BIOCHEMICAL METHODS OF INVESTIGATIONS
CLINICAL BIOCHEMISTRY

is

the laboratory service absolutely essential for medical practice or branch of laboratory medicine in which chemical and biochemical methods are applied to the study of disease.
The results of biochemical tests may be of use in:

- diagnosis and in the monitoring of treatment.
- screening for disease
- in assessing the prognosis.
- research into the biochemical basis of disease
- clinical trials of new drugs
Biochemical tests can be broadly divided into two groups:

In 1. *discretionary or selective requesting*, the tests are carried out on the basis of an individual patient's clinical situation.

In contrast, 2. *screening tests* are used to search for disease without there being any necessary clinical indication that disease is present.
Test selection for the purposes of discretionary testing

- To confirm a diagnosis - Plasma (free T4) and (thyroid-stimulating hormone, TSH) in suspected hyperthyroidism
- To aid differential diagnosis - To distinguish between different forms of jaundice
- To monitor progress - Plasma (glucose) to follow of patients with diabetes mellitus
Screening may take two forms:

1. Well-population screening in which typically a spectrum of tests is carried out on individuals from an apparently healthy population in an attempt to detect presymptomatic or early disease.
2. Case-finding screening programmes perform appropriate tests on a population sample known to be at high risk of a particular disease.
Neonates:

- PKA (phenylketonuria) - Serum [phenylalanine]
- Hypothyroidism - Serum [TSH] and/or [thyroxine]

Pregnancy:

- Diabetes mellitus in the mother - Plasma and urine [glucose]
- Open neural tube defect (NTD) in the foetus - Maternal serum [a-fetoprotein]
Most biochemistry laboratories provide the "core analyses", commonly requested tests which are of value in many patients, on a frequent basis.
Core biochemical tests:

1. Sodium, potassium, chloride and bicarbonate
2. Urea and creatinine
3. Calcium and phosphate
4. Total protein and albumin
5. Bilirubin and alkaline phosphatase
6. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)
7. Glucose
8. Amylase
Specialized tests:

Not every laboratory is equipped to carry out all possible biochemistry requests.

1. Hormones
2. Specific proteins
3. Trace elements
4. Vitamins
5. Drugs
6. Lipids and lipoproteins
7. DNA analyses
The emergency tests:

- All clinical biochemistry laboratories provide facilities for urgent tests. An urgent test is designated as one on which the clinician is likely to take immediate action.

- 1. Urea and electrolytes
- 2. Blood gases
- 3. Amylase
- 4. Glucose
- 5. Salicylate
- 6. Paracetamol
- 7. Calcium
The **biological fluids** employed in the clinical biochemistry laboratory include:

- Blood, urine, saliva, sputum, faeces, tissue and cells, cerebrospinal fluid, peritoneal fluid, synovial fluid, pleural fluid, stones.
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 determinations.
Biochemical investigations can be performed on 4 types of blood specimens –

- whole blood, plasma, serum and red blood cells.
1. **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.
2. **Plasma**, obtained by centrifuging the whole blood collected with an anticoagulant, is employed for the parameters —

- fibrinogen, glucose, bicarbonate, chloride, ascorbic acid etc.
3. **Serum** is the supernatant fluid that can be collected after centrifuging the clotted blood.

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.
4. Red blood cells are employed for the determination of abnormal hemoglobins, glucose 6-phosphate dehydrogenase, pyruvate kinase etc.
ANTICOAGULANTS

1. Heparin (inhibits the conversion of prothrombin to thrombin) is the most widely used anticoagulant for clinical chemical analysis. Heparin is an ideal anticoagulant, since it does not cause any change in blood composition.
Ethylene diamine tetra acetic acid (EDTA) is a chelating agent, and is particularly useful for hematological examination because it preserves cellular components of the BLOOD.
3. Sodium fluoride is usually used as a preservative for blood glucose by inhibiting the enzyme systems involved in the glycolysis.

