Clinical Biochemistry Laboratory, Artificial Intelligence, Quality Control, Automation, Biomedical Waste Etc.

1

The Artificial Intelligence (AI) Speaks

"In labs, I work with precision and grace;
Decoding results at a lightening fast pace;
From patterns in data to diagnostics refined;
I am the future of care where health is aligned."

The ultimate application of the biochemistry subject is for the health and welfare of mankind. Clinical biochemistry (also known as ***clinical chemistry*** or ***chemical pathology***) is the laboratory service absolutely essential for medical practice. The results of the biochemical investigations carried out in a clinical chemistry laboratory will help the clinicians to determine the diseases (diagnosis) and for follow-up of the treatment/recovery from the illness (prognosis). Biochemical investigations hold the key for the ***diagnosis and prognosis of diabetes mellitus, jaundice, myocardial infarction, gout, pancreatitis, rickets, cancers, acid–base imbalance*** etc. Successful medical practice is unimaginable without the service of clinical biochemistry laboratory.

The ***biological fluids*** employed in the clinical biochemistry laboratory include ***blood***, ***urine***, ***cerebrospinal fluid*** and pleural fluid. Among these, ***blood*** (directly or in the form of plasma or serum) is ***frequently used*** for the investigations in the clinical biochemistry laboratory.

On rare occasions, tissue biopsy samples may also be used for diagnosis.

COMMON ABNORMALITIES AND BIOCHEMICAL PROFILES

A large number of conditions, particularly diseases (diabetes mellitus, genetic diseases, MI, organ failure etc.), are associated with abnormal biochemical profiles. A selected list of them is given in ***Table 1***, e.g. cancer can be diagnosed by estimating tumor markers.

REFERENCE RANGE

There exist biological variations between normal and healthy individuals for any analyte/

Table 1 Common abnormalities associated with blood/plasma biochemical profiles (selected examples listed)

Abnormality	Biochemical profile
Organ function failure	***Renal failure***.....serum creatinine and urea elevated ***Liver failure***.....serum bilirubin and ALT increased.
Myocardial infarction	Cardiac troponin I and CPK in serum elevated.
Cancer	Tumor markers elevated e.g. PSA in prostate cancer.
Diabetes mellitus	Increased blood glucose and HbA_{1C}
Genetic diseases	Serum phenylalanine elevated in phenylketonuria; NH_3 increased in urea cycle disorders.
Nutritional disorders	Serum albumin low in kwashiorkor.
Endocrine dysfunction	TSH high in hypothyroidism and low in hyperthyroidism.
Changes in blood pH	Metabolic acidosis–plasma bicarbonate low; metabolic alkalosis–plasma bicarbonate high.
Inflammation/infection	C-reactive protein elevated.
Use of drugs	Increase in serum uric acid in cancer chemotherapy.
Poisons	Decrease in the activity of serum butylcholinesterase in organophosphorus poisoning.
Stress	Increase in serum cortisol and catecholamines.
Altered physiological conditions	Elevated levels of serum hCG in pregnancy; increased blood lactate after strenuous exercise

compound measured in the laboratory. This is referred to as reference range around a mean (or average) which is considered to be normal. The most important reference biochemical values are given elsewhere.

If may be noted that the reference values of some analytes differ between sexes, different age groups, and in different ethnic groups. Further, the biochemical results differ from laboratory to laboratory, due to variations in the methods employed.

TECHNIQUES USED IN CLINICAL BIOCHEMISTRY LABORATORY

The reader may refer laboratory techniques in biochemistry (***Chapter 41***) for a brief knowledge on the tools employed in clinical biochemistry. A summary of major techniques with their applications in clinical biochemistry is given in ***Table 2***.

DRY CHEMISTRY DIPSTICK

For certain assays, the reagents, enzymes or antibodies can be put together on ***specially designed plastic strip*** namely dipstick. Dipsticks are handy devices for use at the bedside in a hospital, doctor's clinic or even at home by individuals. Some commonly used tests employing dry chemistry dipsticks are listed–

- Finger-prick blood glucose measurement by test strip and glucometer.
- Strips to semiquantitatively detect urinary levels of glucose, ketone bodies, proteins etc. (individual dipsticks or multidipsticks are available)
- Dipstick for home pregnancy test to detect human chorionic gonadotropin (hCG) in urine.

COLLECTION OF BLOOD

Venous blood is ***most commonly*** used for a majority of biochemical investigations. It can be drawn from any prominent vein (usually from a vein on the front of the elbow). ***Capillary blood*** (<0.2 ml) obtained from a finger or thumb, is less frequently employed. ***Arterial blood*** (usually drawn under local anesthesia) is used for ***blood gas determination***.

Precautions for blood collection : Use of sterile (preferably disposable) needles and syringes, cleaning of patients' skin, blood collection in clean and dry vials/tubes are some of the important precautions.

ANTICOAGULANTS

Certain biochemical tests require unclotted blood. Anticoagulants are employed for collecting such specimens.

Table 2 A summary of major techniques with their applications in clinical biochemistry laboratory

Laboratory technique	Applications
Centrifugation	Plasma and serum separation Isolation of subcellular components
Microscopy	Urinalysis for sediment examination Identification of crystals (e.g. uric acid, calcium oxalate) Examination of cellular components
Electrophoresis	Serum protein analysis Hemoglobin variant detection Nucleic acid (DNA/RNA) analysis
Spectrophotometry	Enzyme activity measurement (e.g. ALT, AST) Qualification of glucose, cholesterol, and proteins Electrolyte analysis (e.g. calcium, magnesium)
Chromatography	Therapeutic drug monitoring Detection of metabolic disorders (e.g. organic acidurias) Analysis of amino acids, lipids, and carbohydrates
Mass spectrometry (MS)	Newborn screening for inborn errors of metabolism Quantification of drugs and toxins Detection of complex biomolecules
Immunoassays	Hormone measurements (e.g. TSH, cortisol, insulin) Detection of infectious disease markers (e.g. HIV, hepatitis) Tumor marker detection (e.g. PSA, CA-125)
Flow cytometry	Immunophenotyping of cells Biomarker detection in cellular populations Monitoring of immune system functionality
Osmometry	Measurement of osmolality in plasma, serum, or urine Diagnosis of dehydration or overhydration Evaluation of kidney function
PCR and molecular techniques	Genetic mutation analysis Viral load determination (e.g. HIV) Molecular diagnosis of hereditary disorders
Ion-selective electrode (ISE)	Measurement of electrolytes (e.g. sodium, potassium, chloride) Acid–base balance assessment
Automated analyzers	Comprehensive metabolic panels (CMP) Lipid profiles Routine enzymatic assays (e.g. LDH, CK)
Nephelometry and turbidimetry	Measurement of immunoglobulins Detection of inflammatory markers (e.g. CRP) Analysis of clotting factors
Point-of-care testing (POCT)	Rapid glucose monitoring Blood gas analysis On-site electrolyte and hemoglobin testing

Heparin : Heparin (inhibits the conversion of prothrombin to thrombin) is an ideal anticoagulant, since it does not cause any change in blood composition. However, other anticoagulants are preferred to heparin, due to the cost factor.

Potassium or sodium oxalate : These compounds precipitate calcium and inhibit blood coagulation. Being more soluble, potassium oxalate (5–10 mg per 5 ml blood) is preferred.

Potassium oxalate and sodium fluoride : These anticoagulants are employed for collecting blood to estimate glucose. Further, sodium fluoride inhibits glycolysis and preserves blood glucose concentration.

Ammonium oxalate and potassium oxalate : A mixture of these two compounds in the ratio 3:2 is used for blood collection to carry out certain hematological tests.

Ethylene diaminetetraacetic acid (EDTA) : It chelates with calcium and blocks coagulation. EDTA is employed to collect blood for hematological examinations.

