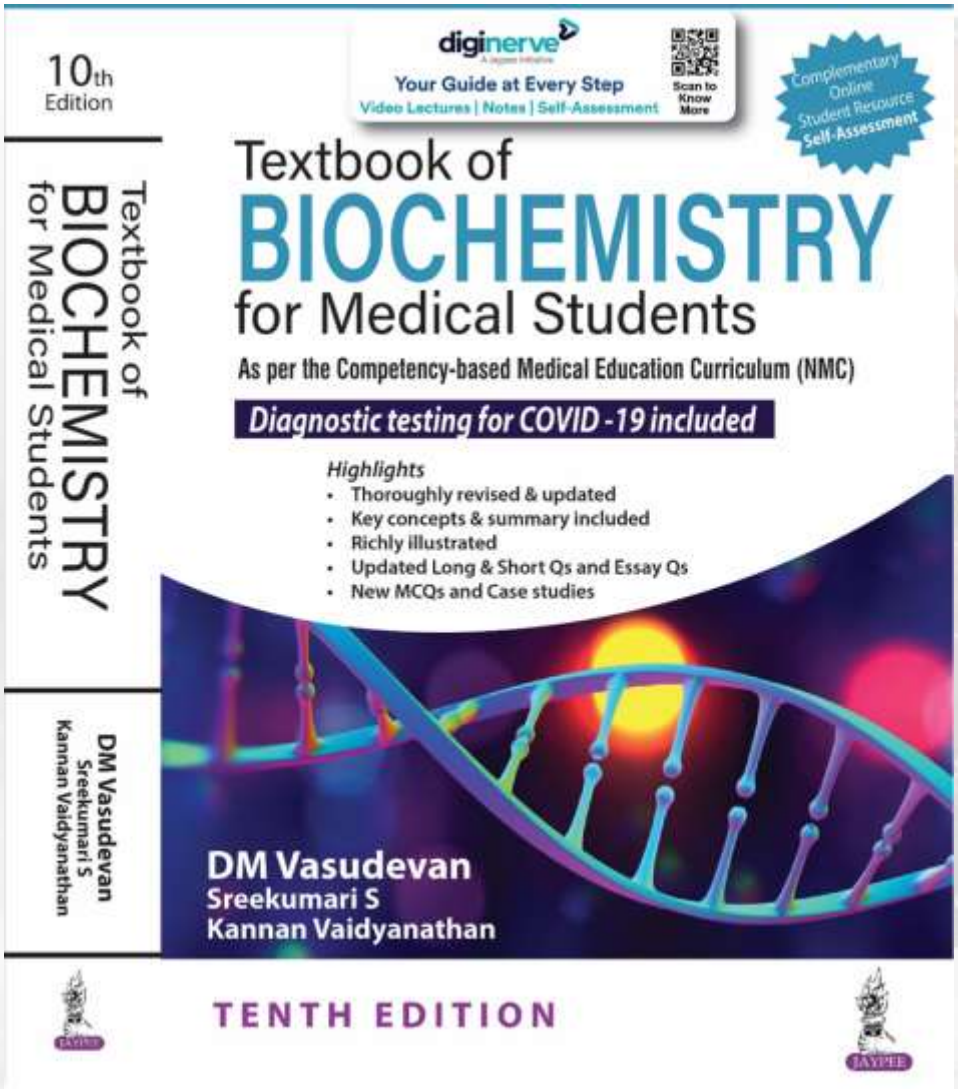
Chapter 39:

Clinical Laboratory



Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

ELECTROPHORESIS

Movement of charged particles when subjected to an electric field.

Positively charged particles (cations) move to cathode and negatively charged ones (anions) to anode.

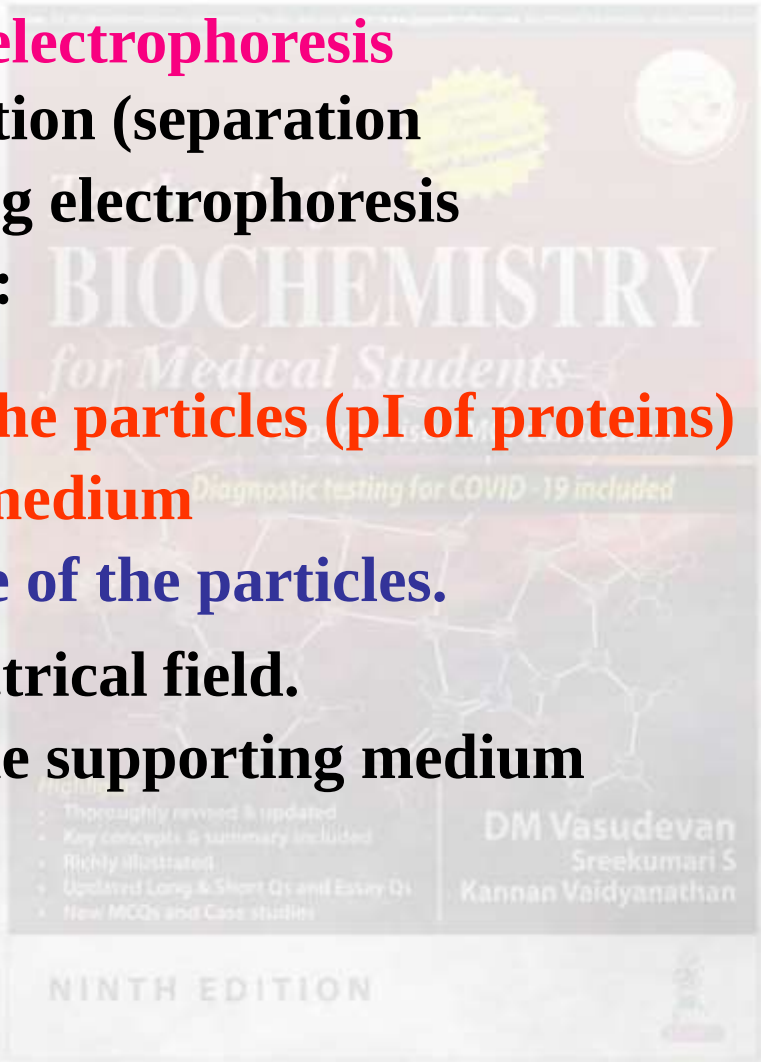
Proteins exist as charged particles.

Widely used for separation of proteins in biological fluids.

Factors affecting electrophoresis

The rate of migration (separation of particles) during electrophoresis will depend upon :

1. **Net charge on the particles (pI of proteins)**
2. **The pH of the medium**
3. **Mass and shape of the particles.**
4. **Strength of electrical field.**
5. **Properties of the supporting medium**
6. **Temperature.**



Support Medium for Electrophoresis

Filter paper : Electro-phoresis for 16–18 hours at a low voltage.

Disadvantages

Long time

Diffusion of particles leading to blurring of margins are the of paper.

Cellulose acetate membrane strips.

Only one hour

Separation without diffusion.

identification of lipoproteins, isoenzymes and haemoglobins.

Agarose gels

heterogeneous polysaccharides
viscous liquid when hot
but solidify to a gel on cooling.

The gel is prepared in the buffer and spread over slides and allowed to cool.
A small sample (few microlitres) of serum or biological fluid is applied.
Electrophoretic run takes 90 minutes.

Serum proteins

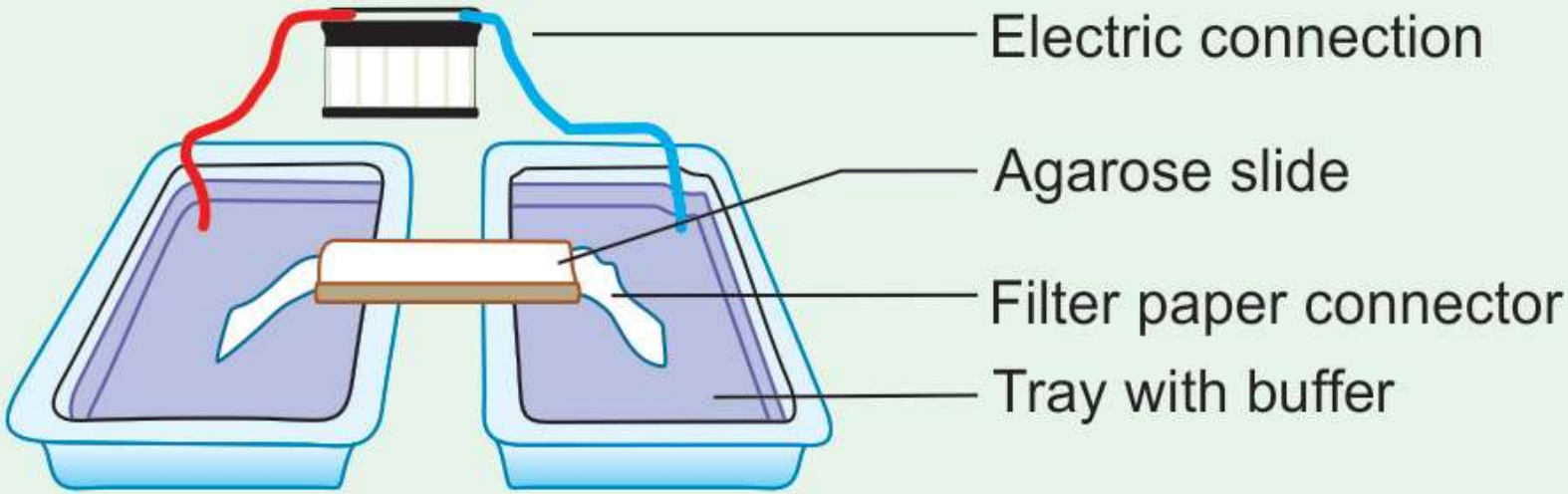
protein mixtures
nucleic acids.

