parameters are looked for in a specific body fluid when a disease is suspected.
From a clinical point of view, one purpose of performing a test could be corroborating a particular diagnosis or ruling it out. Other tests may be done to assess the severity of a disease process or monitor its progress. Still others may evaluate or monitor the effectiveness or potential side effects of a particular therapeutic regimen; and certain tests can give a clue about the prognosis or probable outcome of a disease.

The final interpretation of the results of investigations, whether biochemical or of any other category, should be in total context of the disease process and the clinical profile of the patient.

This chapter deals with common units and abbreviations used; the importance of quality control; and automation in a clinical laboratory. Analysis of the various body fluids and their role in clinical diagnosis is discussed in detail.

Units and abbreviations

1. Metabolites (glucose, urea etc) are expressed as mg / dL or mmol/L.
2. Electrolytes (Na^+, K^+) as mmol/L or meq/L (earlier terminology)
3. Enzymes as Units/L

Enzymes are sometimes expressed in conventional units (e.g. Amylase - Somogyi units, Phosphatase in King-Armstrong Units)

Chemical units

1. **Molar Solutions:** Contain 1gm molecular weight of the solute / L of solution. 1 Molar solution of H_2SO_4 contains 98.08 gm H_2SO_4/L (Mol .Wt of H_2SO_4 = 98.08).

2. **Normal Solution:** Contain 1gm equivalent weight of the solute / litre of solution. 1 mole HCl, 0.5 mole H_2SO_4, 0.333 mole H_3PO_4 in 1000 ml of solution in water are one Normal solutions. No. of moles x valency = No. of equivalents; Molarity x valency = Normality

The following equations define the expression of concentrations:

$$\text{Molarity of a Solution} = \frac{\text{No of moles of solute}}{\text{No of litres of solution}}$$

$$\text{Normality of a Solution} = \frac{\text{No of gm equivalents of solute}}{\text{No of litres of solution}}$$

The units of measure commonly used to express the concentrations of electrolytes in plasma is milliequivalents (mEq)/L or millimoles(mmol)/L.

$$\text{No. of mEq} = \frac{\text{Mass (g)} \times 1000}{\text{equivalent weight (g)}} = \frac{\text{Mass (g)} \times 1000 \times \text{valency}}{\text{MW}}$$

$$\text{No. of mmol} = \frac{\text{Mass (g)} \times 1000}{\text{MW}}$$

mg/100 ml can be converted to mEq/L or mmol/L as follows:

$$\text{mEq/L} = \frac{\text{mg / 100 ml} \times 10 \times \text{valency}}{\text{Atomic or molecular mass}} \qquad \text{mmol/L} = \frac{\text{mg / 100 ml} \times 10}{\text{atomic or molecular mass}}$$

Example: If serum sodium is 322 mg/100 ml [3220 mg/L].
Atomic wt. of Na = 23, Valency = 1
m Eq/L = (322 x 10 x 1) / 23 = 140
Sodium concentration in plasma is also expressed as mmol/L.

Interpretation of results

Value obtained with a particular parameter is interpreted as increased, decreased or within normal (reference) range.

Reference values: Values obtained from individuals who are in good health as judged by other clinical and laboratory parameters, after suitable standardization and statistical analysis, under definite laboratory conditions.

Normal (Reference) Range: Values within which 95% normal healthy person's fall. The cut off values are set as mean reference value +/- N times standard deviation, of a normal healthy population; where N varies between 1, 2 and 3.

Quality Control

A major role of the clinical laboratory is the measurement of substances in body fluids or tissues for the purpose of diagnosis, treatment or prevention of disease, and for greater understanding of the disease process. To fulfil these aims the data generated has to be reliable for which strict quality control has to be maintained. Quality control is defined briefly as the study of those sources of variation, which are the responsibility of the laboratory, and the procedures used to recognize and minimize them.

Quality control involves consideration of a reliable analytical method. Reliability of the selected method is determined by its accuracy, precision, specificity and sensitivity; with major emphasis of QC being laid on monitoring the precision and accuracy of the performance of analytical methods.

Accuracy has to do with how close the mean of a sufficiently large number of determinations on a sample is to the actual amount of substance present and is dependent on the methodology used.

Precision refers to the extent to which repeated determination on an individual specimen vary using a particular technique and is dependent on how rigorously the methodology is followed.

Specificity is the ability of an analytical method to determine solely the analyte it is required to measure.

Sensitivity is the ability of an analytical method to detect small quantities of the measured analyte.

Analytical methods require calibration, the process of relating the value indicated on the scale of the measuring device to the quantity required to be measured. Calibration is done using standard, the solution with which the sample is compared to arrive at the result.
Standard solutions refer to the known amount of a substance in a solution in which its concentration is expressed in terms of moles or in weights per unit volume.

Uses:

1. For preparing a standard calibration graph, for e.g. a glucose standard solution is used in the estimation of glucose in blood, CSF and urine.
2. A standard can also be used to estimate the unknown concentration by comparing the absorbance of standard and test solutions which is measured using a colorimeter concentration of unknown substance

$$= \frac{T - B}{S - B} \text{ X Concentration of Standard}$$

where S, T and B are the absorbance of standard test and blank solutions respectively.

3. Preparation of buffers: Standard buffer components like acid and its conjugate base are prepared as standard solutions and mixed in different proportions to attain the required pH of the standard buffer.
4. Standard solutions of total protein, glucose, urea, creatinine, albumin etc. obtained from commercial sources, are used to calibrate auto analyzers.

Two major types of errors may occur in a laboratory:
Random errors that arise due to inadequate control on pre-analytical variables, patient identity, sample labelling, sample collection, handling and transport, measuring devices etc.

Systemic errors that occur due to inadequate control on analytical variables; e.g. due to error in calibration, impure calibration material, unstable/ deteriorated calibrators, unstable reagent blanks etc.

The performance of a method routinely used in a laboratory must be monitored continuously by quality control techniques in order to detect any change in accuracy or precision and take corrective action. Quality control is of two kinds: internal quality control, the procedure making use of results of only one laboratory for quality control; and external quality control in which the results of several laboratories which analyse the same sample(s) are used.

Internal quality control (QC) programme may be formulated considering the following points:
1. Clinical correlation of test with the disease the patient is suspected to be suffering from.
2. Within-assay variation: The same sample is analysed twice during an assay and the outcome noted. Results should be identical if no error exists; a large variation suggesting one or more errors.
3. Intra-laboratory duplicates: Samples may be analysed in duplicates for 2 days and reproducibility of the four values checked.
4. The results of a test may be compared with the results of the same tests previously conducted on the patient. The values are expected to increase with disease progression and vice versa. A deviation from this pattern indicates error.

External QC programme: The concerned laboratory is provided with vials of controls without reference values for analysis under the conditions of that lab. The results obtained would be sent to the reference laboratory for verification. Internal QC programme is suitable to determine the reproducibility of result (precision). External QC programme is useful to assess the closeness of a result to the actual value (accuracy).

If the result of the presently used method widely deviates from the majority of the other methods which agree with one another, the method should be immediately replaced by another. Revaluation of calibration standards, reagents, pipettes and measuring devices must be considered in case of any kind of deterioration.

Manual vs automation in clinical laboratory

Automation in clinical laboratory is a process by which analytical instruments perform many tests with the least involvement of an analyst. The International Union of Pure and Applied Chemistry (IUPAC) define automation as “the replacement of human manipulative effort and facilities in the performance of a given process by mechanical and instrumental devices that

are regulated by feedback of information so that an apparatus is self-monitoring or self-adjusting". Presently no currently available clinical instrument fully meets this definition, however the term 'automation' is applicable to the individual steps in many analytical processes and modern instrumentation is improvising with more and more intelligence built into new generations of laboratory analyzers to soon come up to the IUPAC definition.

Automated instruments enable laboratories to process a much larger workload without a relative increase in manpower. Automation in clinical laboratories has evolved from fixed automation whereby an instrument performs a repetitive task by itself, and has progressed to programmable automation, which permits it to perform a variety of different tasks. Intelligent automation has recently been introduced into a few individual instruments or systems to enable them to self-monitor and respond appropriately to changing conditions. Instead of resorting to manual means automation leads to reduction in variability of results and error of analysis by doing away with jobs that are repetitive and monotonous for an individual and that can lead to boredom or casual attitude. However, the improved reproducibility attained by automation is not necessarily associated with improved accuracy of test results since accuracy is mainly influenced by the analytical methods used. The significant improvement in quality of laboratory tests in recent years is due the combination of well-designed automated instrumentation with good analytical methods and effective quality assurance programs. Automation may initially incur high costs for procurement of the equipments but is economical in the long run due to the reduction in the manpower required to perform the tasks.