HEMOLYSIS

The rupture or lysis of RBC, releasing the cellular constituents interferes with the laboratory investigations. Therefore, utmost care should be taken to avoid hemolysis when plasma or serum is used for biochemical tests. Use of dry syringes, needles and containers, allowing slow flow of blood into syringe are among the important precautions to avoid hemolysis.

Clinical Applications

Choice of blood specimens

Biochemical investigations can be performed on 4 types of blood specimens—whole blood, plasma, serum and red blood cells. The selection of the specimen depends on the parameter to be estimated.

- ***Whole blood*** (usually mixed with an anticoagulant) is used for the estimation of hemoglobin, carboxyhemoglobin, pH, glucose, urea, non-protein nitrogen, pyruvate, lactate, ammonia etc. (Note : For glucose determination, plasma is preferred in recent years).
- ***Plasma***, obtained by centrifuging the whole blood collected with an anticoagulant, is employed for the parameters—fibrinogen, glucose, bicarbonate, chloride, ascorbic acid etc.
- ***Serum*** is the supernatant fluid that can be ***collected after centrifuging the clotted blood***. It is the most frequently used specimen in the clinical biochemistry laboratory. The parameters estimated in serum include proteins (albumin/globulins), creatinine, bilirubin, cholesterol, uric acid, electrolytes (Na^+, K^+, Cl^-), enzymes (ALT, AST, LDH, CK, ALP, ACP, amylase, lipase) and vitamins.
  It may be noted that plasma is physiologic fluid while serum is prepared in the laboratory.
- ***Red blood cells*** are employed for the determination of abnormal hemoglobin, glucose-6-phosphate dehydrogenase, pyruvate kinase etc.

PRESERVATION OF BLOOD SPECIMENS

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

TYPES OF LABORATORY TESTS

The biochemical investigations (on blood/plasma/serum) carried out in the clinical biochemistry laboratory may be grouped into different types.

1. **Discretionary or on-off tests :** Most common clinical biochemistry tests that are designed to answer specific questions, e.g. does the patient have increased blood urea/glucose concentration? Normally, these tests are useful to support the diagnosis.

2. **Biochemical profiles :** These tests are based on the fact that more useful information on the patients disease status can be obtained by analysing more constituents rather than one, e.g. ***plasma electrolytes*** (Na^+, K^+, Cl^-, bicarbonate, urea); ***liver function tests*** (serum bilirubin, ALT, AST).

3. **Dynamic function tests :** These tests are designed to measure the body's response to external stimulus, e.g. oral glucose tolerance test (to assess glucose homeostasis); bromosulphthalein test (to assess liver function).

4. **Screening tests :** These tests are commonly employed to identify the inborn errors of metabolism, and to check the entry of toxic agents (pesticides, lead, mercury) into the body.

5. **Metabolic work-up tests :** The programmed intensive investigations carried out to identify the endocrinological disorders come under this category.

The term ***emergency tests*** is frequently used in the clinical laboratory. It refers to the tests to be performed immediately to help the clinician for proper treatment of the patient, e.g. blood glucose, urea, serum electrolytes.

PRINCIPLES OF SELECTED LABORATORY INVESTIGATIONS

The ***principles*** involved in some of the laboratory investigations, commonly employed in the biochemistry laboratory are briefly described here. For interpretation of results, disease states etc., the individual chapters must be referred.

1. **Blood glucose estimation**

The quantitative determination of blood (plasma/serum) glucose is of great importance in the diagnosis and monitoring of diabetes mellitus.

2. **Blood urea estimation**

Determination of blood urea (reference range 10–40 mg/dl) is important for the evaluation of kidney (renal) function. Elevation of blood urea is associated with pre-renal (diabetic coma, thyrotoxicosis), renal (acute glomerulonephritis, polycystic kidney) and post-renal (obstruction in the urinary tract, due to tumors, stones) conditions.

Diacetyl monoxime (DAM) method **:** Urea when heated with diacetyl monoxime forms a yellow coloured complex of dioxime derivatives which can be measured at 520 nm.

3. **Serum creatinine estimation**

Estimation of serum creatinine (reference range 0.5–1.2 mg/dl) is used as a diagnostic test to assess kidney function. Serum creatinine is not influenced by endogenous and exogenous factors, as is the case with urea. Hence, some workers consider serum creatinine as a more reliable indicator of renal function.

Alkaline picrate method **:** This method is based on ***Jaffe's reaction***. Creatinine reacts with alkaline picrate to form creatinine picrate, an orange red coloured complex, which can be measured in a colorimeter at 530 nm.

(**Note :** Urinary creatinine can also be determined by employing the same principle given above).

4. **Determination of serum proteins**

The normal concentration of total serum proteins is in the range 6–8 g/dl (albumin 3.5–5.0 g/dl; globulins 2.5–3.5 g/dl; A/G ratio is 1.2 to 1.5:1). The A/G ratio is lowered either due to a decrease in albumin or an increase in globulins.

Serum albumin concentration is decreased in liver diseases, severe protein malnutrition, and excretion of albumin in urine (due to renal damage). Serum globulin concentration is elevated in chronic infections and multiple myeloma.

Biuret method **:** Peptide bonds (—CO—NH) of proteins react with cupric ions in alkaline medium to form a violet colour complex which is measured at a wavelength 530 nm. This method is suitable for total serum proteins with estimation.

Bromocresol green (BCG) dye method **:** This technique is employed for the estimation of serum albumin. BCG dye reacts with albumin to form an intense blue-green coloured complex which can be measured at 628 nm.

5. **Estimation of serum bilirubin**

The total bilirubin concentration in serum is 0.2–1 mg/dl (conjugated ~0.6 mg/dl; unconjugated ~0.4 mg/dl). Elevation in serum bilirubin concentration is observed in jaundice. Unconjugated bilirubin is increased in hemolytic jaundice, conjugated bilirubin in obstructive jaundice, while both of them are increased in hepatic jaundice.

van den Bergh reaction **:** Serum bilirubin estimation is based on van den Bergh reaction. The principle of the reaction is that diazotised sulfanilic acid (formed by mixing equal volumes of sulfanilic acid in HCl and sodium nitrite) reacts with bilirubin to form a purple coloured azobilirubin which can be measured at 540 nm.

6. **Estimation of serum cholesterol**

Serum cholesterol concentration (reference range 150–200 mg/dl) is elevated in atherosclerosis, diabetes mellitus, obstructive jaundice and hypothyroidism. Decreased levels are observed in hyperthyroidism.

Acetic anhydride method **:** Serum cholesterol reacts with acetic anhydride in the presence of glacial acetic acid and concentrated H_2SO_4 to form a green coloured complex. Intensity of this colour is measured at 560 nm.

7. **Estimation of serum uric acid**

Uric acid is the end product of purine metabolism. Its concentration in serum is increased (reference range—men: 4–8 mg/dl; women: 3–6 mg/dl) in gout.

Henry-Caraway's method **:** Uric acid in the protein-free filtrate when treated with phosphotungstic acid in the presence of sodium carbonate (alkaline solution) gives a blue coloured complex which can be measured at 660 nm.

8. **Estimation of serum calcium**

Serum calcium level is elevated (reference range 9–11 mg/dl) in hyperparathyroidism and decreased in hypothyroidism.

O-Cresolphthalein complexone method **:** Calcium reacts with the dye, O-cresolphthalein complexone (CPC) in alkaline solution to form a complex which can be measured at a wavelength 660 nm.

9. **Estimation of serum phosphorus**

Serum phosphate (reference range 3–4.5 mg/dl) is increased in hypoparathyroidism, and decreased in hyperparathyroidism and renal rickets.

For the determination of serum phosphate, serum proteins are precipitated by trichloroacetic acid. The protein-free filtrate containing inorganic phosphate is reacted with molybdic acid reagent to form phosphomolybdate. The latter in turn is reduced to molybdenum blue by treatment with 1-amino-2-naphthol-4-sulfonic acid (ANSA). The intensity of the blue colour is measured at 689 nm.