Polyacrylamide gel electrophoresis (PAGE)

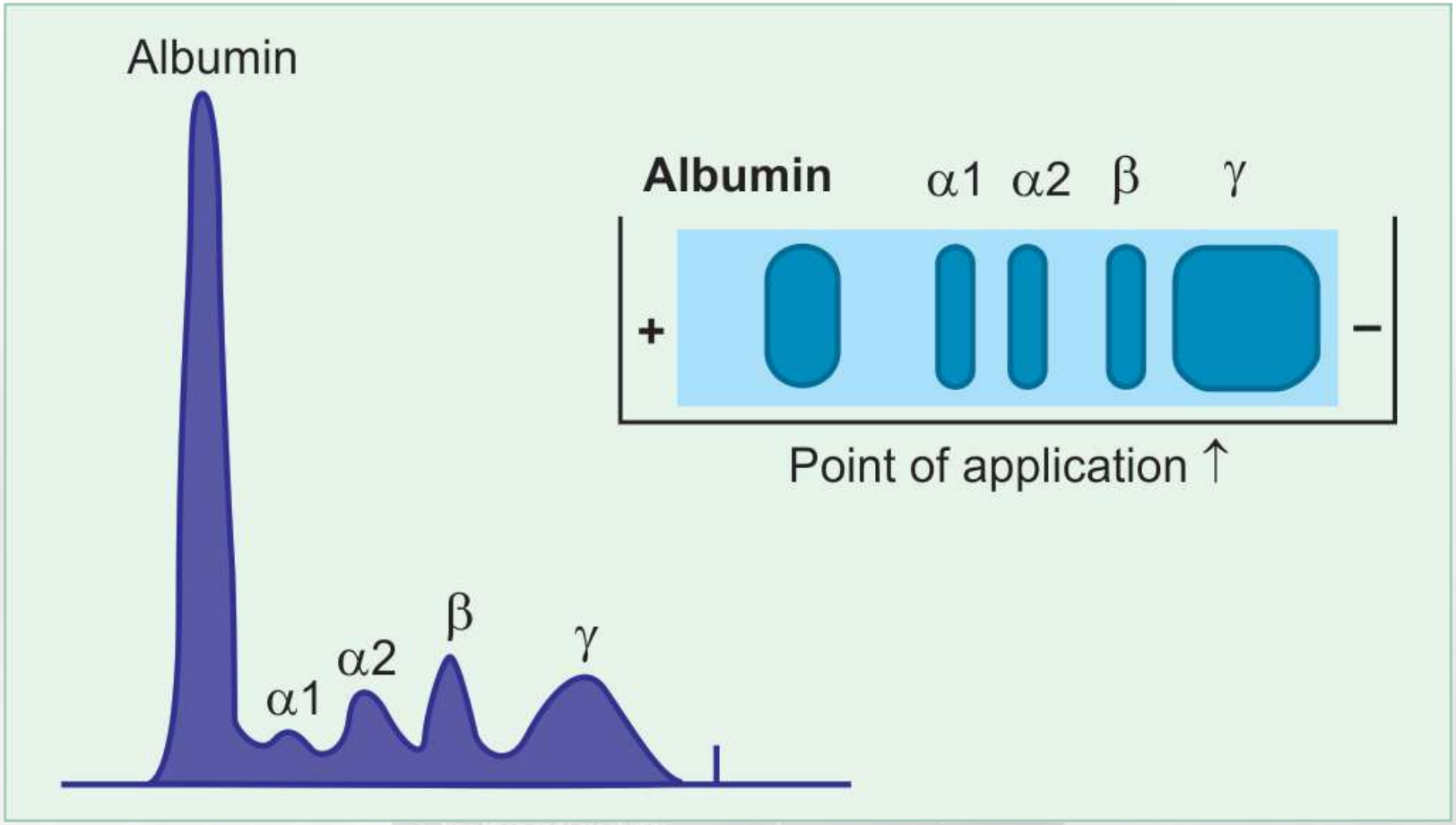
Polymerisation of acrylamide;
cross linkage, Microscopic mesh work

Electrical pull;
Molecular sieving effect;
Separation is very efficient.

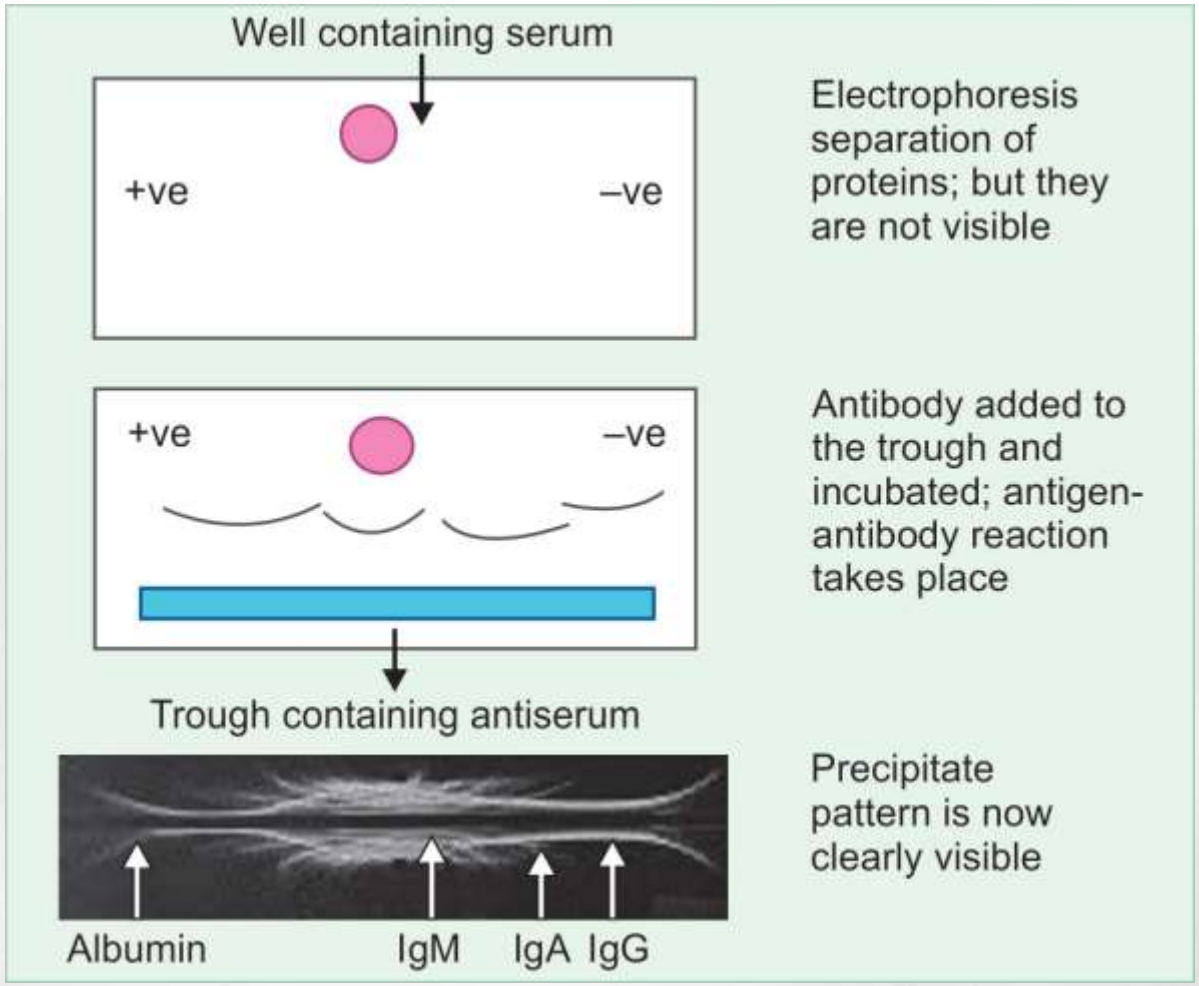
In agar gel electrophoresis,
serum components 5 fractions;
in PAGE, serum will show
more than 20 different bands.



Electrophoresis apparatus



Electrophoresis of normal serum sample.



Immunoelectrophoresis pattern.

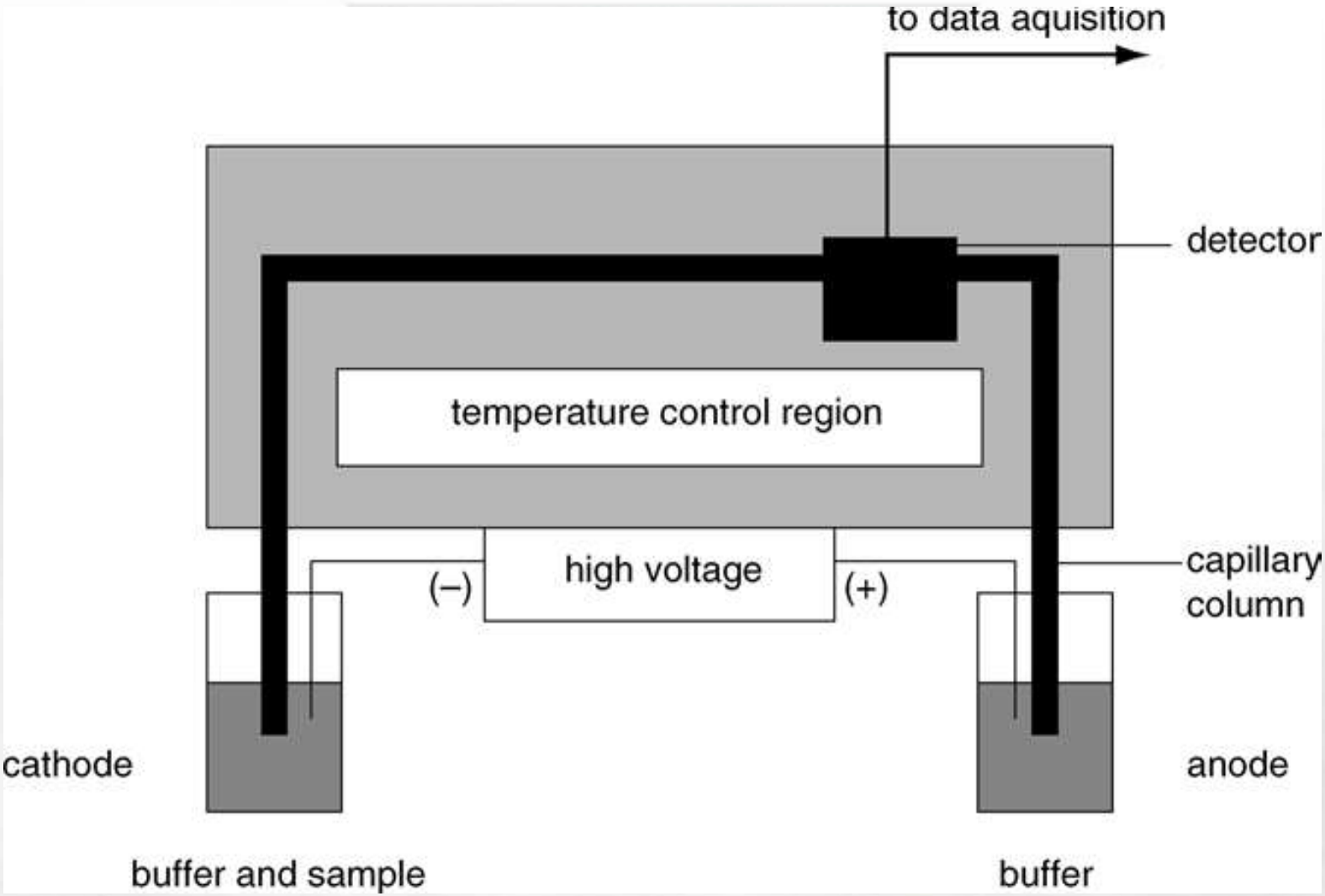
High Voltage Electrophoresis

Since, the separation of molecules depends on the strength of the current, higher voltages (400–2,000 volts) are used. This is called high voltage electrophoresis (HVE). The advantage is that the result could be obtained within half an hour.