Automated analyzers usually include the mechanized versions of basic manual laboratory techniques and procedures, and several ways have been developed for automating them. When initially introduced, automation mimicked manual test procedures and was applied to those tests requested most often. All the individual steps in the procedure are duplicated. Analytical methods, which are quicker and with fewer steps as well as modification of existing protocols are being developed as the manufactures have integrated computer hardware and software into analyzers to provide automatic process control and data processing capabilities.

Types of analyzers

Semi-auto analyzer: Here, the samples and reagents are mixed and read manually (Figs.1 & 2).

Figure-1: Semi-Autoanalyzer

Figure-2: Semi-Autoanalyzer

Batch analyzer: The reagent mixture is mixed and fed automatically. One reagent is stored in the machine at a time enabling one batch of a specifc test to be automatically conducted e.g. RA 100.

Random Access autoanalyzers: These analyzers can store more than one reagent. Samples are placed in the machine and the computer is programmed to carry out any number of selected tests on each sample e.g. Hitachi 912 (Fig. 3).

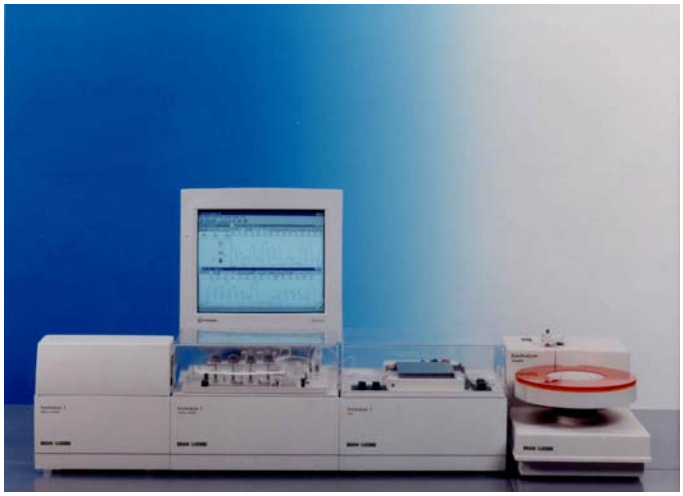


Fig. 3: Autoanalyzer

Some of the terms commonly used in autoanalysers are:
Batch analysis wherein several samples are processed for analysis in the same analytical run.

Sequential analysis, where each sample in the batch enters the analytical process one after another and the results are printed in the sequential order that they are fed.

Continuous flow analysis Here the samples of one batch are sequentially subjected to the same analytical reactions at the same rate, each sample being separated from the previous one by air.

Single channel analysis (single test analysis) each of the samples is analysed by a single process. Result of a single parameter is produced.

Multiple channel analysis(multiple test analysis) Each of the samples is subjected to multiple analytical processes and sets of test results are obtained.

Random access analysis Any sample may be analysed at random by a signal to the processing system. eg. of such systems are Ektachem, Hitachi 912 etc.

The following are the steps in the automated systems:

1. Sample identification: The tube containing each of the samples is labelled at the time of collection of blood or other fluids for analysis. On reaching the lab where it is to be tested, the sample is recorded by computerized procedure after which the samples are processed. (Glass and plastic wares using in laboratories Figs. 4 & 5).

Fig. 4: Vacutainers used for blood collection and storage

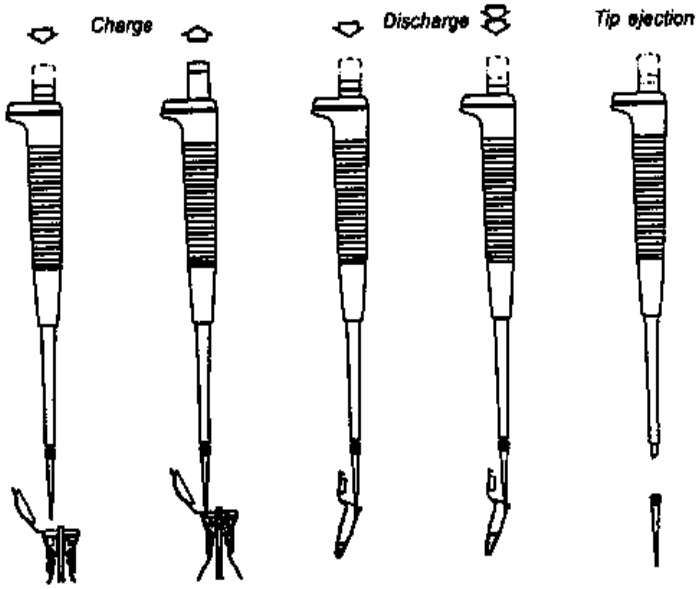


Fig. 5 Pipettes: Demonstration of how to use a pippete

Bar coding: The bar coding technology for sample identification is available in several analytical systems. A bar coded label is placed onto the sample containers and is read by the bar readers placed at key positions in the analytical train. The information that is read by the reader is transfered to and processed by the system software.

2. Sample preparation: The clotting of blood, centrifugation and transfer of serum causes delay in the specimen preparation. To eliminate these problems the use of whole blood for analysis and automation of specimen can be done.
3. Sample handling, transport and delivery: The containers (tubes) holding the samples are kept covered till the time of analysis to avoid evaporation or spillage. For analysis, the sample is loaded on loading zone of the analyzer.
4. Sample processing: Automation of the analysis of analytes requires the capability of removing the interfering substances from blood for the analyte to be tested

5. Reagent handling and delivery: Reagents should be stored in 4°C refrigerator till the assay as per requirement, and the instrument may also be pre-cooled.
6. Chemical reaction: The samples undergo chemical reactions in the analyzers in the presence of the appropriate reagents and optimum conditions set.
7. Measurement, signal processing and microprocessing: The measurements and output signals are automatically processed and the results are made available in form of readings/ graphs as per the requirements input initially.

The demand for increased efficiency and cost effectiveness in health care has led to the production and commercial availability of a number of sophisticated automated analyzers to analyze blood, urine and CSF samples. Depending on the specific requirements and workload, laboratories opt for a combination of automatic, semiautomatic and manual mode of analyses.

Collection and preservation of biological fluids

The different body fluids that are used for biochemical investigations are given below:

Body Fluid	Investigation Performed	Method of Collection
WHOLE BLOOD	Blood gases Glucose Urea	Obtained by arterial or venepuncture; collected with anticoagulants like heparin
PLASMA	Enzymes Metabolites Electrolyte	Blood with anticoagulants centrifuged at 2000 rpm, the supernatant is plasma
SERUM	Enzymes Metabolites Electrolyte	Blood collected in plain glass container, without any anticoagulant, centrifuged at 2000 rpm after clotting, the supernatant is serum
URINE	Sugar Proteins Bile salts Pigments Blood steroids	Directly passed into a glass container, sometimes a catheter is introduced in the bladder
CEREBRO SPINAL FLUID	Sugar, Protein Chloride	Lumbar puncture from Subarachnoid space

Anticoagulants

Chemical agents that prevent coagulation are routinely used when whole blood or plasma is required. Some of the commonly used anticoagulants are:

(1) Heparin (2) Salts of Ethylene diamine tetra acetic acid (EDTA)
(3) Oxalates (4) Sodium Fluoride

1. **Heparin:** It may be considered to be a natural anticoagulant because it is already present in the blood, but in concentrations less than that required to prevent clotting in

freshly drawn blood. Heparin prevents coagulation by increasing the activity of antithrombin III, an inhibitor of thrombin. This anticoagulant is used in a concentration of 0.2 mg / ml of blood and since its molecular weight is large, it produces no change in erythrocyte volume.

2. **Salts of Ethylene diamine tetracetic acid (EDTA):** It is an anticoagulant which acts by virtue of removing calcium ions by chelation. A concentration of 2 mg of the disodium salt/ml of blood is sufficient. Concentrations even greater than this produce no detectable change in erythrocyte volume.