10. **Determination of SGPT and SGOT**

Serum glutamate pyruvate transaminase (SGPT; ***alanine transaminase***) and serum glutamate oxaloacetate transaminase (SGOT; ***aspartate transaminase***) are two important diagnostic enzymes. SGPT activity (reference range 5–40 IU/L) is more specifically increased in liver diseases (hepatic jaundice). SGOT activity is elevated (reference range 5–45 IU/L) in heart diseases (myocardial infarction).

Principle of assay **:** SGPT catalyses the following reaction:

L-Alanine + α-ketoglutarate ⟶
L-glutamate + Pyruvate

SGOT brings about the following reaction:

L-Aspartic acid + α-ketoglutarate ⟶
L-glutamate + Oxaloacetate

The keto acid (pyruvate or oxaloacetate), formed in the above reaction, when treated with 2,4-dinitrophenyl hydrazine forms dinitrophenyl hydrazone (brown colour) in alkaline medium which can be measured at 505 nm.

11. **Determination of serum alkaline phosphatase**

The activity of the enzyme serum alkaline phosphatase (reference range 3–13 KA Units/dl) is elevated in rickets and obstructive jaundice.

Principle of assay **:** Alkaline phosphatase hydrolyses disodium phenylphosphate liberating phenol. On treatment with 4-amino antipyrine in alkaline medium, phenol gives ferricyanide (reddish colour) which can be measured at 520 nm.

12. **Determination of serum amylase**

Serum amylase activity is increased (reference range 80–180 Somogyi Units/dl) in acute pancreatitis.

Principle of assay **:** Amylase acts on starch and hydrolyses to dextrins and maltose. Starch forms blue coloured complex with iodine, a decrease in the colour (measured at 670 nm) is proportional to the activity of amylase.

13. **Analysis of cerebrospinal fluid**

Cerebrospinal fluid (CSF) is the aqueous medium surrounding the brain and spinal cord. From the biochemical perspective, estimation of ***proteins and glucose*** in CSF is ***important***. Increase in protein (reference range 15–40 mg/dl) and decrease in glucose (reference range 50–75 mg/dl) in the cerebrospinal fluid are observed in tuberculosis meningitis.

CSF protein estimation **:** Sulfosalicylic acid (in sodium sulfate solution) precipitates CSF proteins and the turbidity is measured at 680 nm.

CSF glucose estimation **:** Any one of the standard methods employed for the determination of blood glucose can be used for CSF glucose estimation.

URINE IN CLINICAL BIOCHEMISTRY

Urine is a liquid waste product excreted by the kidneys. It plays a critical role in maintaining fluid balance, regulating electrolytes, and elimination of metabolic waste.

PHYSICAL PROPERTIES

The physical properties of urine provide valuable information about the body's hydration status, and potential health conditions.

- ***Colour of urine*** – normally pale yellow to amber; red or pink due to RBC or due to consumption beet roots/berries; milky or cloudy indicates the presence of bacteria or lipids.
- ***Specific gravity*** – normal range 1.005–1.030; low specific gravity (<1.005) due to diabetes

insipidus; high specific gravity (>1.03) indicates glycosuria or proteinuria.

- ***pH*** – normal range 4.5–8.0; Acidic urine (pH <4.5) seen in metabolic acidosis, starvation, high-protein diet; alkaline urine (pH >8.0) indicates respiratory alkalosis, vegetarian diet.
- ***Volume*** – varies with fluid intake, normally 800–2000 ml/day; ***Oliguria*** (<400 ml/day) seen in dehydration, kidney failure; ***Polyuria*** (>3000 ml/day) observed in diabetes mellitus, diabetes insipidus or excessive fluid intake; ***Anuria*** (<100 ml/day) in severe kidney failure.

NORMAL AND ABNORMAL CONSTITUENTS OF URINE

Urine is primarily composed of water and solutes (organic and inorganic substances). About 95% of urine is water. The major organic substances include urea, creatinine, uric acid, ammonia, oxalate and citrate. The inorganic substances are electrolytes (Na^+, K^+, Cl^-, Ca^{2+}), phosphates, sulfates, trace elements (Mg, Zn, Cu). In addition, there are some minor components excreted into urine (***Table 3***).

Abnormal constituents of urine

The presence of abnormal constituents in urine indicates pathological conditions or diseases.

In the ***Table 4***, comprehensive information on abnormal components of urine is given. Some important ones are listed:

- ***Proteins*** (proteinuria) – kidney damage (nephrotic syndrome).
- ***Glucose*** (glucosuria) – diabetes mellitus
- ***Ketone bodies*** (ketonuria) – diabetes ketoacidosis, starvation.
- ***Blood*** (hematuria) – UTI, kidney stones, trauma.
- ***Bilirubin*** (bilirubinuria) – liver dysfunction
- ***Crystals*** (crystalluria) – kidney stones, metabolic disorders.

Urine analysis is useful to detect several diseases, track therapeutic efficacy, besides providing information about hydration, diet and metabolic health.

TESTS FOR NORMAL AND ABNORMAL CONSTITUENTS OF URINE

Normal constituents

1. **Sodium hypobromite test :** This is a test for the detection of ***urea***. Sodium hypobromite decomposes urea to liberate nitrogen. The latter can be identified by brisk effervescence.

2. **Specific urease test :** The enzyme urease (source—horse gram) specifically acts on ***urea*** to liberate ammonium carbonate (alkali). The latter can be identified by a colour change in phenolphthalein indicator (pink colour in alkaline medium).

3. **Benedict's uric acid test :** ***Uric acid***, being a strong reducing agent, reduces phosphotungstate to tungsten blue in alkaline medium.

4. **Murexide test :** ***Uric acid*** is oxidized by nitric acid to give purpuric acid (reddish yellow). This in turn combines with ammonia to form purple red colour ammonium purpurate (murexide).

5. **Jaffe's test :** ***Creatinine*** reacts with picric acid in alkaline medium to form orange red colour complex.

Table 3 A summary of different categories of normal urinary components

Category	Components
Water	~95% of total urine volume
Electrolytes	Sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphate (PO_4^{3-}), sulfate (SO_4^{2-})
Nitrogenous wastes	Urea, creatinine, uric acid, ammonia (NH_3)
Organic compounds	Glucose, amino acids, organic acids (e.g. citric acid, oxalic acid, hippuric acid)
Hormones and enzymes	Small amounts of hormones (e.g. aldosterone, cortisol, sex hormones) and enzymes
Vitamins	Water-soluble vitamins (e.g. vitamin C, B-complex vitamins)
Metabolites and drugs	Metabolites from food, drugs, and other ingested substances
Cells and other particles	Epithelial cells; red blood cells (RBC), white blood cells (WBC), casts (hyaline granular, cellular)
Pigments	Urobilin (yellow colour), bilirubin

Table 4 Abnormal components of urine and the corresponding possible diseases or conditions, they may indicate

Abnormal component	Possible diseases/conditions
Glucose (glucosuria)	Diabetes mellitus
Protein (proteinuria)	Kidney disease (e.g. glomerulonephritis, nephrotic syndrome)
Blood (hematuria)	Urinary tract infection (UTI), kidney stones, trauma, tumors
Leukocytes	Urinary tract infection (UTI), inflammation of the urinary tract
Nitrites	Bacterial infection (typically UTIs)
Ketone bodies	Diabetic keto acidosis, starvation
Bilirubin	Liver disease (e.g. hepatitis, cirrhosis), biliary obstruction
Urobilinogen	Liver disease, hemolytic anemia
Casts	Various kidney diseases (e.g. hyaline casts in dehydration, RBC casts in glomerulonephritis)
Crystals	Kidney stones, metabolic disorders
Bacteria	Urinary tract infection (UTI)
Yeast	Fungal infection (e.g. Candida)
Parasites	Parasitic infection (e.g. Schistosomiasis)
Myoglobin	Muscle injury (e.g. rhabdomyolysis)
Hemoglobin	Hemolytic anemia, severe burns, transfusion reactions
Excessive creatinine	Muscle disease, high muscle mass, acute kidney injury
Elevated calcium	Hyperparathyroidism, malignancy
Elevated phosphate	Kidney disease, hypoparathyroidism
Drugs and metabolites	Drug overdose, poisoning

Abnormal constituents

1. **Sulfosalicylic acid test :** ***Proteins*** get precipitated by sulfosalicylic acid by forming protein-sulfosalicylate.