Capillary Electrophoresis

Here the gel is taken in a capillary tube of small bore (50–100 microns) with 100–200 cm in length. Nanoliter range of sample is injected into the tube. A high voltage of 25,000 volt is applied. Within a few minutes, components are separated. Amino acids, proteins, drugs, vitamins, carbohydrates, and nucleotides could be separated by this method.

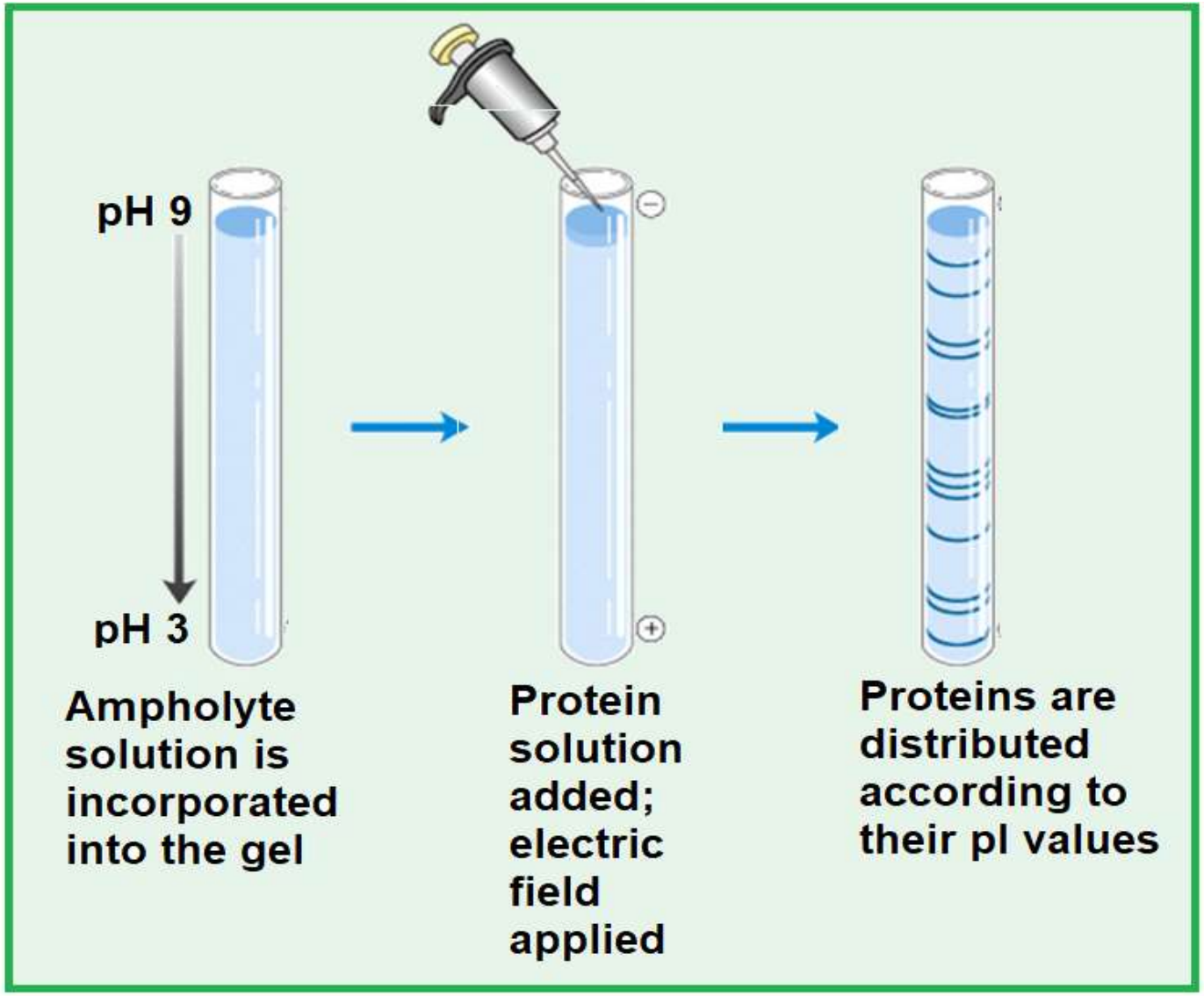
Capillary electrophoresis



Advantage of Capillary electrophoresis

- **Separation time needed is only a few minutes.**
- **The quantity of sample required for the separation is in the nanogram range.**