3. **Oxalates:** Lithium, sodium and potassium oxalates act as anticoagulants by removing calcium ions essential for blood coagulation. Potassium oxalate ($K_2C_20_4.H_20$) is commonly used. 1-2 mg of salt / ml of blood is required.

   The disadvantage of the use of oxalate is the alteration of concentrations of plasma components. Shrinkage of erythrocytes results from a water shift from the erythrocytes to plasma. This shift increases with increasing anticoagulant concentration, and if used in the same concentration on a weight basis, all anticoagulants will have this effect inversely proportional to their molecular weight. Aside from the water shift there may be alteration of erythrocyte permeability, which may explain the varied and inconsistent effects of oxalates and other salt anticoagulants on certain plasma constituents. Because of the difficulty, at times, in obtaining satisfactory preparation of heparin commercially, Heller and Paul introduced in 1934, a balanced oxalate mixture for use in hematocrit and sedimentation rate determinations. It consists of three parts by weight of ammonium oxalate, which causes swelling of the erythrocytes, balanced by two parts of potassium oxalate which causes shrinkage. NH_4^+ & K^+ oxalate mixture in the ratio of 3:2, and 2 mg / ml of blood is the required amount.

4. **Sodium Fluoride:** It is used when blood is collected for glucose estimations. In the erythrocytes (RBC), it specifically inhibits the enzyme enolase of the glycolytic pathway, preventing the consumption of glucose by the RBC's if blood is left standing at room temperature. Though it has a weak anticoagulant action, it is usually combined with another anticoagulant such as potassium oxalate.

Preservation of samples

Alteration in the concentration of a constituent in a stored specimen can result from various processes such as:

1) Adsorption on to the glass container
2) Evaporation if the constituent is volatile
3) Water shift due to the addition of anticoagulants
4) Metabolic activities of the erythrocytes & leucocytes (accelerated by haemolysis) Inducing O_2 consumption and CO_2 production, hydrolysis, glycolysis and finally degradation.
5) Microbial (fungal / bacterial) growth

Changes in concentration of volatile substances such as O_2 and CO_2 are prevented or at least hindered by collection and storage of samples under anaerobic conditions.

The problem of microbial growth appears when the sample is to be stored for longer than one day either at room or refrigerator temperature. This can be solved by four alternative courses of action:

a) Collection and storage under sterile conditions
b) Freezing of the sample
c) Extreme alteration of pH
d) Addition of an antibacterial agent.

Lyophilized samples are stable with respect to many constituents for periods of at least as long as ten years.

Samples can be stored at room temperature 18-37^{O}C, refrigerator temperature (4^{O}C) and frozen state (-10^{O}C or lower). With few exceptions, lower the temperature, greater the stability. Further, microbial growth is considerably less at refrigerator temperature than at room temperature and is completely inhibited in the frozen state. Even in the frozen state, however, some components of plasma deteriorate.

Chemical preservatives

They can be classified into two groups:

1) For prevention of chemical changes such as glycolysis
2) For prevention of microbial growth.

Sander in 1923 introduced the combination of 10 mg Sodium fluoride + 1 mg Thymol / ml of blood. The presence of Thymol effectively controlled microbial growth so that non-sterile specimens were stable for all determinations (except non-protein nitrogen) for at least two weeks.

Monochlorobenzene and monobromobenzene have also been coupled with fluoride and have been claimed to be superior to thymol.

Antibiotics can be used to prevent bacterial growth 1 mg of streptomycin base / 10 ml of blood has been used for preservation of blood for Haemoglobin and Urea determinations.

The common preservatives for urine specimen are formaldehyde, thymol, toluene and chloroform. All these act primarily as antimicrobial agents.

Safety

Safety is each person's responsibility even in a small clinical laboratory. Even then every clinical laboratory must have a formal safety program. It is a good practice to assign a specific person the title of safety officer with the duties of administering the safety program and keeping it current.

It should be ensured that laboratory environment meets the accepted safety standards (Fig. 6) which should include, but not be limited to attention to such items as:

1) Proper labelling of chemicals
2) Types and location of fire extinguishers
3) Hoods that are in good working condition
4) Proper working and grounding of electrical equipment
5) Providing means for proper handling and disposal of bio-hazardous materials including all patient specimen.

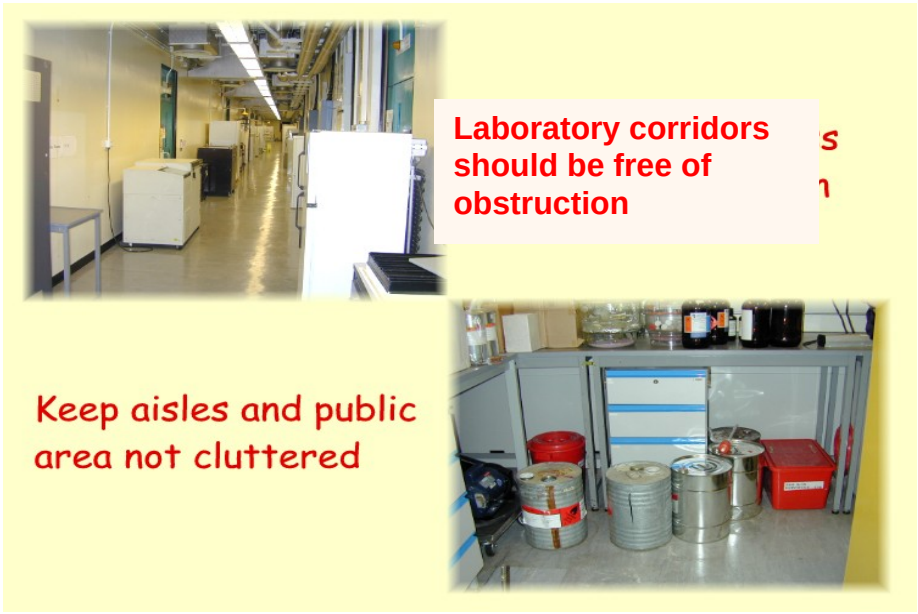


Fig. 6: Safety measures to avoid hazards

To prevent chemical, electrical and biological hazards following universal precautions should be followed:

1) Proper storage and use of chemicals is necessary to avoid chemical hazards. Thus knowledge of the properties of chemicals in use and of proper handling procedures greatly reduces dangerous situations.
2) All the electrical equipment should be grounded using three-point plugs and use of the extension cord should be prohibited.
3) Every laboratory should have the necessary equipment to put out a fire in the laboratory, as well as to put out a fire on the clothing of an individual. Easy access to safety showers should be made.
4) Biological Hazards can be avoided by following precautions called universal precautions. (Figs. 7 & 8).

All specimens should be treated as if they are potentially infectious:

a. Avoid performing mouth pipetting and never blow out pipettes that contain potentially infectious material, for example serum.
b. Do not mix potentially infectious material by bubbling air through the liquid, which leads to aerosol formation.
c. Barrier protection such as gloves, mask and protective eyewear and gowns must be

available and used when drawing blood from a patient. This includes removal and handling of all patient specimens. Disposable, non-sterile latex or vinyl gloves provide adequate protection.

d. Wash hands whenever gloves are changed.

e. Facial barrier protection should be used if there is a significant potential for the spattering of the blood or body fluid.

f. Avoid re-using syringes and dispose off needles in rigid containers without touching these, using one-handed technique.

g. Dispose off all sharp objects appropriately.

h. Wear protective clothing, which serves as an effective barrier against potentially infective materials. When leaving the laboratory, protective clothing should be removed.

i. Make a habit of keeping your hands away from your mouth, nose, eye and any other mucous membranes. This will reduce the possibility of infection.

j. Minimize spills and spatters.

k. Decontaminate all surfaces and reusable devices after use with appropriate registered hospital disinfectant.

l. No warning labels are to be used on patient specimens.

m. Before centrifuging tubes, inspect them for cracks. Inspect the inside of caps for signs of erosion or adhering matter. Be sure that rubber cushions are free from all bits of glass.

n. Never leave a discarded tube or infected material unattended or unlabelled.

o. Periodically clean out freezer and dry ice chests to remove broken ampules and tubes of biological samples. Use rubber gloves and respiratory protection during this cleaning.