2. **Heat coagulation test :** This is a test for the detection of ***albumin*** and/or ***globulins*** in urine. Heat coagulation test is based on the principle of denaturation of proteins, followed by coagulation.

(**Note :** Small amounts of dilute acetic acid are added to dissolve the phosphates and sulfates that get precipitated on heating.)

3. **Benzidine test :** This test detects the presence of ***blood***. Hemoglobin (acts like peroxidase) decomposes hydrogen peroxide to liberate nascent oxygen (O^-) which oxidises benzidine to a green or blue coloured complex.

(**Note :** Pus cells of urine possess peroxidase activity which interferes with benzidine test. This can be eliminated by boiling the urine prior to the test to inactivate the enzyme).

4. **Benedict's test :** This is a ***semiquantitative test*** for the detection of urine reducing sugars (primarily glucose). Benedict's test is based on the principle of reducing property of sugars (described in detail in ***Chapter 2***). Colour of the precipitate formed indicates the approximate amount of ***glucose*** present in urine. Thus, green turbidity = traces; green precipitate = 0.5%; yellow precipitate = 1%; orange precipitate = 1.5% ***brick red precipitate*** = ***2%***. (**Note :** Benedict's test is not specific to glucose, since it can be answered by any reducing substance like glucuronides, salicylates, homogentisic acid, vitamin C).

5. **Glucose oxidase test :** This is a strip test for the ***specific detection of glucose***. The enzyme glucose oxidase oxidizes glucose to liberate hydrogen peroxide which in turn is converted to nascent oxygen (O^-) by peroxidase enzyme. The compound O-diansidine combines with nascent oxygen to form a coloured (yellow to red) complex.

6. **Rothera's test :** Nitroprusside in alkaline medium reacts with keto group of ***ketone bodies***

(acetone and acetoacetate) to form a purple ring. This test is not given by β-hydroxybutyrate.

7. **Hay's test :** This test is based on the surface tension lowering property of ***bile salts*** (sodium glycocholate and sodium taurocholate). Sulfur powder sprinkled on the surface of urine containing bile salts sinks to the bottom.

8. **Petternkofer's test :** This test is employed for the detection of ***bile salts***. The furfural derivatives (by reacting sugar with concentrated H_2SO_4) condense with bile salts to form a purple ring.

9. **Gmelin's test :** Nitric acid oxidizes the bile pigment bilirubin to biliverdin (green) or bilicyanin (blue). Gmelin's test gives a play of colours and is used for the identification of ***bile pigments***.

10. **Fouchet's test :** It is also employed for the detection of ***bile pigments***. Bile pigments are adsorbed on barium sulfate. Fouchet's reagent (containing ferric chloride in trichloroacetic acid) oxidizes bilirubin to biliverdin (green) and bilicyanin (blue).

CLINICAL BIOCHEMISTRY LABORATORY IN HEALTH AND DISEASE

The importance of biochemistry in health and disease states, in relation to normal and abnormal biochemical data have been described in this book. The reader must refer the following chapters for examples of laboratory tests, and their clinical applications:

- Chapter 6 for enzyme alterations in disease states.
- Chapter 19 for hormonal tests.
- Chapter 20 for organ function tests.
- Chapter 21 for acid-base abnormalities.
- Chapter 37 for tumor markers.

For ready reference, the most common reference biochemical values are given on inside back cover.

BASELINE, DIAGNOSTIC, PROGNOSTIC AND DISCHARGE INVESTIGATIONS

Clinical biochemistry investigations are pivotal in patient healthcare. They are employed at various stages of patients' clinical journey to establish baseline, diagnosis, predict outcomes, and evaluate recovery and readiness to discharge.

1. **Baseline investigations**

They are performed at the start of patient's clinical evaluation to establish values for future comparison. They are useful to understand the patients normal physiological or biochemical state, and to detect any probability of abnormalities.

2. **Diagnostic investigations**

These are carried out to confirm or exclude a suspected disease or condition. They are useful to identify the cause of clinical symptoms, and provide evidence for a particular disease.

3. **Prognostic investigations**

These investigations assess the likely outcome of a disease, and help to guide treatment planning. Prognostic investigations are also useful to predict disease progression, and understand the potential complications.

4. **Discharge investigations**

To evaluate patient's readiness to leave the healthcare facility, and ensure that the recovery is adequate, discharge investigations are carried out. They are useful to guide follow-up, and monitor potential complications of relapse.

PROFILES OF INVESTIGATIONS

The laboratory investigations to be done for baseline, diagnostic, prognostic and discharge largely depend on the patient's clinical history and manifestations.

The common investigations are listed

- ***Liver function tests*** – ALT, AST, bilirubin, albumin
- ***Renal function tests*** – urea, creatinine
- ***Lipid profile*** – total cholesterol, LDL, HDL and triglycerides
- ***Thyroid function tests*** – TSH, Free T_3 and free T_4
- ***Electrolyte panel*** – Na^+, K^+, Cl^- and bicarbonate
- ***Blood glucose and HbA$_{1c}$*** – screen for diabetes or prediabetes
- ***Urine analysis*** – protein, glucose, ketone bodies

In addition to the above, cardiac markers, tumor markers, arterial blood gas analysis, coagulation

profile, inflammatory markers etc., may also be done based on the patient's clinical evaluation and health status.

NEWBORN SCREENING

Newborn screening (**NBS**) is a public health program, designed to identify disorders in newborns that can affect their long-term health or survival. Most of these conditions may not show any symptoms at birth, but can lead to severe consequences, if left undiagnosed and untreated. Early detection certainly helps for timely intervention, improving health outcomes, and in some cases, preventing disability, and death.

The purpose of newborn screening is ***early diagnosis*** of disorders, ***timely treatment***, improved quality of life and reduction in healthcare costs. The first NBS was done by Robert Guthrie in 1960 for phenylketonuria.

Blood collection for newborn screening

Newborn screening is a multistep process, usually conducted within the first ***24 to 48 hours, after birth***. A few drops of blood are collected from baby's heel and placed on a filter paper card. The dried blood spots are then sent to specialized laboratories for analysis.

Disorders screened

The specific disorders or conditions screened vary from country to country, depending on the resources and health priorities. Some of the commonly screened conditions are listed.

- ***Metabolic disorders –*** e.g. phenylketonuria (PKU), maple syrup urine disease (MSUD).
- ***Hemoglobinopathies –*** e.g. sickle-cell disease.
- ***Endocrine disorders*** – e.g. congenital hypothyroidism, congenital adrenal hyperplasia.

BIOCHEMISTRY LABORATORY TESTS FOR SCREENING

The laboratory tests are carried out on dried blood spots, collected through a heel prick. The common biochemistry tests are aimed to detect abnormal levels of metabolites, enzymes, or other biomarkers, to identify the disorders.

1. **Enzyme activity assays**

Direct measurement of enzyme activities.

- ***Galactosemia*** – essay of enzyme galactose 1-phosphate uridyltransferase.
- ***Gaucher's disease*** – β-glucocerebrosidase assay.