Isoelectric focussing



CHROMATOGRAPHY

ADSORPTION

PARTITION

PAPER

THIN LAYER

GAS LIQUID

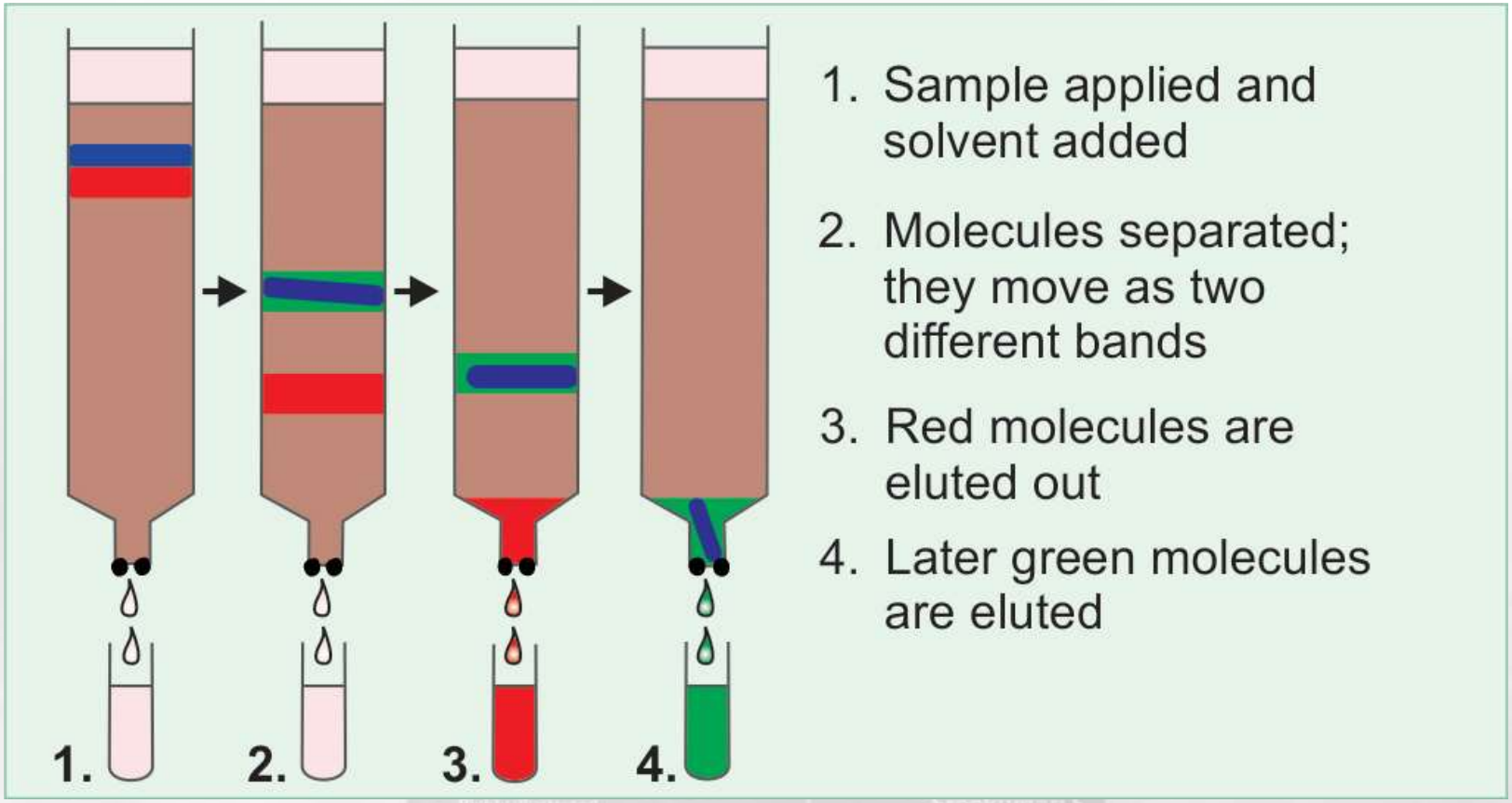
ION-EXCHANGE

GEL FILTRATION

AFFINITY

Adsorption Chromatography

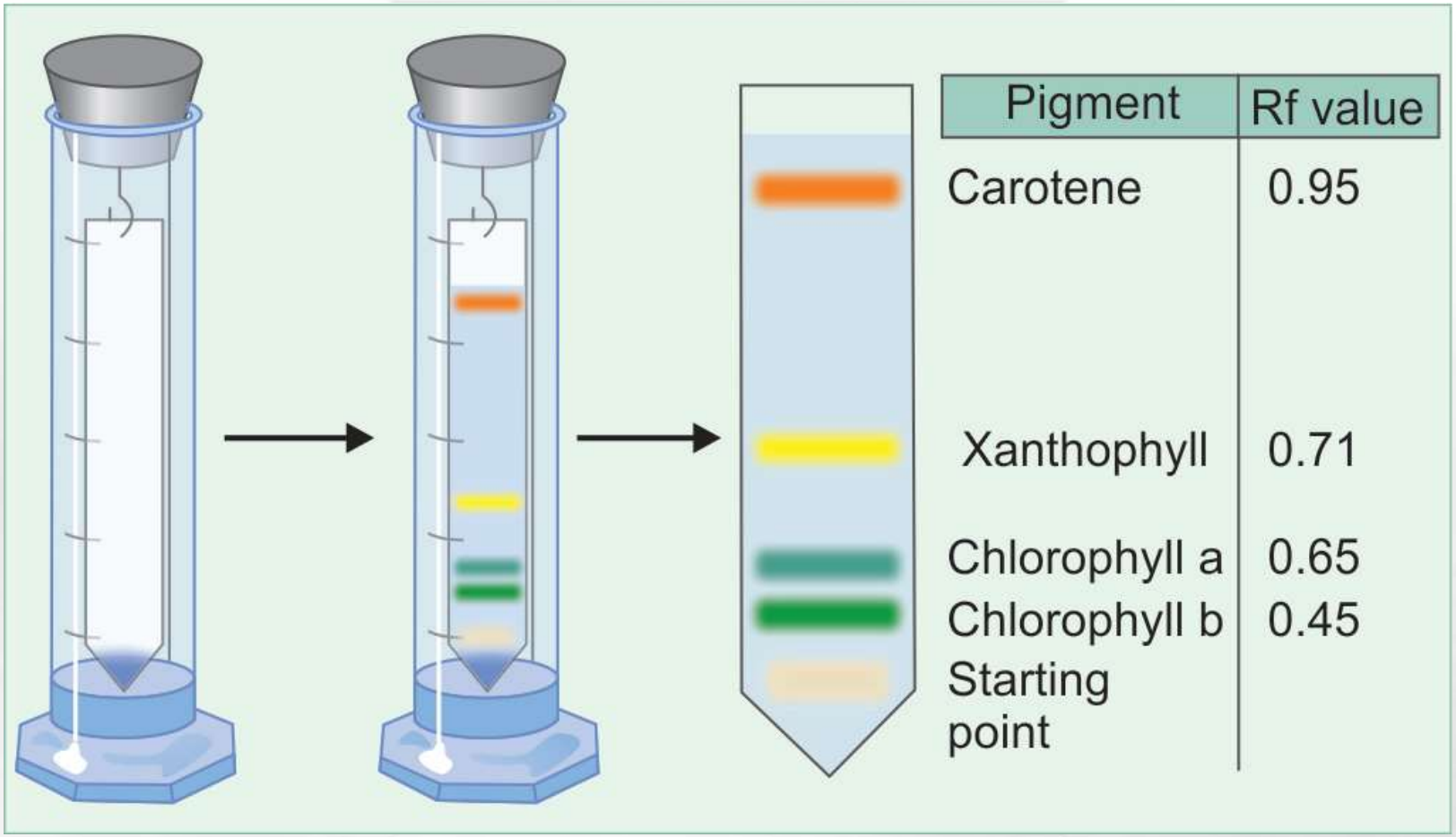
The separation is based on differences in adsorption at the surface of a solid stationary medium. The common adsorbing substances used are alumina, or silica gel. These are packed into columns and the mixture of proteins to be separated is applied in a solvent on the top of the column. The components get adsorbed on the column of adsorbent with different affinity. The fractions slowly move down; the most weakly held fraction moves fastest; followed by others, according to the order of tightness in adsorption. The eluent from the column is collected as small equal fractions and the concentration of each is measured.



Adsorption chromatography.

Paper Chromatography

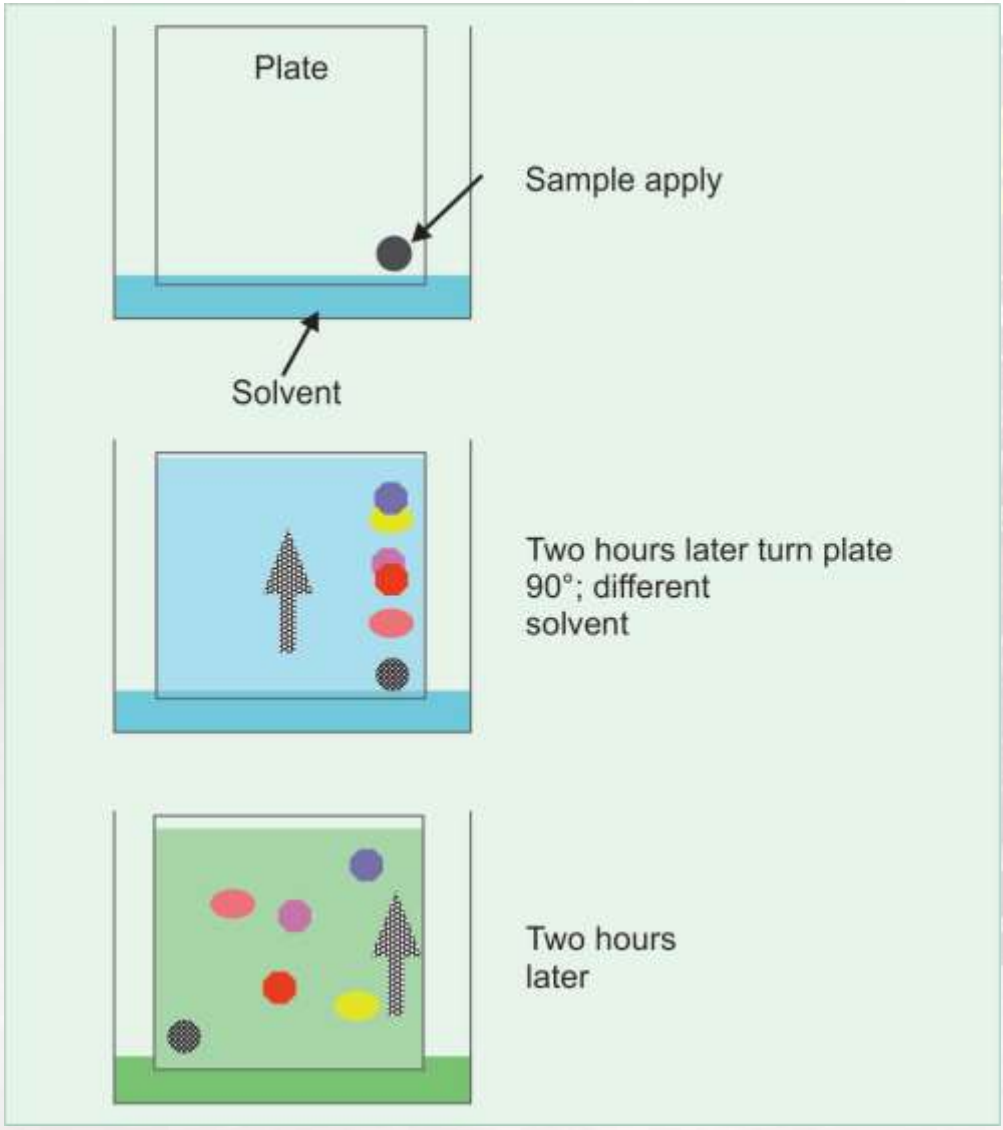
The **stationary phase** is water held on a solid support of filter paper (cellulose). The **mobile phase** is a mixture of immiscible solvents which are mixtures of water, a nonpolar solvent and an acid or base, e.g. butanol-acetic acid-water or phenol-water-ammonia. Either ascending or descending type of chromatography can be done with the mobile phase being applied from the bottom (**ascending**) or at the top (**descending**). In ascending chromatography, the paper is placed in a glass trough containing the solvent which ascends up the solid support medium. The components of the mixture to be separated are carried up with the solvent.



Paper chromatography.

Thin Layer Chromatography

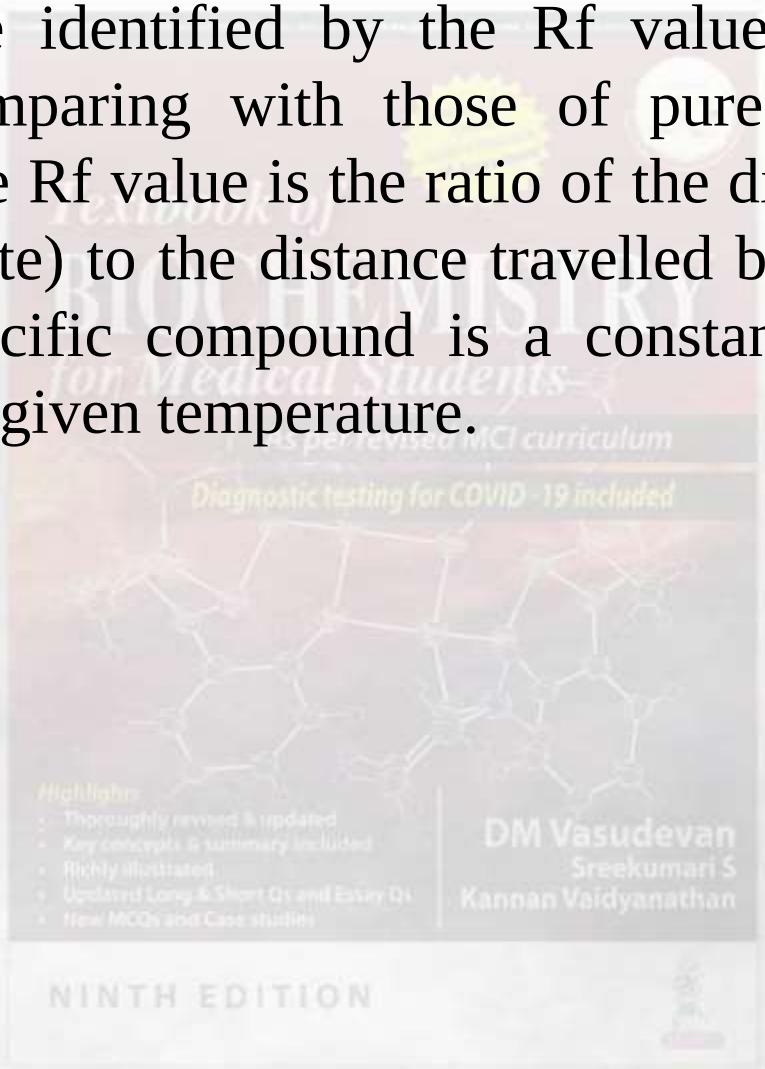
This is another version of liquid–liquid chromato-graphy. A thin layer of silica gel (Kieselguhr) is spread on a glass plate; biological sample is applied as a small spot; the plate is placed in a trough containing the solvent. The stationary water phase is held on the silica gel and mobile phase of nonpolar solvent moves up. In the case of paper chromato-graphy, it takes 14–16 hours for separation of components to be separated. But in the case of thin layer chromatography (TLC), it takes only 2–4 hours. That is a distinct advantage for TLC. TLC can be performed as two dimensional also.