Fig.7: Biosafety measures

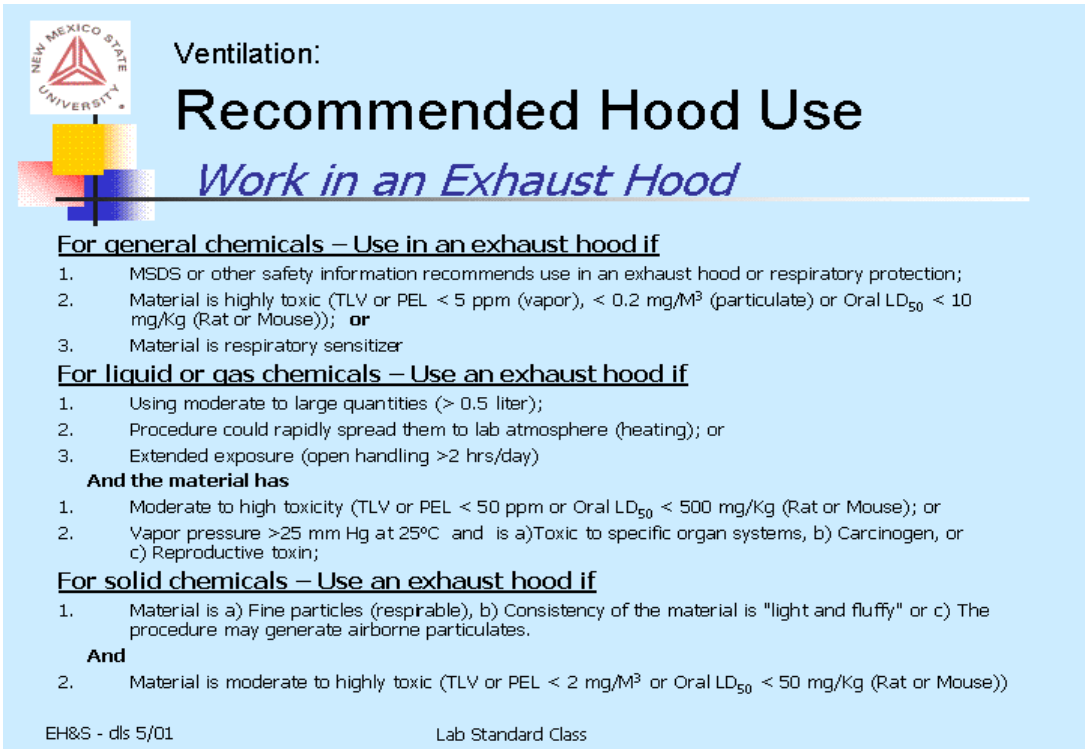


Fig. 8: Use of exhaust hood for biosafety measures

Chemical composition of blood

Introduction

Blood is a suspension of cells. Erythrocytes, leucocytes and platelets in fluid plasma, circulating virtually in a closed system of blood vessels. The cellular fraction constitutes 45% of the volume of blood and plasma constitutes 55% of the volume of blood.

Normal pH of arterial blood is 7.4. The various chemical constituents of blood include the proteins (albumin, globulin, fibrinogen, lipids, glucose, amino acids, urea, uric acid, creatinine, hormones and vitamins and the electrolytes Na^{+}, K^{+}, Ca^{++}, Mg^{++}(among cations) and CI^{-}, HCO^{-}_{3}, HPO^{--}_{4}(among anions))

Collection of blood

Venous blood is collected usually from antecubital vein or some other prominent veins of the forearm under aseptic conditions. Arterial blood is required rarely. This may be collected from radial, brachial or femoral artery. Capillary blood may be collected from the tip of the thumb or finger or from the ear lobe.

Experiments

Centrifuge a sample of oxalated blood. Observe that the cells are sedemented and plasma is separated. What is the color of the plasma? Normally it is pale yellow.

1. Add 10 drops of 2.5% $CaCI_2$ solution to 10ml-oxalated blood. Mix and let stand. The

blood clots. Calcium is clotting factor. Oxalate removes it as insoluble calcium oxalate, preventing clotting. When additional calcium ions are added, clotting occurs. Keep the clotted blood for an hour. A fluid separates while the clot retracts. Transfer the fluid to a centrifuge tube and briefly centrifuge. The clear supernatant is serum, note its color. Normally, it is light yellow. Plasma and serum are chemically same except that serum lacks fibrinogen.

2. Test for proteins
   a) **Globulins**: To 2 ml serum, add 2 ml saturated $(NH_4)_2SO_4$ solution. (Half saturation)
   b) The globulins are precipitated. Filter. Test the filtrate by Biuret reaction using 40% NaOH. The test is positive. The filtrate contained serum albumin that was not precipitated by half saturation.
   c) **Albumin**: Fully saturate with ammonium sulphate crystals the filtrate of the above experiment. Precipitation will be observed which is due to albumin.
   d) **Fibrinogen**: Mix 0.5 ml plasma, 15 ml water and 0.5 ml of 2.5 $CaCI_2$ solution in a small beaker. Allow it to stand for 20 mts at 37°C in the incubator. After incubation, insert a tapered glass rod into the solution and feel the transparent clot formed and collect it by pressing against the walls of the beaker. The clot is fibrin, the insoluble form of fibrinogen. Suspend the fibrin clot normal saline to remove the adhering proteins and dissolve in 5 ml of 5% NaOH. Perform biuret test with the solution. It is positive. Fibrin is a protein.

Test for-inorganic constituents

Deproteinisation of serum: Take 10 ml serum in a test tube. Add a few drops of 2% acetic acid. Keep in boiling water for 5 mts. The proteins coagulate, Filter. The filtrate is the protein free of serum containing all the inorganic constituents except proteins. Use this filtrate for the following experiments:

1. **Test for chlorides**: To 1 ml filtrate, add 2 drops of conc. HNO_3 and 2 drops of 3% AgNO3. A white precipitate of AgCI shows presence of chlorides in serum.
   Principle: chlorides react with silver nitrate forming a white precipitate of silver chloride. Nitric acid prevents the precipitation of salts other than chlorides.
2. **Test for Phosphates**: To 2 ml filtrate and 2 ml of ammonium molybdate reagent and a few drops of conc. HNO3. Warm, if necessary, to get a canary yellow color which is due to the presence of phosphates in serum.
   Principle: in the presence of nitric acid, phosphours reacts with ammonium molybdate to form a yellow ppt of ammonium phosphomolybdate.
3. **Test for Calcium**: To 2 ml filtrate add 1 ml of saturated ammonium oxalate. A white cloudiness is due to the precipitation of calcium oxalate, which indicates presence of calcium.
   Principle: ammonium oxalate reacts with calcium to form a white precipitate of calcium oxalate.

Test for organic constituents in the filtrate

(a) **Test for Glucose**: To 0.5 ml filtrate add 1 ml of Folin's alkaline copper sulphate solution mix and keep in boiling water for 5 mts. Coll. Add 5 ml of phosphomolybdic acid reagent. A deep blue color shows the presence of glucose.
(b) **Test for urea**: To 0.6 ml filtrate add 0.2 ml of aqueous horsegram suspension (10%).

Mix and keep in warm water for 10 mts. Add 5 ml water, mix and filter. To the filtrate add 2 ml Nesseler's reagent. A brownish yellow color indicates presence of urea.
Principle: The enzyme urease present in horsegram converts urea in to ammonium carbonate, which gives color with Nesseler's reagent.

(c) **Test for Uric acid**: To 0.5 ml filtrate add 1 ml of 10% Na2CO3 and 1 ml of phosphotungstic acid, dilute (Folin & Denis). A blue color develops due to uric acid.

(d) **Test for Creatinine**: To 1 ml filtrate add 0.5 ml of 1% picric acid followed by 0.5 ml of 10% NaOH. The yellow color changes to orange due to the presence of creatinine. (This is known as Jaffe's reaction, which is quantitative for the estimation of creatinine in serum and urine).

Chemical analysis of urine

Urine is an excretory product of the body and presence of certain substances in the urine reflects the metabolic state of the body. Since it can be easily collected and examined, routine and microscopic examination of urine are preliminary and important in diagnosis of various pathological conditions.