2. **Tandem mass spectrometry (TMS)**

This technique detects multiple metabolites in a single test. Some conditions identified are listed

- ***Amino acid disorders*** – elevated phenylanine (phenylketonuria); increased branched chain amino acids (maple syrup urine disease).
- ***Organic acidemias*** – elevated methylmalonyl-CoA (methylmalonic acidemia); increased propionylcarnitine (propionic acidemia).
- ***Fatty acid oxidation disorders*** – increased acylcarnitines (medium chain acyl-CoA dehydrogenase deficiency).

3. **Immunoassays**

This technique employs antibodies to measure specific analytes (proteins, hormones, antigens etc.) often using fluorescence or chemiluminescence.

- ***Congenital hypothyroidism*** – test for thyroxine (T_4), or thyroid stimulating hormone (TSH).
- ***Congenital adrenal hyperplasia*** – test for 17-hydroxyprogesterone.
- ***Cystic fibrosis*** – test for immunoreactive trypsinogen.

4. **High-performance liquid chromatography (HPLC)**

HPLC is based on the principle of separation and identification of molecules, based on their retention time.

- ***Hemoglobinopathies*** – identifies abnormal hemo-globins, e.g. sickle-cell disease.
- ***Phenylketonuria*** – measures phenylalanine levels.

5. **Gas chromatography–mass spectrometry (GC-MS)**

This technique separates and identifies organic compounds, based on their mass spectra. It is usually done as a diagnostic follow-up for abnormal initial screening. e.g. organic acidemias.

MOLECULAR BIOLOGY TECHNIQUES FOR NEWBORN SCREENING

With advances in laboratory sciences, molecular biology techniques complement the

traditional-biochemical methods for precise identification and diagnostic confirmation of NBS. These are particularly useful for identification of mutations causing disorders. Some molecular techniques employed in newborn screening are briefly described.

1. **Polymerase chain reaction (PCR)**

PCR involves amplification of specific DNA regions for detection of mutations.

- ***Cystic fibrosis*** – identification of mutations in CFTR gene.
- ***Sickle-cell disease*** – detection of point mutation in β-globin gene.

2. **Real-time PCR**

Quantitative analysis of DNA or RNA using PCR, with fluorescence – based detection.

- ***Severe combined immunodeficiency (SCID)*** – quantities T-cell receptor excision circles indicating T-cell function.
- ***Spinal muscular atropy*** – detection of SMNI gene deletions.

3. **Microarray technology**

This technique involves simultaneous analysis of multiple DNA or RNA targets. The principle is based on the hybridization of nucleic acid probes immobilized on a solid surface.

- ***Chromosomal abnormalities*** – detects duplications and microdeletions.
- ***Cystic fibrosis*** – a screening panel to identify CFTR mutations.
- ***Thalassemias*** – detection of common deletions in α- and β-globin genes.

4. **DNA sequencing**

The exact nucleotide sequence of DNA can be determined. DNA sequencing identifies the variations in the order of nucleotides. Although this technique is useful for a comprehensive detection of mutations (including novel variants), it is very expensive and has longer processing time.

Artificial intelligence (AI) in newborn screening

AI algorithms can analyse patterns of metabolic profiles, and genetic profiles to improve diagnostic accuracy. AI is also useful to streamline sample processing and result interpretation.

Overview of inborn errors of metabolism

The inborn metabolic errors can be broadly classified as follows.

- ***Aminoacidopathies,*** e.g. PKU, MSUD
- ***Organic acidemias,*** e.g. methylmalonic acidemia
- ***Fatty acid oxidation disorders,*** e.g. medium chain acyl-CoA dehydrogenase deficiency
- ***Urea cycle disorders,*** e.g. Orginosuccinic aciduria
- ***Lysosomal storage disorders,*** e.g. Gaucher's disease

In the ***Table 5***, different types of inborn errors (with examples), corresponding biochemical markers and screening techniques are given.

PRENATAL SCREENING

Prenatal screening primarily aims to assess the health of the fetus, and to detect potential genetic, chromosomal and structural abnormalities. It plays a critical role in ensuring maternal and fetal well-being by guiding clinical decisions, and preparing families for potential outcomes Prenatal screening helps the expectant parents and clinicians to make informed decisions about pregnancy management.

Objectives of prenatal screening

1. ***Detection of abnormalities :*** The prenatal screening identifies the pregnancies at increased risk for conditions like chromosomal abnormalities (e.g. Down syndrome) and structural abnormalities (neural tube defects).

2. ***Early intervention :*** It helps to do timely diagnostic testing, counseling (of parents, families), and medical and surgical interventions.

3. ***Informed decision-making :*** With the critical information available, the parents are prepared for potential outcomes, including specialized neonatal care.

Types of prenatal screening

Tests of prenatal screening are non-invasive, and categorized into first-trimester, second-trimester non-invasive prenatal testing, and integrated/sequential screening approaches.

1. **First-trimester screening :** It is performed between 11-14 weeks of gestation

Table 5 A summary of different types of inborn errors (with examples), corresponding biochemical markers and screening techniques

Inborn error type	Example(s)	Biochemical marker(s)	Screening technique(s)
Aminoacidopathies	Phenylketonuria (PKU) Maple syrup urine disease (MSUD) Homocystinuria	Phenylalanine Leucine, isoleucine, valine Homocysteine, methionine	Tandem mass spectrometry (MS) Amino acid analysis (HPLC) Gas chromatography-MS (GC-MS)
Organic acidurias	Methylmalonic acidemia (MMA) Isovaleric acidemia	Methylmalonic acid, propionylcarnitine Isovalerylcarnitine, 3-hydroxyisovaleric acid	Organic acid analysis (GC-MS) MS/MS, GC-MS
Fatty acid oxidation disorders	Medium-chain Acyl-CoA dehydrogenase deficiency (MCAD) Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)	Octanoylcarnitine (C8) Hydroxyacylcarnitines	MS/MS MS/MS Acylcarnitine profiling
Urea cycle disorders	Ornithine transcarbamylase deficiency Argininosuccinic aciduria	Ammonia, citrulline Argininosuccinic acid, citrulline	Plasma ammonia measurement, amino acid analysis Amino acid analysis (HPLC or MS/MS)
Carbohydrate metabolism disorders	Galactosemia Glycogen storage disorders	Galactose-1-phosphate Glucose, lactate, pyruvate	Enzyme assay, MS/MS Genetic testing, enzyme assay
Lysosomal storage disorders	Gaucher's disease Pompe's disease	Glucocerebrosidase activity Acid α-glucosidase activity	Enzyme assay (fluorometric or MS) Enzyme assay, genetic testing
Peroxisomal disorders	Zellweger syndrome Refsum disease	Very long chain fatty acids Phytanic acid	MS/MS, GC-MS GC-MS
Mitochondrial disorders	Pyruvate dehydrogenase deficiency	Lactate, pyruvate	Lactate/pyruvate analysis, enzyme assay

(HPLC-High-performance liquid chromatography; MS/MS-Tandem mass spectrometry)

- ***Maternal serum markers*** – free β-human chorionic gonadotropin (hCG) elevated in Down syndrome; pregnancy – associated plasma protein-A (PAPP-A) lower levels in Down syndrome.
- ***Nuchal translucency (NT) ultrasound*** – it measures the thickness of fluid at the back of the fetal neck. Increased NT is associated with trisomy 21, trisomy 18, and certain congenital heart defects.

2. **Second-trimester screening :** It is performed between 15-20 weeks of gestation.

- ***Quadruple test*** measures alpha-fetoprotein (AFP) hCG, estriol and inhibin A. High AFP suggests neural tube defects. Low AFP, high hCG and low estriol are associated with Down Syndrome. (**Note :** Quadruple test is an extension of ***triple marker test***, with estimation of an additional analyte, inhibin A)

3. **Non-invasive prenatal testing (NIPT) :** For NIPT, cell-free fetal DNA (cfDNA) from maternal blood is used. It detects common sex chromosome abnormalities and common aneuploidies, e.g. trisomy 21, trisomy 18, trisomy 13.