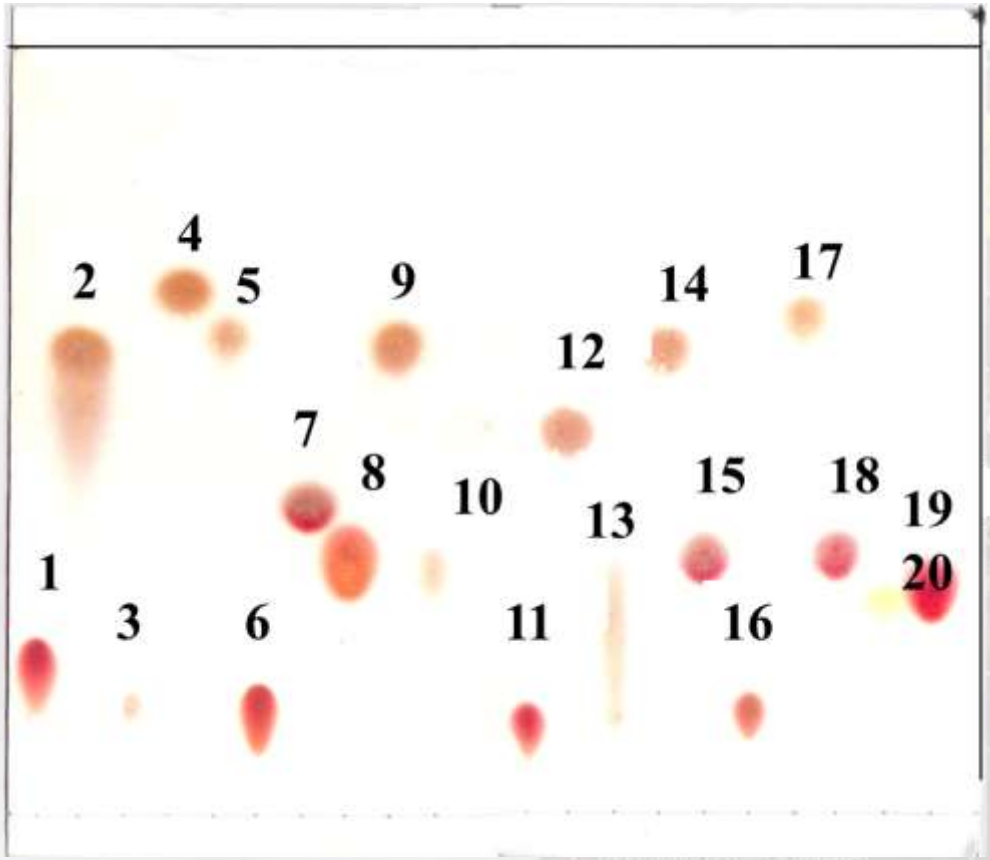


Thin layer chromatography (two-dimensional)

The Rf Value

The spots may be identified by the Rf value of the unknown substance and comparing with those of pure standards (Rf = ratio of fronts). The Rf value is the ratio of the distance traveled by the substance (solute) to the distance travelled by the solvent. The Rf value of a specific compound is a constant for a particular solvent system at a given temperature.





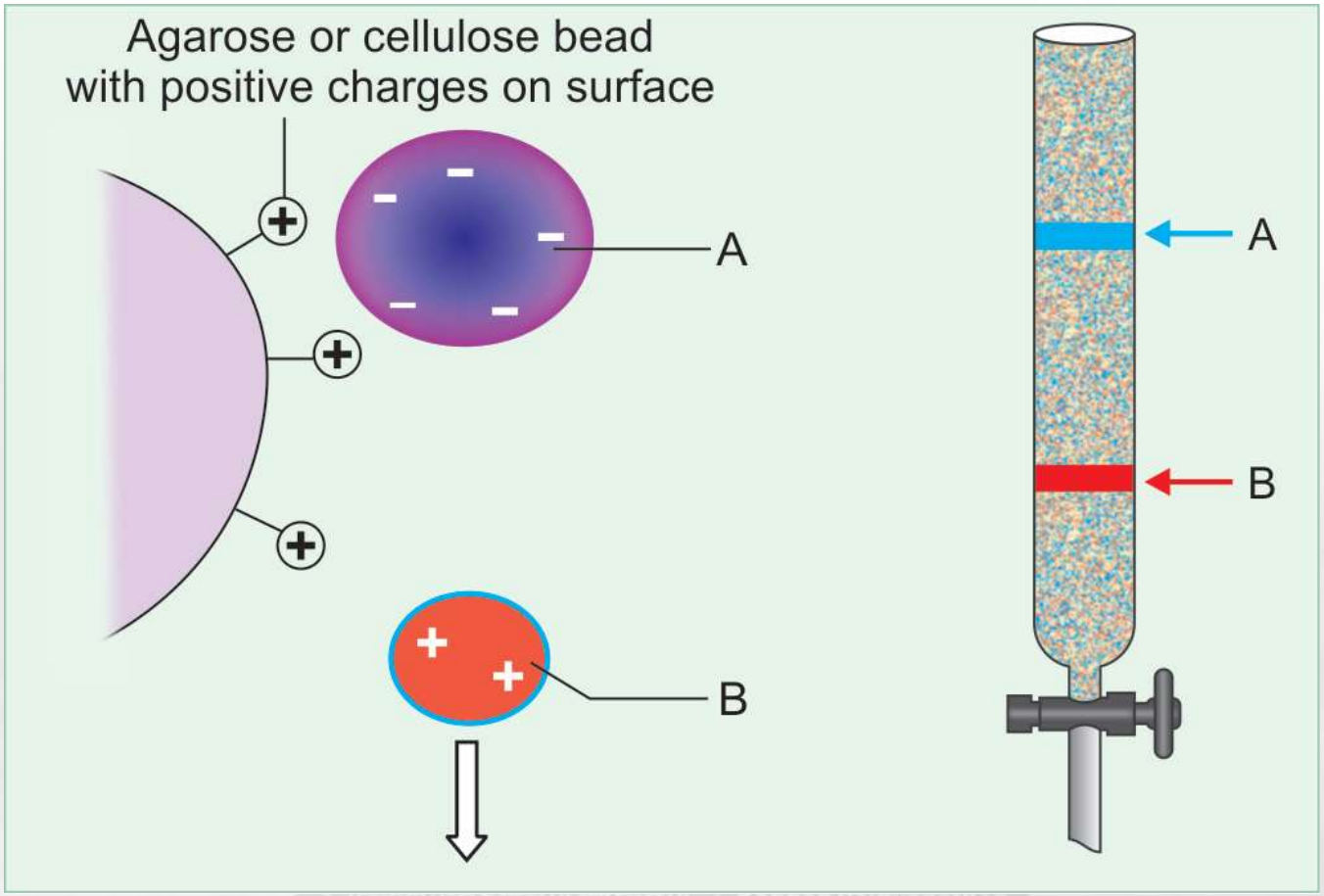
1. Arginine
2. Methionine
3. Cystine
4. Leucine
5. Tyrosine
6. Lysine
7. Alanine
8. Glycine

Thin layer chromatography (TLC) separation of amino acids on silica gel. **Solvent medium: Butanol / Acetic acid / Water; 8/2/2 (v/v), Location reagent: Ninhydrin**

9. Phenylalanine
10. Aspartic acid
11.Ornithine
12.Valine
13.Cysteine
14.Isoleucine
15.Threonine
16.Histidine
17. Tryptophan
18.Glutamic acid
19.Proline
20.Serine

Ion Exchange Chromatography

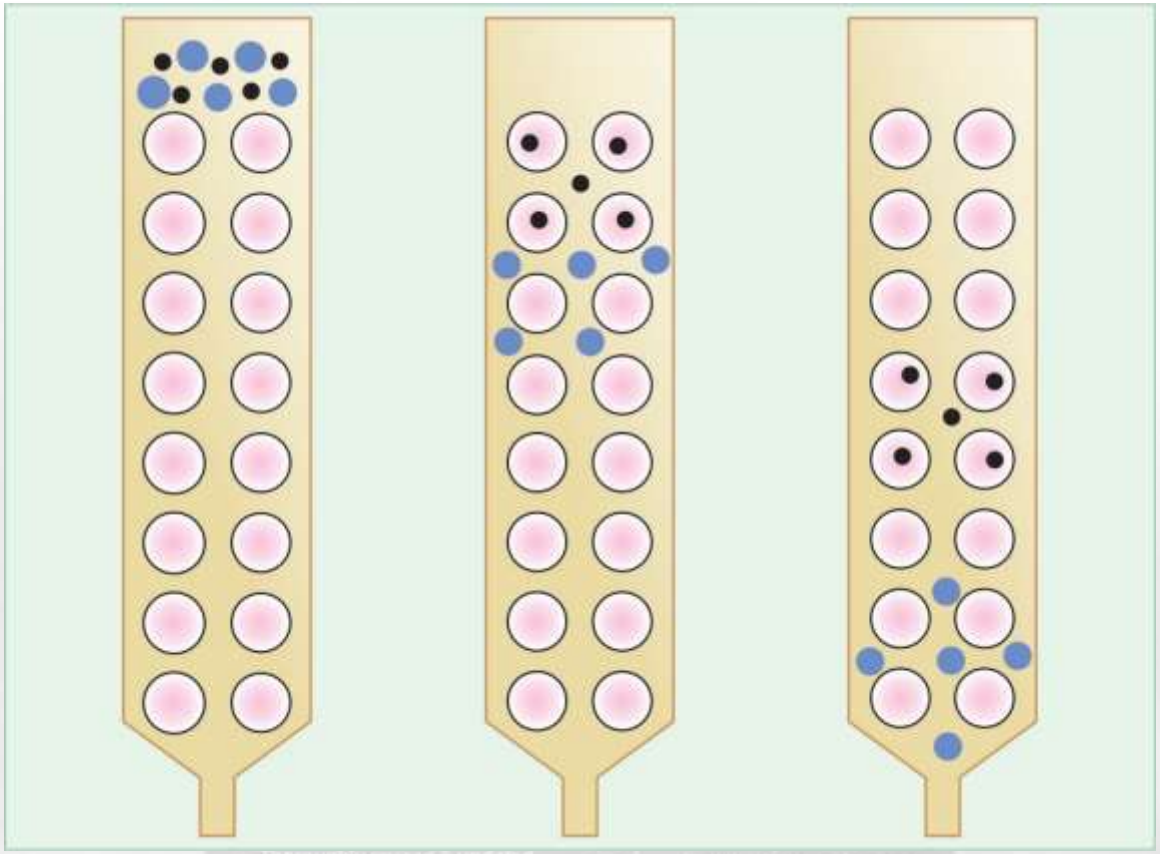
In this method, the separation is based on **electrostatic attraction** between charged biological molecules to oppositely charged groups on the ion exchange resins. The resins are either cation exchange resins or anion exchange resins. The separation is based on the ionic character of proteins and amino acids (isoelectric point). The **cation exchange** particles carry acid groups, e.g. COO–.Na+. When cations (C+) are passed through the column, Na+ in the resin is replaced by C+; thus C+ particles are adhered in the column, while negatively charged particles are eluted out easily.



Ion-exchange chromatography. A = Negatively charged molecules attach with the beads and so move slowly. B = Positively charged molecules repel with the beads, so move faster in the column.

Gel Filtration (Size Exclusion) Chromatography

It is also called molecular sieving. Hydrophilic cross-linked gels like acrylamide (Sephacryl), agarose (Sepharose), and dextran (Sephadex) are used for separation of molecules based on their size. The gel is packed in a column. The gel particles are porous in nature. These pores will allow small molecules enter into the gel. But larger molecules could not enter into pores of the gel and so are excluded. Suppose a mixture of insulin plus immunoglobulin is passed through the column. The small molecule (insulin) has to travel a long distance inside the gels. Thus, small molecules are held back. But the large immunoglobulin molecules cannot enter the pores; so they move in the column rapidly. In short, **larger molecules will come out first, while smaller molecules are retained in the column**.