Collection of specimen and its preservation

Like all biological specimens, urine has to be collected and adequately preserved to prevent contamination and bacterial overgrowth since it is a very good culture medium. The type of urine specimen to be collected is determined by the test to be performed:

1) A clean, early morning, fasting specimen is generally the most concentrated specimen and preferred for microscopic examination and for detection of abnormal amounts of constituents e.g. protein.
2) A clean, timed specimen is one obtained at specific times of the day or during certain phases of the act of micturition.
   -First 10ml of urine voided is most appropriate to detect urethritis.
   -Midstream specimen is best for bacteriological study.
3) Catheter specimens are used for microbiological examination in critically ill patients or in urinary tract obstruction, only.

Preservatives used

1) The most satisfactory form of preservation is refrigeration at 4^0C combined with chemical preservation.
2) Commonly used forms of preservation used earlier were formalin (2 drops of 40% in 30 ml of urine) or Thymol (0.1mg per 100ml of urine sample). Nowadays tablets containing a mixture of chemicals are widely used. They act by lowering the pH and by releasing formaldeyde.
3) For Ketone bodies: Investigation is to be done immediately or within 2 hours of collection or it should be refrigerated with adequate preservative.

Physical examination of urine

Colour

Normally the urine is colourless to straw coloured (due to urochrome).
Deep Yellow: Mild to severe dehydration, Jaundice, B complex therapy (due to

riboflavin).
Red to brown: Haematuria, haemoglobinuria, myoglobinuria, porphyria (erythropoetic type).
Brown to black: Alkaptonuria, methaemoglobinuria.

Appearance

Normal urine is perfectly clear and transparent when freshly voided. It may become turbid if exposed for long time due to the urea being acted upon by bacteria and converted into ammonium carbonate or due to separation of mucoproteins.

Turbidity

Phosphate excretion in alkaline urine
Pus cells

Specific gravity

Measured by Urinometer - implies the capacity of kidney to concentrate urine.
Normal value: 1.002-1.028
Depends upon - State of hydration & solute load

Values more than 1.028 imply	- Severe dehydration
	- DM (diabetes mellitus)
	- Adrenal insufficiency
Values less than 1.002 indicates	- Increase water intake
	- Diabetes insipidus
	- Chronic Nephritis

Important: A low fixed specific gravity even on fluid restriction denotes loss of concentrating ability by the kidneys and is usually found is Chronic Renal Failure (CRF). The specific gravity is fixed at 1.010, a condition known as isosthenuria.

Volume

Normal value- 700 - 2000 mL / day
Depends upon - Fluid intake
- Solute load
- Loss of fluid by skin or otherwise.
-Climatic condition

Some important terms:
Polyuria: More than 3L/day
Conditions- Diabetes Mellitis, Diabetes Insipidus, Recovery from acute renal failure (ARF), Diuretic therapy
Oliguria: Less than 500 ml/day.
Conditions- ARF, Vomiting, Fever, Burns.
Anuria: Less than 50 ml per 12 hours.

*p*H

Normal range is 4.5 to 8.5. Average 6.0 in 24 hrs sample.
pH 8.5 or more found- After heavy meals, Proteus infection
pH 4.5 or less found- After heavy exercise, Metabolic Acidosis, Chronic Respiratory acidosis.

Chemical Examination

1. Proteins

Normal adults excrete upto 150mg proteins/day (5 to 15mg Albumin, 50 to 70mg Tamm Horsfall mucoproteins, a product of epithelial lining of the tubules, and the rest different plasma proteins or glycoproteins).

Nephrotic Syndrome is a clinical condition when kidney loses more than 3.5gm proteins/day/1.73 m^2 with hypoalbuminemia, edema, hyperlipidemia, lipiduria and hypercoagulability.

Microalbuminuria: It is a condition characterized by urinary albumin excretion rates between 20-200 μg/min or 30-300 mg per 24 hours. This is shown to precede the overt renal disease and is an indicative of increased risk for development of diabetic nephropathy.

2. Glucose / Reducing Substances

Normal urine has reducing sugars in the concentration of 1-1.5 g / L. Of this, Glucose is 200-300mg.

Glycosuria: Glucose in the urine beyond the normal range.

Renal threshold: In normal persons, so long as the blood glucose is less than 160-180mg/dL, glucose is not excreted by the kidney in amounts which are detected by the routine tests used. This level is termed the renal threshold for glucose.

Renal glycosuria: It is a condition in which the renal threshold for glucose decreases so that glucose is present in urine in the presence of normal blood sugar levels. *Physiological renal glycosuria is seen in pregnancy*.

3. Ketone Bodies

Ketone Bodies are- β-OH butyrate (78%),
- Acetoacetate (20%) and
- Acetone (2%)

Normal blood levels 0.5-1.5mg%.
Normal amounts in urine are 50mg/day.
Ketonuria seen in- Diabetic ketoacidosis, Starvation, Severe vomiting, Glycogen storage disorders, high fat diet.

4. Blood

Presence of RBC in urine is called *Haematuria*.
Hematuria seen in
- Nephrolithiasis (Stones in the urinary tract)
- Malignant hypertension
- Sickle Cell Anemia
- Coagulation abnormalities
- Malignancy of Kidney, Urinary tract, and Bladder.

Haemoglobinuria: Presence of free Hb in urine, seen in intravascular hemolysis when the binding capacity of Haptoglobin is exceeded.

Myoglobinuria: Presence of Myoglobin in urine, seen in crush injuries and Muscular disorders.

5. Bilirubin

A freshly voided specimen is required since bilirubin is very unstable. Unconjugated bilirubin does not cross the Glomerular basement membrane as it is non polar and water insoluble. Conjugated passes freely as it is water-soluble.

6. Urobilinogen

It is a colourless compound formed in the intestine by the action of gut flora on bilirubin and is reabsorbed by enterohepatic circulation and excreted in urine.

Increased in: Haemolytic jaundice and Hepatocellular jaundice.

Decreased in: Broad-spectrum antibiotic therapy (by killing gut flora) and Obstructive jaundice.

Microscopic Examination

1. Cells

a. RBC - ≥2/HPF is abnormal unless it is collected by catheterization.
b. WBC or Pus cells - ≥2/HPF is abnormal and is found in UTI (urinary tractinfection).
c. Epithelial cells - Normal desquamation from urinary tract.
d. Bacteria, if any.

2. Crystals

Associated with renal calculi, commonly is Oxalate in acidic urine and Phosphate in alkaline urine.

3. Casts

These are masses of agglutinated proteins in the form of cylindrical moulds of tubular lumen. Many types are found but commonly seen are:

a. Hyaline casts: Contain mainly proteins and no cells. Cylindrical and transparent, seen in Pre-renal causes of acute renal failure.
b. Cellular casts: Casts coated with various cells
    i. RBC casts are diagnostic of acute glomerulonephritis.
    ii. WBC casts are seen in chronic pyelonephritis.
    iii. Epithelial casts are seen due to desquamation of tubular epithelial cells. As the cellular casts travel the tubules they are degraded to granular and then to waxy casts, which may be seen in diseases, associated with tubular stasis.

Tests

1. For Proteins

a) Heat test: Based on the principle of heat coagulation and precipitation of proteins.
Procedure: Fill half the test tube with urine and heat the top 1/2 of the sample. Look for any turbidity at the upper part of the tube by comparing with the lower part of the tube. If

any turbidity appears, add 2 drops of 33% acetic acid. (Acidification is necessary because in alkaline medium heating may precipitate phosphates). If the precipitate is due to proteins, it will increase on acidification and if it is due to phosphates, it will dissolve again.

Observation	**Semiquantitative**
Barely visible turbidity	5mg/100ml
Distinct turbidity	10-30mg/100ml
Moderate turbidity	40-100mg/100ml
Heavy turbidity	200-500mg/100ml

Bence Jones proteins are light chains of IgG that are excreted in the urine in cases of multiple myeloma. On heating a sample of urine at 60°C turbidity appears and again disappears on further heating.

b) TCA test: Add 1ml of 3% of TCA to 5 ml of clear urine. Protein appears as a white precipitate.

2. For Sugars

Benedict's Tests: It is a semiquantitative test based on reduction of Cu^{++} ions by reducing sugars in hot alkaline medium.

$$Cu^{2+} \xrightarrow[\text{Reducing sugar}]{\text{Hot alkaline medium}} Cu^{+} \longrightarrow Cu_2O \text{ (ppt.)}$$

Procedure: To 5ml of Benedict's reagent add 8 drops of urine. Heat to boiling point for 3 minutes, keep on stand for 2 minutes and note the colour of precipitation formed.