4. **Integrated and sequential screening :** It combines first and second-trimester tests to provide a more comprehensive risk assessment. This approach of integrated screening gives a single risk result after completing both phases.

Abnormalities identified in prenatal screening

1. **Chromosomal abnormalities :** Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13).

2. **Structural abnormalities :** Neural tube defects (e.g. spina bifida), abdominal wall defects (e.g. omphalocele).

3. **Genetic disorders :** Cystic fibrosis, sickle-cell anemia.

4. **Maternal-fetal conditions :** Preeclampsia risk management through markers like placental growth factor.

Follow-up for abnormal screening results

1. **Diagnostic testing :** Performed on ***chorionic villus sampling (CVS)*** between 10–13 weeks of gestation to confirm chromosomal or genetic abnormalities. ***Amniocentesis*** carried out after 15 weeks for detailed chromosomal analysis and neural tube defects.

2. **Ultrasound evaluation :** High-resolution ultrasound can detect structural anomalies of the fetus.

3. **Genetic Counseling :** Prenatal screening helps the parents to understand the implications of abnormal results.

Technological advancements in prenatal testing

1. **Artificial intelligence (AI) :** It enhances the accuracy of biochemical analysis and ultrasound tests to detect subtle abnormal patterns.

2. **Next-generation sequencing :** It expands the range of conditions detectable under non-invasive prenatal testing.

3. **Point-of-care testing :** Development of portable devices for on-site prenatal screening.

Ethical and social considerations of prenatal screening

1. **Informed consent :** Parents must know the purpose, benefits and limitations of prenatal screening.

2 **Reproductive decisions :** Screening results will influence the decisions about continuing or termination of pregnancy.

Prenatal screening is a cornerstone of modern prenatal care to know the fetal health. Proper counseling and ethical considerations are essential to ensure that prenatal screening results benefit families, and aligns with their expectations and values.

ONLINE MENDELIAN INHERITANCE IN MAN (OMIM)

OMIM is a ***catalogue of human genes and genetic disorders*** with a focus on gene-phenotype relationship. The present OMIM Website (omim.org) has been developed and maintained by John Hopkins University School of Medicine (JHUSOM). OMIM database has been designed for use by physicians, healthcare professionals and researches dealing with genetic disorders. Further, OMIM database is freely available to public.

OMIM classification system

There is a unified index for genetic diseases with a specific numbering system that is widely used in medical literature.

Each OMIM entry is given a unique 6-digit identifying number as summarized below.

Autosomal loci or phenotypes (created before May 15, 1994)	100000–299999
X-linked loci or phenotypes	300000–399999
Y-linked loci or phenotypes	400000–499999
Mitochondrial loci or phenotypes	500000–599999
Autosomal loci or phenotypes	600000 and above

Some examples of OMIM are given

Phenylketonuria type I (enzyme defect-phenylalanine hydroxylase)	261600
Alkaptonuria (Enzyme defect-homogentisate oxidase)	607474
Galactosemia (Enzyme defect-galactose 1-phosphate uridyltransferase)	230400

QUALITY CONTROL

Quality control in clinical biochemistry laboratory refers to the reliability of investigative service. Any error in the laboratory will jeopardize the lives of patients. It is therefore utmost important that the laboratory errors are identified and rectified.

Quality control comprises of four interrelated factors namely precision, accuracy, specificity and sensitivity.

Precision refers to the reproducibility of the result when the same sample is analysed on different occasions (replicate measurements) by the same person. For instance, the precision is good, if the blood glucose level is 78, 80 and 82 mg/dl on replicates.

Accuracy means the closeness of the estimated result to the true value, e.g. if true blood urea

level is 50 mg/dl, the laboratory reporting 45 mg/dl is more accurate than the one reporting 35 mg/dl.

Specificity refers to the ability of the analytical method to specifically determine a particular parameter, e.g. glucose can be specifically estimated by enzymatic glucose oxidase method.

Sensitivity deals with the ability of a particular method to detect small amounts of the measured constituent.

TYPES OF QUALITY CONTROL

Internal quality control refers to the analysis of the same pooled sample on different days in a laboratory, the results should vary within a narrow range.

External quality control deals with the analysis of a sample received from outside, usually from a national or regional quality control centre. The results obtained are then compared.

Common errors in clinical brochemistry laboratory

The errors that occur in clinical biochemistry laboratory are broadly of three types

1. **Pre-analytical errors**
   - Improper sample collection, labeling or storage
   - Hemolysis, lipemia or sample contamination
2. **Analytical errors**
   - Instrument malfunctioning
   - Improper calibration or use of expired reagents
3. **Post-analytical errors**
   - Errors in data transcription
   - Delayed reporting of results

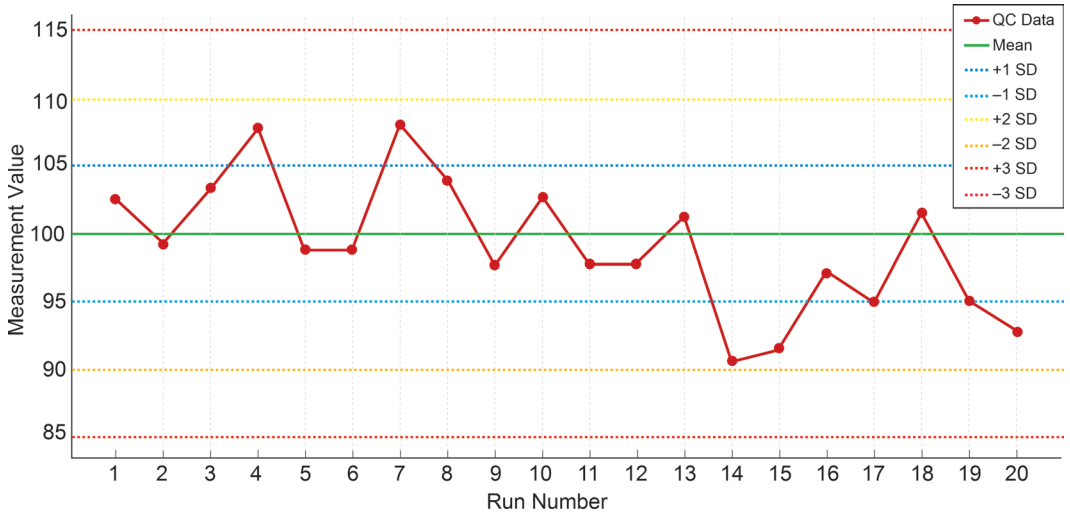
Monitoring of quality control

Levey-Jennings (L-J) charts are most commonly used in laboratory quality control to monitor the performance of analytical processes. L-J charts are useful to ensure accuracy, precision and consistency in clinical biochemistry.

An L-J chart consists of X-axis, representing time (e.g. daily or batch runs) and Y-axis representing QC result of a specific parameter (e.g. glucose, creatinine). In addition, L-J chart also has a mean time with the average value of QC material, and control limits. The control limits are categorized as

- ±1SD (standard deviation) – indicates expected variability of the results within a normal range
- ±2SD-acceptable range, values beyond this area are considered warnings.
- ±3SD-indicates action limits, values beyond this indicate significant errors.

Levey-Jennings charts are essential to maintain high standards in clinical biochemistry practice.



Levey-Jennings chart to monitor quality control.

They provide data to detect and address errors, ensuring the reliability and accuracy of laboratory results.

AUTOMATION IN CLINICAL BIOCHEMISTRY LABORATORY

Automation in clinical biochemistry has revolutionized laboratory medicine, enhancing accuracy and efficiency in the analysis of biochemical parameters. It involves the use of advanced equipment and systems to perform tests with minimal human intervention. Automation significantly improves the quality of healthcare delivery.