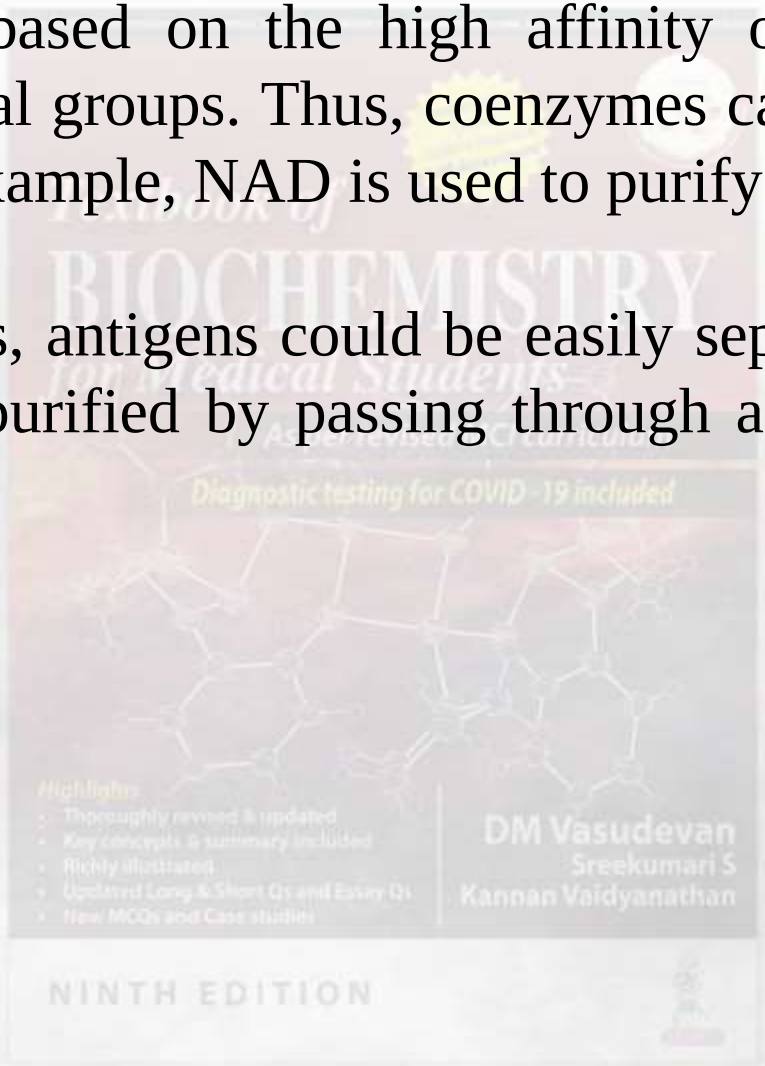


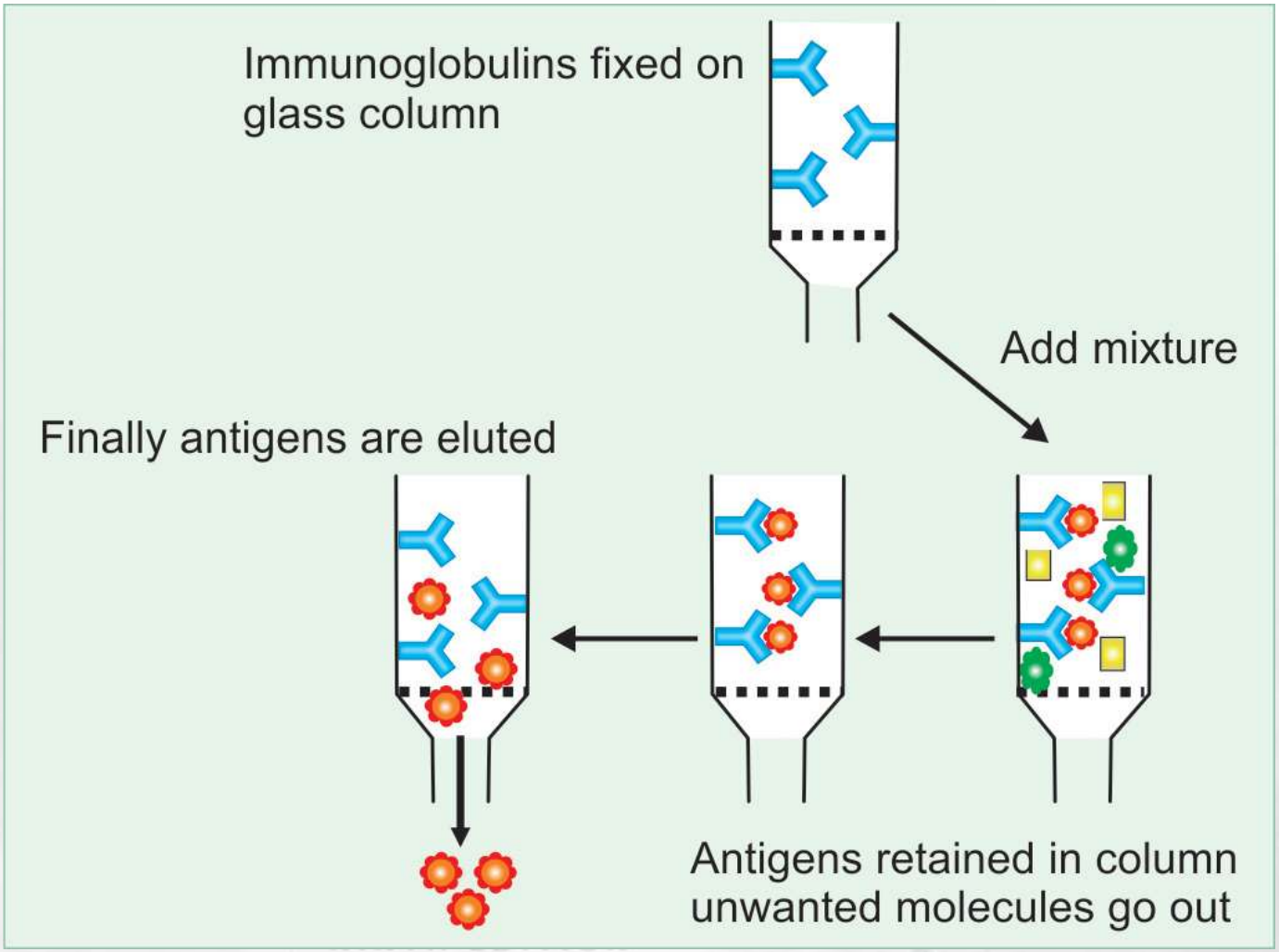
Sephadex (gel filtration) chromatography. (A) Protein solution is added on the top of the column. (B) Small proteins get inside the beads, and so takes a longer time to reach the bottom. (C) Larger molecules cannot enter into the beads, so travels quickly, and reaches the bottom faster.

Affinity Chromatography

The technique is based on the high affinity of specific proteins for specific chemical groups. Thus, coenzymes can be used to purify the enzymes. For example, NAD is used to purify dehydrogenases.

By using antibodies, antigens could be easily separated. Conversely, antibodies can be purified by passing through a column containing the antigen.



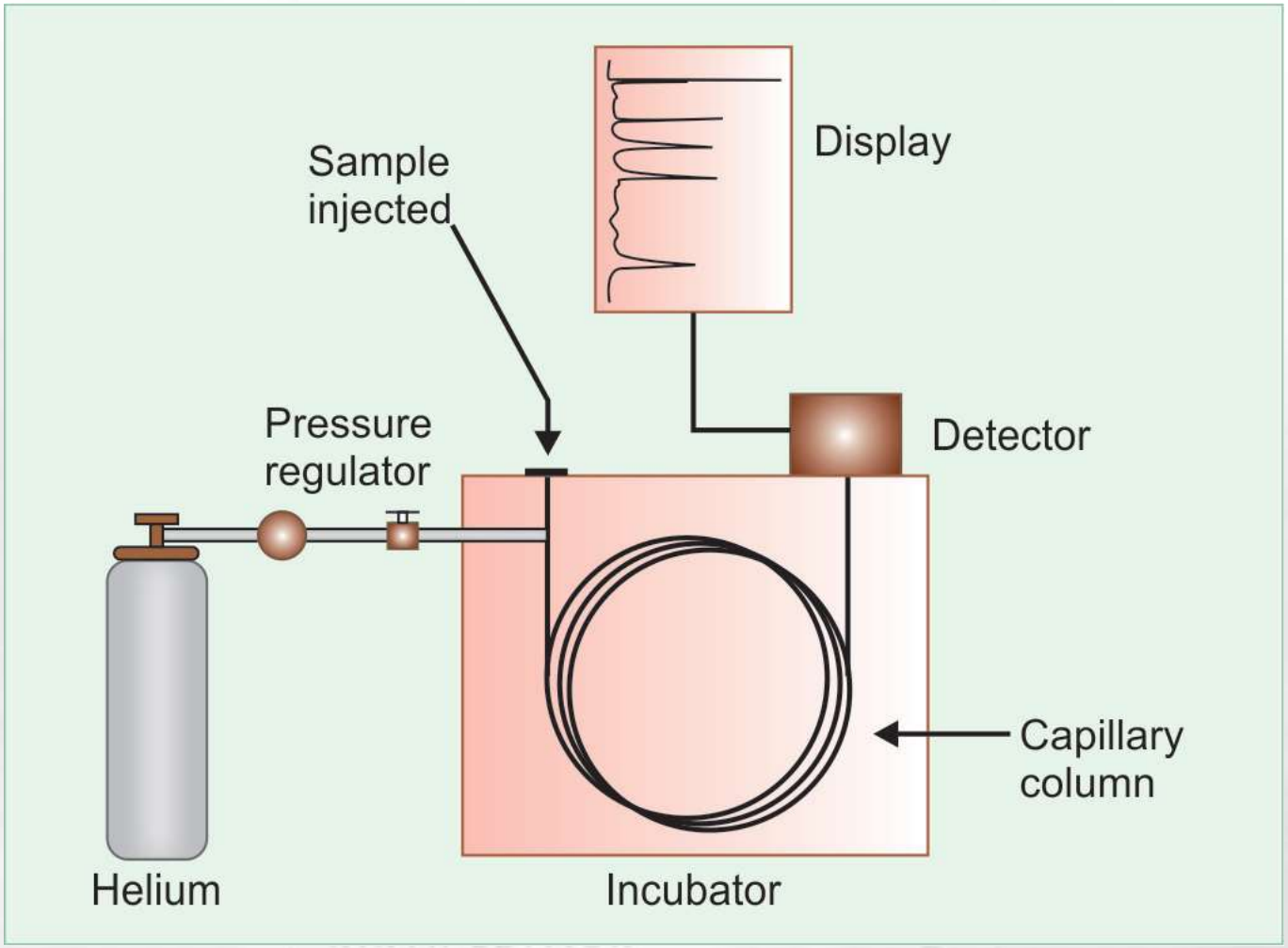


Affinity chromatography.