Observation	**Report**	**Interpretation**
Clear blue/green	Nil	-
Green ppt.	1+	100-300mg%
Yellow ppt.	2+	300-1000mg%
Brown ppt.	3+	1-1.5g%
Orange-red ppt.	4+	1.5g%

A false positive test with Benedict's reagent is found with thymol, formaldehyde, Chloroform, Lactic acid, Vitamin C, Dextrin.

3. For Blood/Haemoglobin

Benzidine test

Procedure: Add 2ml of urine and 1ml of 3% H_2O_2 to 3ml of fresh saturated solution of benzidine in glacial acetic acid. Blue colour within 10 min is suggestive of occult blood.

Principle: H_2O_2 is catalysed by Hb to give (O_2), which oxidizes benzidine to a coloured derivative.

4. For Ketone bodies

a) Rothera's nitroprusside test

Procedure: Saturate 5ml of urine with solid ammonium sulphate and add 0.2ml freshly prepared sodium nitroprusside solution. Mix well and slowly add 0.5ml of ammonia. A purple ring at the junction of the liquids indicates the presence of Ketone bodies.

Principle**:** In alkaline media (by ammonia and ammonium sulphate), freshly prepared Sodium nitroprusside soln. forms a purple colored compound in reaction with acetoacetic acid and acetone (α-ketones). *Note*: *β-Hydroxybutyrate does not give a positive Rothera's test.*

b) Gerhardt's Test

Procedure: Add 10% $FeCl_3$ solution drop by drop to 5 ml of urine in a test tube until no more ppt is formed. A purplish colour is given by acetoacetic acid. Similar colour is given by salicylate, phenol and antipyrine. If urine is heated and then tested, there is no colour if the original colour was due to ketone bodies.

5. For Bile Salts

Hay'sTest

Principle: Bile salts are surface tension lowering agents. So in presence of bile salt sulphur powder will sink.

Procedure: Sprinkle a little dry sulphur powder on the surface of fresh clean urine taking distilled water as control. If the particles sink, bile salts are present in urine

6. For Bile Pigments

Fouchet'sTest

Procedure: Add a pinch of $MgSO_4$ to 10 ml of urine followed by addition of 5ml of 10% $BaCl_2$, solution to 10ml urine and filter. $BaSO_4$ acts as absorbent for bilirubin and helps in concentrating it. Dry the filter paper and add a drop or two of Fouchet's reagent (25g of Trichloroacetic acid, 10ml of 10% $FeCl_3$ and 90ml water) at the edge of the ppt. A greenish blue color denotes the presence of bilirubin (due to oxidation of bilirubin to biliverdin by $FeCl_3$).

Analysis of Cerebrospinal Fluid

Cerebrospinal fluid (CSF) is a clear, colourless fluid filling the ventricles and subarachnoid space. CSF production is a result of the combined processes of diffusion, pinocytosis and active transfer. The majority is produced by selective dialysis of blood plasma by a specialized sponge-like structure called the "choroid plexus" of third, lateral and fourth ventricles but about 30% comes from' other brain capillaries and seeps into the system via the extracellular fluid.

The anatomy of the ventricular system allows for movement of CSF in and around all the major structures of the brain. From the lateral ventricles located within the cerebral hemisphere, it circulates through the foramina of Monro into the third ventricle. At its caudal end, the third ventricle is connected by aqueduct of Sylvius to the fourth ventricle. CSF then flows into the basal cisterns and subarachnoid space by two lateral foramina of Lusckha and

median foramina of Magendie. From the cisterns the CSF flows / throughout the subarachnoid space and over the hemispheric convexities and around the spinal cord. The total volume of CSF is about 150 ml and the rate of CSF production is about 550 ml per day thus, turnover rate is about 3.7 times a day. CSF is reabsorbed into the venous system by numerous microscopic arachnoid villi and larger but less common arachnoid granulations (pacchinian bodies). Villi and granulations represent outpouchings of the arachnoid membrane that penetrate gaps in the dura and protrude within the venous sinuses. These projections act as valves, which permit single direction bulk, flow (direct flow) of CSF into the venous blood about 500 ml per day with additional amounts through diffusion into cerebral blood vessels and through the cribriform plate of ethmoid bone into the nose. The reabsorption of CSF occurs along the entire neuraxis. In the SA space CSF comes in contact with perivascular spaces around the blood vessels entering and leaving the brain where cells and protein leak during inflammation. It must be remembered that there is no lymphatic drainage system in the central nervous system CNS), hence only 2 pathways are available for the elimination of wastes -capillary drainage and excretion via CSF. CSF secretion is an active process overall but production is independent of intraventricular pressure and resorption is proportional to it. A blood CSF barrier exists for many substances like bilirubin and certain drugs, so that their concentration in CSF is lower than in plasma.

Composition

The composition of CSF is essentially same as that of brain ECF and is largely determined at the cell surfaces on which it is produced (choroid plexus), where it is absorbed (arachnoid villi and pacchinian granulation). Its ionic composition is the same as that of plasma for some components and different for others. In general CSF glucose concentration is 60% of serum, sodium chloride and magnesium are same or greater than serum but potassium, calcium and glucose are lower than serum. Active transport in and out of the CSF space is probably responsible for maintaining this difference.

Total volume	=	150 ml (30ml within cerebral ventricles, 120ml in SA space), (85 ml in spinal part and 35 ml in cranial part)
Specific gravity	=	1.006-1.008
pH	=	7.31 -7.40 (7.33)
Normal pressure	=	110-130 mm Ringer's solution, or 7-10mm Hg
Color	=	colorless
Transparency	=	clear, free of clots ; Osmolarity = 292-297mOsm/l
Cellularity	=	nil or less than 5 lymphocyte or monocyte / mm3
Glucose	=	50-80 mg/dl
Protein	=	15-50 mg/dl
Bilirubin	=	nil
Na+	=	138-150 mEq/L
CI -	=	116-122 mEq/L ; HCO3 = 20-24 mmol/L

The normal A:G ratio in CSF proteins is 3:1.
Ratio of serum: CSF protein is 200:1.

Functions

1. mechanical support (cushion effect)
2. removal of waste metabolic products

3. transport of biologically active compounds which may function as chemical
4. maintenance of the chemical environment of brain

Pathological states in which examination of CSF may be required

A wide range of disorders can produce change in CSF composition and the type and extent and extent of change is often not specified for a single pathological condition.

Cerebral dysfunction

I. Infections: Meningitis (purulent, aseptic)
Encephalitis
Neurosyphylis (acute)
TB meningitis
2. Cerebrovascular
Sub-arachnoid haemorrhage
3. Dementia and degenerative
Alzheimer's disease
4. Neoplastic
Meningial carcinomatosis
Secondary deposits
5. Demyelinating
Multiple sclerosis
6. Autoimmune Sarcoid
7. Others Normal pressure hydrocephalus
Pseudotumour cerebri

Cranial nerve dysfunction

1. Miller Fischer variant of GBS
2. Lyme's disease

Motor neuron:
Amyotrophic lateral sclerosis (ALS)

Cerebellar: Cerebellitis

Sensory dysfunction

Neuropathy: Diabetic
CIDP (Chronic Inflammatory Demyelinating Polyradiculopathy)

Trauma

Head injury
Fractured vertebrae

Acquisition

Quinke first developed the technique of LP or spinal tap in 1891. CSF is collected by lumbar puncture in which a fine bore needle (22 or 24 L.P needle) is passed between the 3rd and 4th lumber vertebrae into the subarachnoid space with the patient lying in the lateral position and the fluid allowed to flow automatically. The bevel of the needle should be parallel to the long axis of the spine. The whole procedure is done under strict asepsis. The first few drops of the

fluid are discarded and the rest of the fluid is “collected in sterile containers. There are specific indications and contraindications for lumbar puncture.

The specimen is divided into 3 aliquots for:

a) Chemistry and Serology
b) Bacteriology
c) Microscopy

Protocol for investigation:

1. Pressure (Opening and closing pressure)
2. Appearance
   color
   turbidity
   coagulum
3. Cytological examination
   direct examination
   staining of the centrifuged deposit (e.g.Leishman's stain)
4. Microbiological investigations
   staining of centrifuged deposit (gram stain, AFB)
   culture and sensitivity
5. Biochemical investigations
   Total proteins (Lowry method or turbidometry)
   Qualitative test for gamma globulin (Pandy's test)
   Quantitation of glucose
   Quantitation of chloride
   Misc. enzymes (LDH, CK),
   bicarbonates, urea, calcium, copper, folate.