Phases of automation

Automation in laboratory practice involves the use of computerized systems and robotics to streamline the pre-analytical, analytical and post-analytical phases of laboratory testing.

- ***Pre-analytical phase*** – deals with sample identification (barcoding), centrifugation (automated), aliquoting (sample division into multiple aliquots) and sample transport (through robotic arms).
- ***Analytical phase*** – autoanalyzers can integrate multiple technologies (photometry, nephelometry, chemiluminescence etc.), and simultaneously perform wide range of tests (enzymes, electrolytes, metabolites, hormones etc.).
- ***Post-analytical phase*** – involves results validation, (normal or abnormal) report generation and results archiving (storing for future use)

Benefits of automation

The benefits of automation and clinical biochemistry laboratory are listed

- ***Enhanced efficiency*** – processing large number of samples simultaneously in a short period.
- ***Improved accuracy and precision*** – automation minimizes human errors in pipetting, calculations and sample handling. Inbuilt QC programs ensure reliable results.
- ***Standardization*** – automated protocols ensure uniform testing conditions and comparability across different laboratories.
- ***Cost-effectiveness*** – although initial investment cost is high, automation reduces long-term labour costs, and decreased reagent waste.
- ***Increased safety*** – automation reduces direct manual handling of samples, thereby minimizing exposure to infectious agents and hazardous materials.

Automation has transformed clinical biochemistry laboratory practice, and significantly improved efficiency and accuracy. It is expected that automation will continue to evolve and address challenges that include personalized medicine, public health and research.

ARTIFICIAL INTELLIGENCE (AI)

The ***simulation of human intelligence by machines*** is referred to as artificial intelligence (AI). These machines are designed to learn, think and perform tasks that typically require human cognition. AI systems can process information, recognize patterns and make decisions, based on the available data, often surpassing human capabilities in speed and efficiency.

The advantages of AI include increased efficiency and productivity, reduction in human errors in critical tasks, effective handling of vast databases, and finding solutions to complex problems.

AI IN CLINICAL BIOCHEMISTRY LABORATORY PRACTICE

Artificial intelligence is transforming clinical biochemistry laboratory practice through automation of tasks, improving diagnostic accuracy, and enhancing operational efficiency. The different types of AI with their applications in clinical biochemistry laboratory are given in ***Table 6***. Some important aspects are described hereunder.

1. **Automated data analysis :** AI algorithms, by using machine learning (ML) and deep learning (DL) can analyze complex biochemical data faster and more accurately than traditional methods. The advantages are listed

- Detection of patterns of large datasets
- Identification of abnormal results for further investigation.
- Providing predictive insights into patient outcomes.

Table 6 A summary of different types of artificial intelligence (AI) and their specific applications in clinical biochemistry

Type of AI	Description	Applications in clinical biochemistry
Machine learning (ML)	Algorithms that learn patterns from data	Biomarker discovery for diseases Predictive diagnostics (e.g. diabetes, CKD) Quality control monitoring for instruments
Deep learning (DL)	Neural networks for complex data analysis	Image analysis (e.g. gel electrophoresis, histology) High-throughput data analysis (e.g. mass spectrometry) Disease classification
Natural language processing (NLP)	Understanding and generating language	Extracting biochemical data from EHRs Automating laboratory report generation Literature mining for diagnostic insights
Expert systems	Rule-based AI mimicking human expertise	Decision support for diagnostic tests Interpreting abnormal test results Optimizing laboratory workflows
Computer vision	AI for visual data interpretation	Analyzing cell morphology in microscopy Detecting defects in processed samples Enhancing digital pathology
Robotics and automation	Automating repetitive tasks	Automated sample handling and sorting High-throughput biochemical analysis Reducing human error in laboratory processes
Predictive analytics	Predicting trends and outcomes	Monitoring disease progression via biomarkers Forecasting laboratory workload Predicting treatment responses
Reinforcement learning	Learning optimal actions dynamically	Optimizing laboratory workflows Prioritizing test scheduling Adapting resource allocation based on workload
Federated learning	Collaborative AI with decentralized data	Sharing diagnostic insights across institutions Developing universal QC standards Privacy-preserving collaboration on patient data
Hybrid AI systems	Combining multiple AI types	Integrated diagnostics combining biochemical, genomic, and clinical data Personalized medicine workflows

2. **Decision support systems :** AI-powered decisions help the clinicians to appropriately interpret biochemical test results.

- Detailed insights based on patient history.
- Recommendations for treatments and follow-up.
- Alters any misinterpretation or overlooked conditions.

3. **Disease prediction and screening :** AI systems help in identifying biomarkers for diseases, and aiding early diagnosis and risk stratification.

- Identifying metabolic disorders or specific enzyme defects.
- Screening for chronic conditions like CHD, diabetes mellitus.

4. **Personalized medicine :** AI can integrate biochemical data with patient's personal information (e.g. lifestyle, genetics). This is useful in many ways

- Tailored diagnosis of the individual.
- Personalized therapeutic interventions.
- Improved treatment efficiency and monitoring of diseases.

5. **Quality control (QC) management :** With the support of AI, QC can be better managed and improved by

- Regularly monitoring instrument performance.
- Detecting deviations in analytical procedures, that might go unnoticed.
- Predicting potential errors or failures, that might go undetected.

6. **Optimization of work flow :** AI streamlines clinical laboratory operations by

- Automating sample processing and analysis.
- Reducing turnaround times for test results.
- Predicting workload peaks for better resource allocation.

7. **Integration with electronic health records (EHRs) :** AI integrates laboratory data with EHRs to provide a holistic view of patient health. Such integraton supports

- Comprehensive clinical decision making.
- Population health management.
- Policy formulations, based on the data.

8. **Handling of huge data :** AI can handle volumes of data and help in research by

- Identifying trends, and correlations in large datasets.
- Facilitating biomarker discovery.

AI ENHANCES ACCURACY IN CLINICAL BIOCHEMISTRY LABORATORY

AI significantly improves accuracy in clinical biochemistry laboratories through advanced data analysis, process optimization and error detection mechanisms. Some of the advantages of AI are listed

- Improved data interpretation
- Error reduction in analytical processes
- Optimization of diagnostic algorithms
- Enhancing pre-analytical and post-analytical accuracy
- Enhanced precision in complex assays.
- Reduction in human bias
- Application in critical diagnostics (early disease detection, monitoring)
- Improved maintenance of laboratory equipment

AI IN QUALITY CONTROL IN CLINICAL BIOCHEMISTRY LABORATORY

AI plays a significant role in enhancing quality control (QC) programs in clinical biochemistry laboratories by ensuring reliability, accuracy and consistency. Some aspects of AI contribution to QC are described.

1. **Automated QC data analysis**

- Use of advanced statistical techniques and analysis for a comprehensive assessment of laboratory performance.
- Recognition of patterns by AI can be done more appropriately which humans might overlook.

2. **Continuous monitoring and error detection**

- Tracks instruments performance and environmental conditions (temperature, humidity) and identifies deviations, if any.
- AI detects even subtle irregularities (equipment malfunction, reagent degradation) and gives automated alerts.

3. **Reduction in human errors**

- AI records and organizes QC data systematically, minimizing errors in manual documentation.
- AI enforces adherence to standard procedures, reducing human errors.

4. **Improved QC sample management**

- AI ensures that QC samples are properly prepared and handled correctly to minimize the risk of pre-analytical errors.
- QC samples are properly utilized balancing cost and accuracy.

5. **Dynamic QC rules and decision making**

- AI can adjust QC rules dynamically, based on the history to improve sensitivity and specificity.
- AI can validate the test results comparing historical QC data.

6. **Predictive maintenance**

- AI can predict equipment maintenance or calibration, based on the performance.
- Reagent stability, degradation and expiration can be monitored by AI.
- AI can forecast potential QC issues to take appropriate action.