Gas–Liquid Chromatography

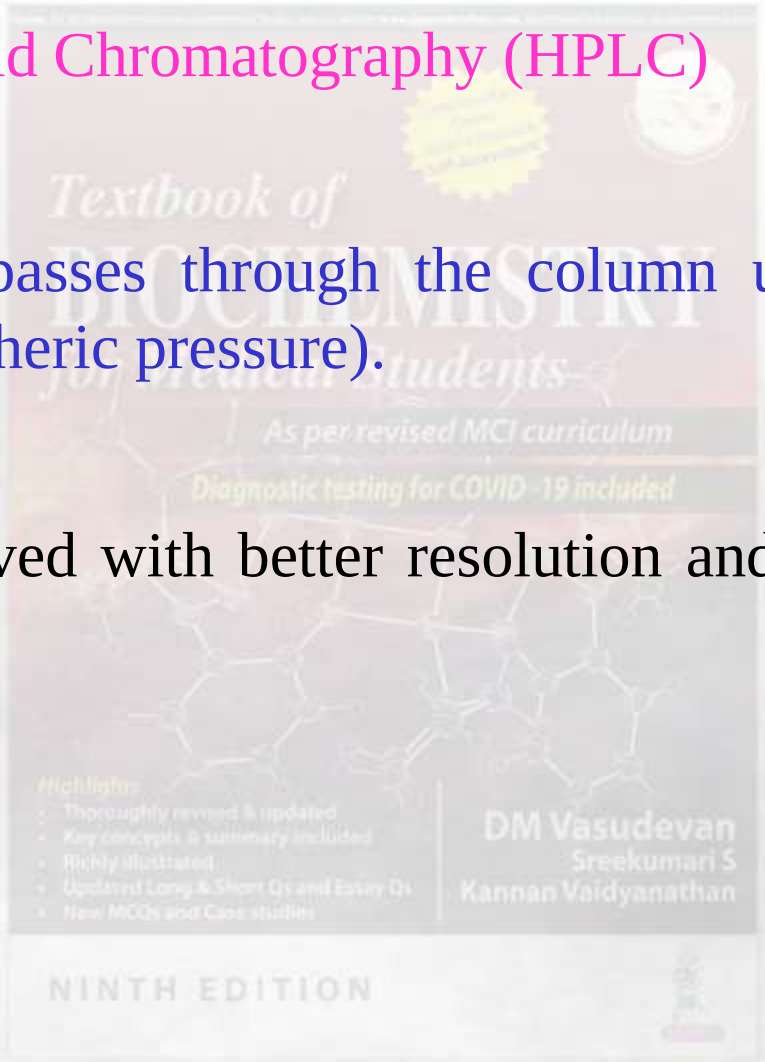
GLC is another type of partition chromatography where the stationary phase is a liquid and the mobile phase is gas. The stationary liquid phase is supported by a column of inert material such as silica in a long narrow column. The mixture of substances to be separated is made volatile at one end of the column and the vapors are swept over the column by an inert carrier gas like argon or nitrogen. The fractions emerging from the column are detected and quantitated by a detecting device.

This is more suitable for compounds (e.g. lipids) which resist degradation at high temperature.



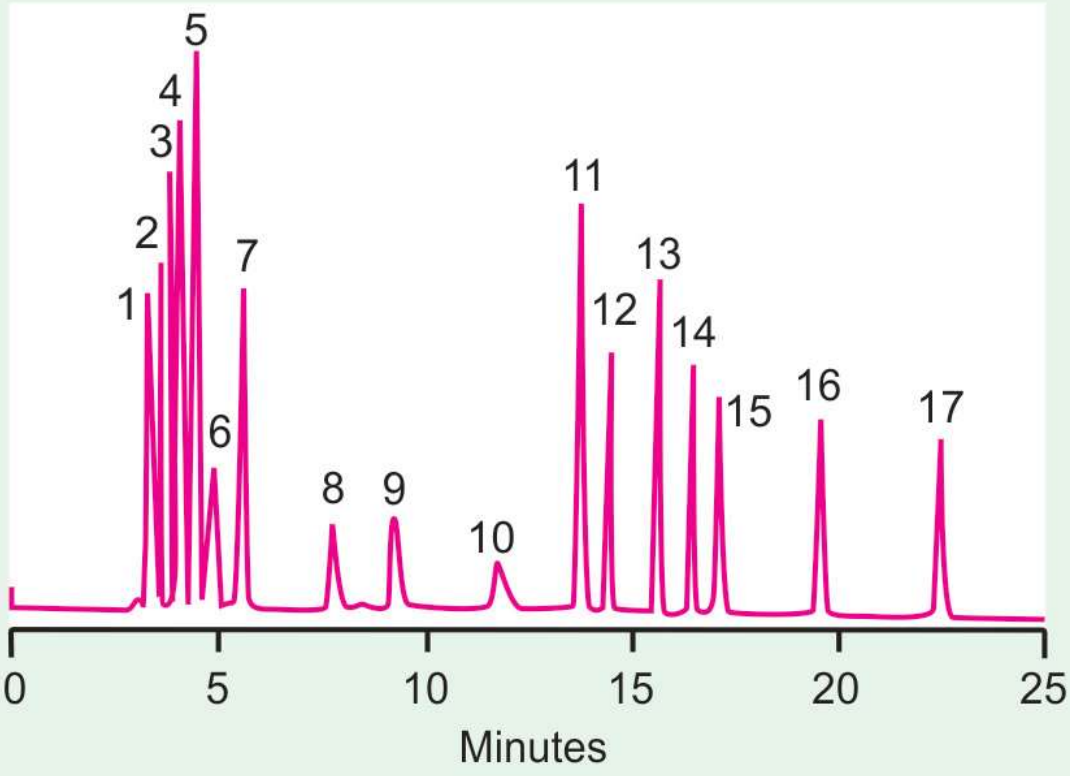
Gas–liquid chromatography.

High Pressure Liquid Chromatography (HPLC)



The liquid phase passes through the column under high pressure (1000 times atmospheric pressure).

Separation is achieved with better resolution and high speed (within minutes)



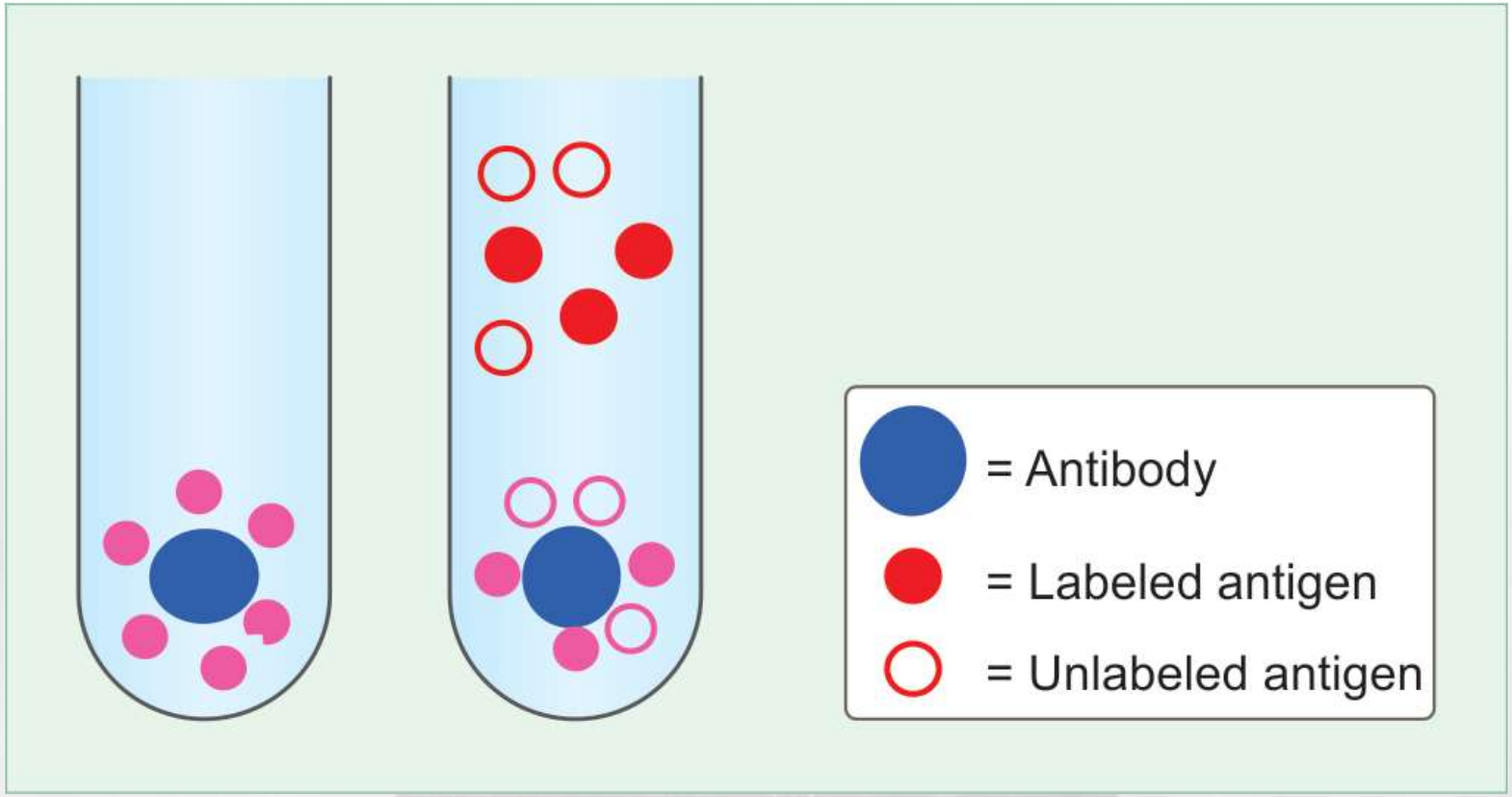
Amino acid profile in high pressure liquid chromatography. 1 = Gly; 2 = Ser/Asn; 3 = Asp; 4 = Gln; 5 = Ala/Thr; 6 = Glu; 7 = Cys/Lys; 8 = His; 9 = Pro; 10 = Arg; 11 = Val; 12 = Met; 13 = Tyr; 14; Ile; 15 = Leu; 16 = Phe; 17 = Trp.