It must be borne in mind that CSF samples must always be centrifuged prior to analysis in order to precipitate any cells otherwise falsely high values for CSF protein will be obtained. The utmost caution must be exercised while pipetting and handling CSF samples.

Changes in CSF in diseased states

Physical Analysis

Pressure: Normally 60-150 mm of water in recumbent position.
Low opening pressure: 10- 20 cm H2O normal- CSF leak or spinal SA obstruction. Elevated opening pressure: More than 20 cm H2O Mass occupying lesion, diffuse cerebral inflammation.

Appearance: Normal CSF is clear and colorless and gives no coagulum or sediment on standing.

Color: Changes only in pathological conditions, whereas the term xanthochromia means yellow colour. It has been used for the presence of other colours as well
Yellowish tinge --markedly increased protein >200%.
Yellowish --bilirubin
Blood may be present due to bleeding from L.P. site, pathological subarachnoid hemorrhage, ventricular hemorrhage, or neurosurgical operations. When hemolysis occurs in CSF the hemoglobin liberated is converted to bilirubin and that gives a yellow coloration to the CSF

(more visible after centrifugation) called xanthochromia. Bilirubin is detected after 10 hours of subarachnoid bleeding.

Turbidity: CSF may occasionally clot if the ratio of blood to CSF is high. Usually due to fibrin clot (e.g., tubercular meningitis a cobweb coagulum appears by keeping CSF for 12-15 hours). Turbidity can also be due to microscopic fat globules (fat embolism).

Cell count: Normal CSF should contain no more than 5 lymphocytes or monocytes / mm3. Nature and number of cells are noted. Presence of RBCs indicate hemorrhage. Presence of WBCs predominantly polymorphs indicate bacterial meningitis. In viral infection and chronic infections a lymphocytic response is obtained.

Biochemical analysis

1. **Proteins**: found in CSF ordinarily originate from serum and reach the cerebral space by endocytosis across the capillary endothelium. An increase in total proteins is the commonest chemical abnormality in CSF and results from a breakdown of the blood CSF and brain-CSF barriers usually as a consequences of an inflammatory reaction but on occasion, if the flow of CSF is obstructed. Albumin is the predominant protein to be increased, globulins appear in varying amount. If the permeability of the barriers is markedly increased, fibrinogen is present which in the test tube forms a clot or coagulum. High protein content accompanied by xanthochromia is referred to as Froin' s syndrome (associated with tumours and spinal compression).
   Examination of CSF protein is done mainly to detect:
   a. Increased blood-brain barrier permeability to plasma protein (80%)
   b. Increased intrathecal IgG secretion (20%).

   Increase in CSF protein occurs in

   a. Hemorrhage (trauma, neoplasm, ruptured aneurysm).
      Note: A false result may occur from a "bloody tap" -rupture of a blood vessel during LP (presence of 1000RBCs -increase protein I mg / ml).
   b. Inflammation, meningitis especially bacterial (meningococcal), may be as high as 2000mg/dl.
   c. Other causes: encephalitis, polio. Decrease in CSF protein occurs in
      Children (6 months -2 years) -Pseudotumour cerebri

   Tests for globulins

   Pandy‘s test: 2 drops of CSF are added to 2ml of reagent (10g phenol + 150ml water) and the degree of opalescence is noted-slight opalescence, opalescence, marked opalescence or turbidity.
   Normal CSF remains clear (no opalescence). Marked opalescence is observed in multiple sclerosis and neurosyphilis.

2. **Glucose**
   CSF glucose concentration is 60% of the normal plasma glucose. Blood and CSF glucose equilibrate only after a lag period of 4hours so that CSF glucose at a given time reflects blood glucose level during the past 5 hr. When glucose determination is critical. LP and blood glucose should be obtained only after the patient has been fasted for the last 4 hr.

Equilibrated CSF glucose is definitely abnormal when it *is* less than 40% of simultaneously determined blood glucose-values of 40mg/dl are almost always abnormal.

Decreased CSF glucose (Hypoglycorrachia)

Markedly decreased in pyogenic meningitis (e.g., 10-20 fig/dl); in tuberculous meningitis *it is* 30-50mg/dl; in viral meningitis it *is* normal.

Note that intrathecally administered *streptomycin* used in the treatment of tuberculous meningitis can reduce the alkaline copper reagent often used *in* glucose determination.

Specialised Tests:

1. Increased lactate	Bacterial meningitis
2. Increased glutamine	Hepatic encephalopathy
3. Increased LDH	Bacterial meningitis,
4. Increased CK-BB	Parenchymal damage
5. Increased adenosine deaminase	Tuberculous meningitis

Protocol for Protein Estimation in CSF

Add I ml each of test CSF, standard and distilled water in respective tubes. Then add 4ml coomassie Brilliant Blue G-250 colour reagent (commercially available). Add 2.5ml of 1M NaOH to sample in all tubes (NaOH need not be added if you use commercially available colour reagent). Mix. keep for 10 mins. Read at 595nm Standard protein concentration - 50mg/dl.

Note: Either use commercially available Brilliant Blue G-250 or prepare by dissolving 100mg of coomassie Brilliant Blue G-250 in 50ml ethanol. To this add 100 ml of phosphoric acid (85% w/v) and dilute to 1 litter with water.

Protocol for estimation of CSF glucose

Place 0.1ml of CSF into 7.8ml of isotonic solution in a centrifuge tube. Mix well. Add 0.1ml of sodium tungstate solution. Mix and centrifuge at 2000 rpm for 10 min. Take 2ml each of the supernatant, standard and isotonic solution in the respective tubes. Then add 2ml of alkaline copper sulphate reagent in all tubes. Mix well and heat in boiling water for 10 min, cool and add 2ml of arsenomolybdic acid reagent in all tubes. Mix and wait for 5 min. Read at 540 nm.
Concentration of standard: 1.25 mg/dl.

Renal Function Test

Renal function tests are specialized tests and are advised when medical history, examination and routine tests like urine analysis are suggestive of some renal disease. Estimation of renal function is important in a number of clinical situations, including assessing renal damage and monitoring the progression of renal disease. Renal function should also be calculated if a potentially toxic drug is mainly cleared by renal excretion. The dose of the drug may need to be adjusted if renal function is abnormal.

I. Complete medical history, physical examination, routine tests including urine analysis.

II. **Renal Function tests**: Renal function tests may be grouped into (a) those which assess the glomerular function, and (b) those, which study the tubular function.

Glomerular function tests	Tubular function tests
Blood Urea	Urine concentration test
Blood creatinine	Urine dilutional test
Inulin clearance	Urine acid excretion
Creatinine clearance	Amino acids in urine
Urea clearance	
PAH clearance	
Proteins in urine	

III. **Tests for structural integrity**

a.) Renal biopsy

b) Renal imaging

Plain abdominal X-Ray, Intravenous pyelogram (IVP)
Renal angiogram, Renal ultrasound, Computerized tomography (CT scan)
Magnetic resonance imaging (MRI)/angiogram (MRA)
Radionuclide renal scan

Renal function tests are only for the analysis of the functional capacity of kidneys. Renal function tests do not give any information about the structural integrity or the structural pathology. For structural details renal imaging and biopsy has to be done.

The functional unit of the Kidney is the nephron, which is composed of the Glomerulus, Proximal convoluted tubules (PCT), loop of henle, Distal convoluted tubules (DCT) and the collecting tubules.

Quantitation of overall function of the kidneys is based on the assumption that all functioning nephrons are performing normally and that a decline in renal function is due to complete functional loss of nephrons rather than to compromised function of nephrons.

Tubular function

Renal tubules make up 95% of the renal mass, do the bulk of the metabolic work and modify the ultrafiltrate into urine. They control a number of kidney functions including acid-base balance, sodium excretion, urine concentration or dilution, water balance, potassium excretion and small molecule metabolism (such as insulin clearance). Measurement of tubular function is impractical for daily clinical use (performed only when there are specific indications) therefore tubular function tests are not discussed further in this chapter.