It should not be used for enzyme assays, as well as when the test involves enzymatic analysis.
Plain tube: no anticoagulant
Clot forms
- General

Plain tube: contains SST gel
- General

EDTA anticoagulant
- Whole blood analysis
- Red cell analysis
- Lipids and lipoproteins

Lithium heparin anticoagulant
- General

Fluoride oxalate
- Glucose
- Lactate

Heparinized syringe
- Arterial blood sampling
COLLECTION OF URINE:

For biochemical investigations, urine can be collected as a single specimen or for 24 hours. Single specimens of urine, normally collected in the morning, are useful for qualitative tests e.g., sugar, proteins.
Urine specimens tend to deteriorate.

The changes include:

1. destruction of glucose by bacteria;
2. conversion of urea to ammonia, by bacteria, with fall in \([H^+]\) and precipitation of phosphates;
3. oxidation of urobilinogen to urobilin and porphobilinogen to porphyrins.
**Preservatives for urine**: The preservatives are used (1) to reduce bacterial action; (2) to minimise chemical decomposition, and (3) to decrease atmospheric oxidation of unstable compounds.

- Formalin, thymol, chloroform, toluene, concentrated HCl and glacial acetic acid are the commonly used urine preservatives.
Biological factors affecting the interpretation of results:

- Sex of the patient.
- Age of the patient.
- Effect of diet.
- Time when sample was taken.
- Stress and anxiety.
- Posture of the patient.
- Effects of exercise.
- Medical history.
- Pregnancy.
- Menstrual cycle.
- Drug history.
QUALITY CONTROL

Quality control in clinical biochemistry laboratory refers to the reliability of investigative service.

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.
Fig. 2 Precision and accuracy.
**Common tests on blood performed away from the laboratory**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Used when investigating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood gases</td>
<td>Acid–base status</td>
</tr>
<tr>
<td>Glucose</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Urea</td>
<td>Renal disease</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Renal disease</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Neonatal jaundice</td>
</tr>
<tr>
<td>Therapeutic drugs</td>
<td>Compliance or toxicity</td>
</tr>
<tr>
<td>Salicylate</td>
<td>Detection of poisoning</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Detection of poisoning</td>
</tr>
<tr>
<td>Glucose</td>
<td>Diabetic monitoring</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Coronary heart disease risk</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Fitness to drive/confusion, coma</td>
</tr>
</tbody>
</table>
Common tests on urine performed away from the laboratory

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Used when investigating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketones</td>
<td>Diabetic ketoacidosis</td>
</tr>
<tr>
<td>Protein</td>
<td>Renal disease</td>
</tr>
<tr>
<td>Red cells/haemoglobin</td>
<td>Renal disease</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Liver disease and jaundice</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>Jaundice/haemolysis</td>
</tr>
<tr>
<td>pH</td>
<td>Renal tubular acidosis</td>
</tr>
<tr>
<td>Glucose</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>hCG</td>
<td>Pregnancy test</td>
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</tbody>
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* A: A positive result indicates a specific condition.
* B: A negative result indicates a specific condition.
The tests commonly performed away from the laboratory can be categorized as follows:

A. Tests performed in medical or nursing settings.
B. Tests performed in the home, shopping centre or clinical setting.
C. Alcohol tests.
1. A urine sample is applied to the test strip.

2. Urine saturates absorbent pad and begins to move along test strip.

3. hCG binds to monoclonal antibody–blue bead complex, which then moves along the plate as the urine diffuses.
The hCG—antibody—blue bead complex binds to a 2nd hCG specific antibody fixed to the plate along a straight line. This produces a blue line on the plate.

Excess of the monoclonal antibody—blue bead complex in the urine binds to a third antibody forming another blue line. This signals the test is complete.

A positive result is shown by 2 blue lines; a negative result is shown by 1 blue line.

Fig. 2 How a pregnancy test kit works.