7. **Integration with laboratory information systems (LIS)**

- AI integrates QC data with laboratory information systems for tracking and reporting.
- Regulatory compliance with accreditation standards (e.g. ISO, NABL) is better achieved through AI.

BIOMEDICAL WASTE (BMW)

The waste generated during medical, surgical and research activities involving humans or

animals is referred to as biomedical waste (BMW). Appropriate management of BMW is very essential to minimize health risks and environmental hazards.

CLASSIFICATION OF BIOMEDICAL WASTE

1. **General non-hazardous waste**
   - ***Non-hazardous waste*** –packaging material, office waste. This poses no health risk.
2. ***Hazardous waste***
   - ***Chemical waste*** – laboratory reagents, disinfectants
   - ***Infectious waste*** – all waste contaminated with blood, other body fluids and pathogens.
   - ***Pathological waste*** – human body parts (tissues, organs).
   - ***Sharp waste*** – syringes, needles, broken glass.
   - ***Pharmaceutical waste*** – unused or expired medicines, drugs, vaccines.
   - ***Radioactive waste*** – all waste with radioactive material.
   - ***Cytotoxic waste*** – waste with cancer treatment drugs.

According to WHO, normally biomedical waste consists of 10-25% hazardous waste, while the remaining is non-hazardous waste.

SOURCES OF BIOMEDICAL WASTE

- ***Healthcare institutions*** – hospitals, clinics, laboratories, nursing homes.
- ***Research organizations*** – medical and biological research laboratories.
- ***Veterinary sources*** – animal hospitals and research facilities.
- ***Domestic healthcare*** – waste from home-based treatments and medications.

RISKS OF BIOMEDICAL WASTE

1. **Health hazards**
   - Exposure to pathogens can cause infections (HIV, hepatitis B).
   - Inhalation of toxic chemicals may lead to respiratory complications.
2. **Environmental pollution**
   - Air pollution and water pollution common due to improper disposal of BMW.
3. **Public hazards**
   - Biomedical waste in open places increases community exposure with high risk of infectious diseases.

BIOMEDICAL WASTE MANAGEMENT

The process of BMW management involves a series of systematic steps – segregation, transportation, treatment, disposal etc.

1. **Waste segregation**

BMW can be segregated at the point of generation, using colour-coded containers

- ***Black*** – non-hazardous general waste
- ***White*** – sharp materials (needles, syringes)
- ***Blue*** – glassware and metallic implants
- ***Red*** – recyclable materials (contaminated plastics)
- ***Yellow*** – pathological waste, infectious waste

2. **Waste collection and transportation**

The above waste has to be collected in leak-proof and labeled containers. It must be ensured that the containers are not overfilled to prevent spills.

The waste has to be transported using closed, designated vehicles to disposal sites. Manual handling of waste during transportation should be avoided.

Medical Concepts

- The biological fluids used in clinical biochemistry laboratory include blood, urine and CSF.
- Newborn screening helps to detect many disorders e.g. phenylketonuria, sickle-cell disease, congenital hypothyroidism
- Prenatal screening identifies chromosomal abnormalities (e.g. Down syndrome) and to take appropriate decision about continuing or termination of pregnancy.
- Automation coupled with AI have helped to revolutionize clinical biochemistry laboratory practice - rapidity, accuracy, quality control etc.
- Abnormal urinary constituents (glucose, protein), are useful for preliminary screening, diagnosis and prognosis of several disorders (e.g. diabetes, nephrotic syndrome).

3. **Waste treatment**

The methods employed for treatment depend on the type of waste.

- ***Encapsulation*** – sharp and hazardous materials are encapsulated in plastic or cement bags before final disposal.
- ***Chemical disinfection*** – use of chemicals (bleaching powder) to treat liquid waste and sharps.
- ***Microwave treatment*** – used to disinfect infectious waste.
- ***Autoclaving*** – uses high-pressure steam to sterilize infectious waste.
- ***Incineration*** – hazardous waste can be burnt to ash at high temperature.

4. **Final disposal**

- ***Recycling*** – plastics and glass that are recyclable can be sterilized and reused.
- ***Landfilling*** – non-hazardous and treated waste can be finally disposed through landfilling.
- ***Deep burial*** – pathological waste can be disposed through deep burial in remote areas to prevent human or animal contact.

5. **Regulatory guidelines**

There are guidelines at the international (WHO) or national levels for the management of biomedical waste. ***In India, Biomedical Waste Management Rules, 2016*** are applicable.

6. **Best ways of BMW management**

- Minimizing waste generation
- Training healthcare personnel for proper handling of BMW
- Use of personal protection equipment (PPE) – gloves, masks, aprons, safety goggles
- Compliance with regulations

7. **Importance of proper BMW management**

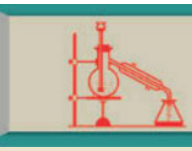
Proper BMW management is a social responsibility, besides protecting environment and preventing the spread of infectious diseases.

A summary of different biomedical waste (with examples), and common methods of disposal is given in ***Table 7***.

Table 7 A summary of different biomedical waste with examples, and common methods of disposal

Type of biomedical waste	Examples	Methods of disposal
Infectious waste	Blood-soaked bandages, used gloves, surgical dressings, and cultures	Autoclaving, incineration, or microwave treatment
Pathological waste	Human tissues, organs, body parts, and animal carcasses	Incineration or deep burial in approved sites
Sharp waste	Used needles, syringes scalpel blades, and broken glass contaminated with biohazards	Autoclaving followed by shredding or encapsulation in puncture-proof containers before disposal in landfills
Pharmaceutical waste	Expired or unused medicines, contaminated drugs and vaccine vials	Incineration or disposal in high-temperature cement kilns
Chemical waste	Laboratory reagents, cleaning solutions, and disinfectants	Neutralization (for non-hazardous chemicals) or incineration for hazardous chemicals
Cytotoxic waste	Chemotherapy drugs and materials contaminated with cytotoxic agents	Incineration in specialized high-temperature incinerators
Radioactive waste	Used radioisotopes, contaminated glassware, and syringes	Decay in secure facilities until radiation levels are safe; then dispose of as general waste or incineration
General non-hazardous waste	Paper, packaging, and non-contaminated materials	Recycling or disposal in municipal landfills
Liquid biomedical waste	Body fluids, blood, and laboratory liquid waste	Chemical disinfection followed by discharge into an effluent treatment plant
Contaminated plastics	IV bags, tubing, and disposable syringes	Autoclaving or chemical disinfection followed by shredding and recycling or incineration
Glassware	Contaminated glass slides, vials, and broken lab equipment	Autoclaving followed by disposal in designed sharp/glass containers or recycling after decontamination

SUMMARY



- *Clinical biochemistry laboratory service is absolutely essential for medical practice.*
- *Biochemical investigations hold the key for the diagnosis and prognosis of various diseases (e.g. diabetes, jaundice, MI, cancers).*
- *Many laboratory techniques (e.g. spectrophotometry, electrophoresis, chromatography) are employed in the clinical biochemistry laboratory.*
- *Newborn screening is useful for early diagnosis and timely treatment.*
- *Prenatal screening aims to assess the health of the fetus, and to detect abnormalities.*
- *Artificial intelligence (AI) is transforming clinical biochemistry laboratory practice through improving diagnostic accuracy and enhancing operational efficiency.*
- *Quality control in laboratory is essential for the reliability of the results with special reference to precision, accuracy, specificity and sensitivity.*
- *Automation in clinical biochemistry has revolutionized laboratory medicine, enhancing accuracy and efficiency in the analysis of biochemical parameters.*
- *Abnormal constituents (proteins, glucose, ketone bodies) of urine are useful to detect diseases (nephrotic syndrome, diabetes mellitus) and track therapeutic efficacy.*
- *Biomedical waste (BMW) generated during medical, surgical and research activities has to be carefully managed and disposed to avoid health risks and environmental hazards.*