Glomerular Filtration Rate: Glomerular filtration rate (GFR) is the rate (volume per unit of time) at which ultrafiltrate is formed by the glomerulus. Expressed in ml/min ≈125ml/min

Renal Plasma Flow: Volume of plasma flowing through the kidney per min. Expressed in ml/min. 25% of the total cardiac output.

Filtration fraction: Fracion of renal plasma flow which is filtered through glomeruli. Expressed as percentage.

Clearance: Clearance of a substance is the volume of plasma cleared of the substance per unit time. It is expressed in ml/min.

$$\text{Clearance (C)} = \frac{\text{U (mg/dL) X V (ml/min)}}{\text{P (mg/dL)}}$$

where U is the urinary concentration of a marker x, V is the urine flow rate and P is the average plasma concentration of x.

Substance filtered neither (reabsorbed nor secreted)	Clearance = GFR	Inulin
Substance filtered (reabsorbed and secreted)	Clearance ≈ GFR	Uric acid
Substance filtered (partially reabsorbed)	Clearance < GFR	Urea
Substance filtered (secreted and not reabsorbed)	Clearance > GFR	PAH

Glomerular Function tests

GFR is the most sensitive and reliable parameter to assess the glomeular function.

1. ***Exogenous markers***
   (a) Inulin - inulin clearance is accurate reflection of GFR
   (inconvenient-requires intravenous infusion)
   (b) Iothalamate nuclide - gold standard for GFR in clinical research

2. ***Endogenous markers***
   (a) Creatinine
   - derived from muscle creatine; production is usually constant
   - plasma concentration is stable for a given individual
   - creatinine clearance (C_{cr}) ≈ GFR when GFR is close to normal.

$$\text{Creatinine clearance } (C_{cr}) = \frac{U_{cr} \text{ X } V \text{ (ml/min)}}{P_{cr}} \approx GFR$$

Identical plasma creatinine concentrations in two separate patients may reflect very different GFR.

Case A: P=1.5mg/dl, V=2ml/min, U=90mg/dl

Case B: Pt who is older and lean and thin
has lower steady-state creatinine production and excretion
P=1.5mg/dl, V=2ml/min, U= 60mg/dl

- *When GFR is low, C_{cr} overestimates the GFR*

(a) Creatinine clearance test

Procedure for creatinine clearance test: Give 500ml of water to the patient to promote urine flow. After about 30 minutes ask patient to empty bladder and discard the urine. Exactly after 60 minutes, again avoid the bladder and collect the urine, and note the volume. Take one blood sample creatinine level in blood and urine are tested and calculated. Reference value for creatinine clearance is 90-130ml/min.

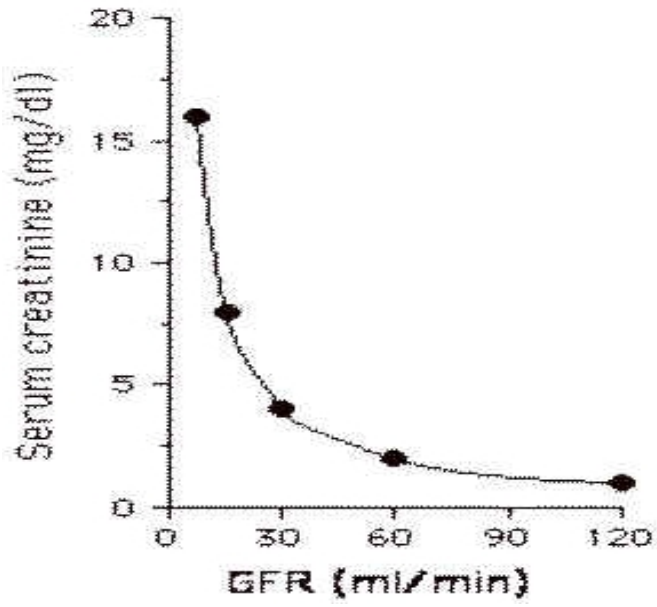
Interpretation of creatinine clearance

A decreased creatinine clearance is a very sensitive indicator of reduced glomenilar filtration rate. A creatinine clearance value upto 75% of the average normal value may indicate adequate renal function. In older people the clearance is decreased.

Significance of determining creatinine clearance is in the early detection of functional impairment of kidney without overt signs and symptoms. Small changes in plasma creatinine which may not apparently indicate abnormal function may show gross changes in the value of clearance. For example, the plasma creatinine level is 1mg/dl and the clearance is 100ml/minute, a rice in plasma creatinine to 2mg/dl will decrease the clearance value by 50%.

Creatinine clearance test is useful in long-term monitoring of patients with renal insufficiency under a protein restricted diet, creatinine clearance is altered by body muscle mass, drugs, age, sex and nature of diet.

Modified creatinine clearance



Corrected GFR

Uncorrected GFR has a +ve correlation body wt, height, BSA and male gender and
- ve correlation with age.
Corrected GFR correlates with age alone

$$= \frac{U_{cr}\ (mg/dL)\ X\ V\ (ml/min)}{P_{cr}\ (mg/dL)}\ X\ \frac{1.73}{BSA}$$

Calculated creatinine clearance
The most well-known formula is the ***Cockcroft-Gault formula***, which is relatively simple to use and reasonably accurate.

$$\frac{(140\text{-age in years})\ X\ 2.12\ X\ Weight\ (Kg)\ X\ K}{P_{cr}\ (mg/dL)\ X\ BSA\ (m^{2})}$$

K= 0.85 for women and 1 for men
BSA = Body surface area

(b) Urea Clearance: Urea clearance is the number of ml of blood which contains the urea excreted in a minute by the kidneys. Since 40% of urea is reabsorbed by the tubules after filtration, the clearance of urea is highly dependent on urine flow rate. Therefore, urea clearances (C_{urea}) is not useful for estimation of GFR by itself. For example in the hydrate state, urine floe rate is high and the C_{urea} may be >70% of GFR whereas in a dehydrated state, C_{urea} may be <30% of GFR.

Maximum Urea Clearance

$$\text{Urea clearance} = U\ X\ \frac{V}{P}$$

Where U=mg of urea per ml of urine; P= mg of urea per ml of plasma and V= ml of urine excreted per minute. This is the maximum urea clearance and the reference range is 60-100ml/minute.

Standard Urea Clearance
Urea clearance value is decreased when the volume of urine (V) is less than 2ml/minute. It is then called standard urea clearance, where the normal value is found to be 54ml/minute.

Interpretation of urea clearance value: If the value is below 75% of the normal, it is considered to be abnormal. The values fall progressively with increasing renal failure. Urea is reabsorbed from renal tubules (urea clearance < GFR) and therefore tubular function also affect urea clearance. Hence creatinine clearance test is more preferred.

BUN (Blood Urea Nitrogen): Plasma Creatinine (P_{cr}) ratio

Normal BUN/P_{cr} ratio ≈ 12-16 : 1

High BUN/P_{cr} ratio	**Low BUN/P_{cr} ratio**
prerenal azotemia	severe liver failure
high protein diet	low protein diet
catabolic states (e.g. sepsis)	anabolic states
gastrointestinal bleeding	rhabdomyolysis
medications (e.g. corticosteroids)	

Renal plasma flow
- It can be estimated by clearance of PAH

- > 90 % of the PAH is removed from the kidney by tubular secretion and glomerular filtration.
PAH is infused to constant plasma conc. and its clearance is calculate.
Nowadays it is estimated using I^{131} iodohippurate or other radionuclides ex. diodrast.

Filtration fraction: it is the ratio of filtered plasma out of total renal plasma flow.
Normal Values:

- Plasma creatinine – 0.9-1.3 mg/dl (men), 0.6-1.1mg/dl (women)
- Plasma Urea – 15-40 mg/dl
- Inulin clearance-

  Men- 125 $\pm$ 25ml/min
  Women- 119 $\pm$ 12 ml/min.

- Creatinine clearance- 90-130 ml/min
- Urea clearance (when V=2ml/min) = 60-100 ml/min
- PAH clearance

  Men - 650 $\pm$ 160 ml/min
  Women- 590 $\pm$ 100 ml/min

Suggested Reading

1. Tiets text book Clinical Chemistry, fourth edition, Ed: Carl A. Burtis and Edward R. Ashwood.