Radioimmunoassay (RIA)

- can analyse picogram levels of drugs, steroids, polypeptide hormones

  Advantages: sensitivity, specificity, simplicity of operation

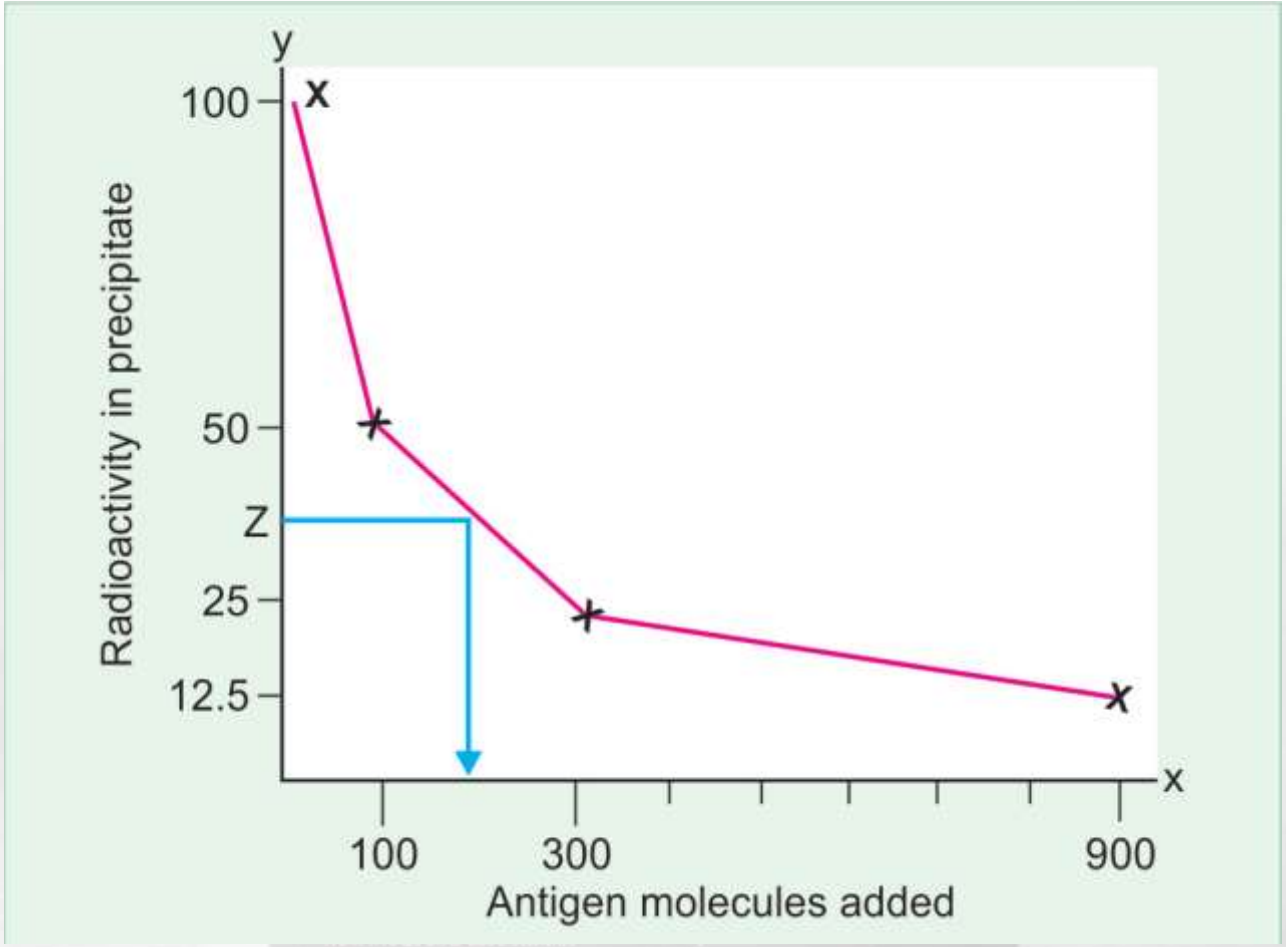
- Disadvantages:
  - cross-reactions with other molecules similar to analyte
  - Shelf life very low
  - Radioactivity
- Because of these reasons, the RIA is rarely done in general clinical laboratory practice.



Principle of radioimmunoassay.

Radioimmuno assay

Tube No.	No. of molecules of anti-body	No. of molecules of labeled hormone	No. of molecules of unlabelled hormone added	Label-led molecules in super-natant activity	Radio-activity in precipitate
1	2	3	4	5	**6**
1	100	100	0	0	**100**
2	100	100	100	50	**50**
3	100	100	300	75	**25**
4	100	100	900	90	**10**
5	**100**	**100**	**Patient's serum.**	**A**	**Z**



From this calibration curve, the value of Z is extrapolated to X-axis, when the hormone in the patient's serum is quantitated.

ELISA Test

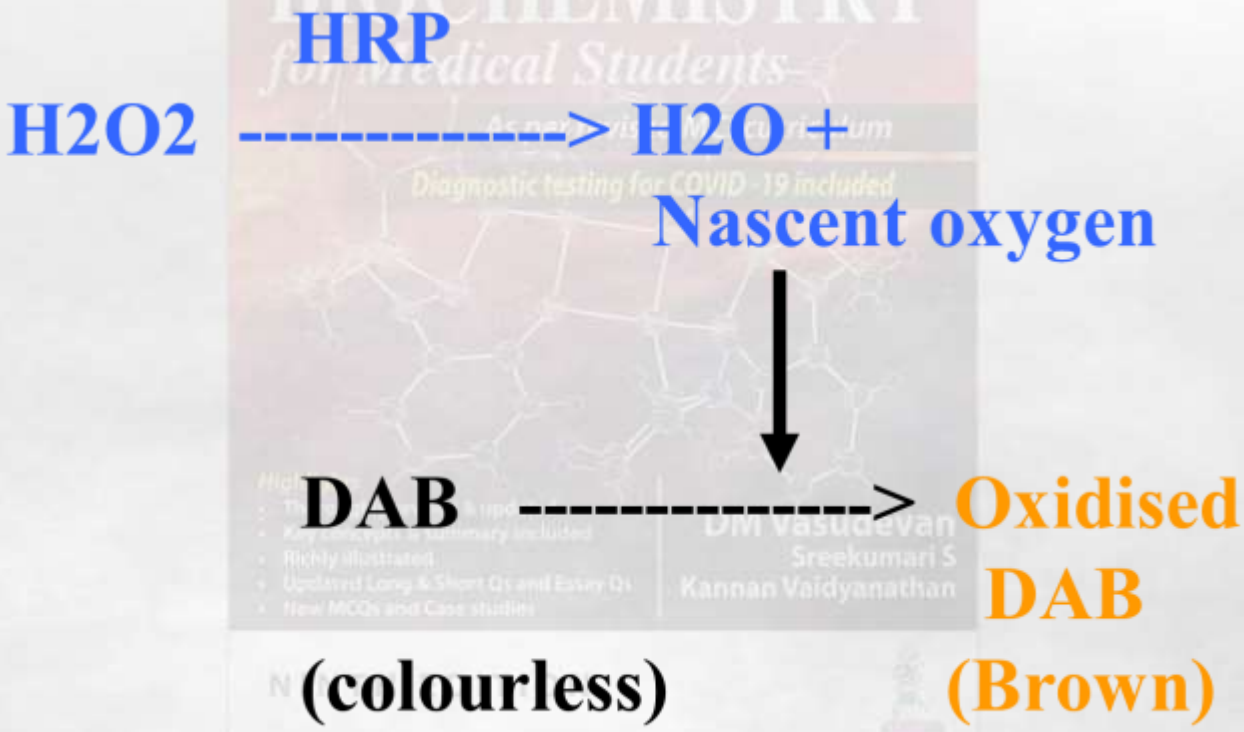
ELISA is the abbreviation for **enzyme-linked immunosorbent assay**. The **advantage is** that there is no radiation hazard. So, the test could be done in any clinical laboratory. The shelf-life is also more. Instead of the radio label, an enzyme is tagged with the antibody. The enzyme labels commonly used are alkaline phosphatase (ALP) and **horse radish peroxidase** (HRP). This test is commonly employed to detect antigens or antibodies present in very small quantities in tissues or blood, such as hormones, growth factors, tumor markers, bacteria, and virus.

ELISA Test

Colour developing reagent

Hydrogen peroxide

Diamino benzidine (DAB)



Elisa

Advantage

Shelf life more

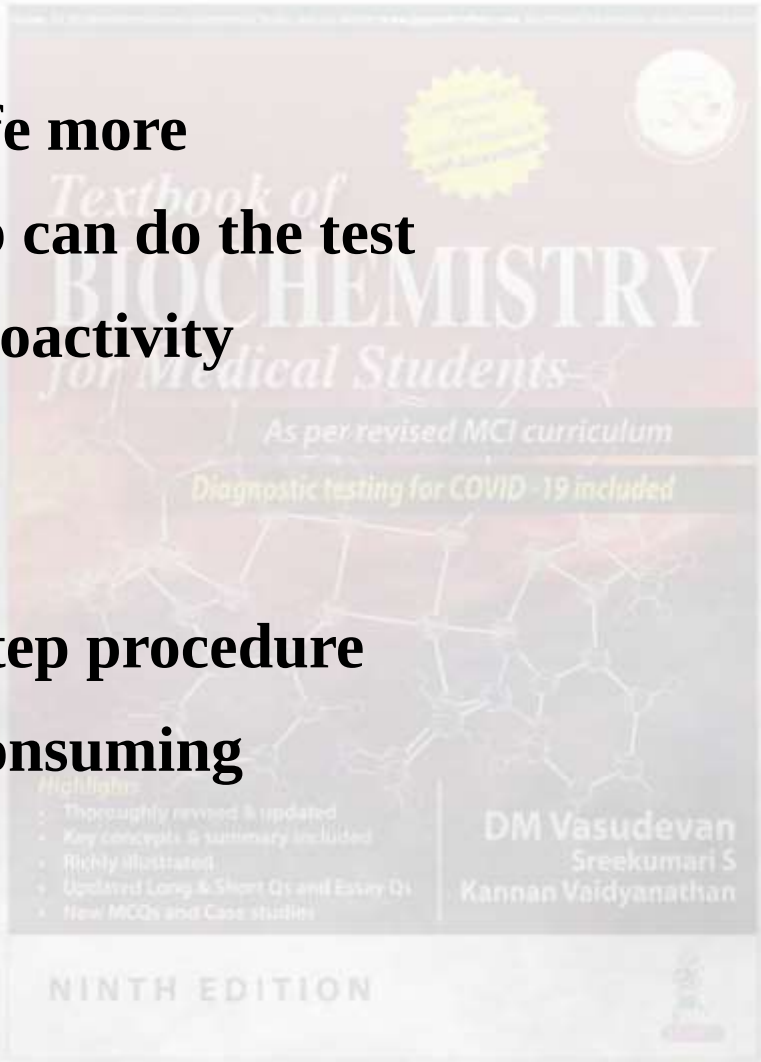
Any lab can do the test

No radioactivity

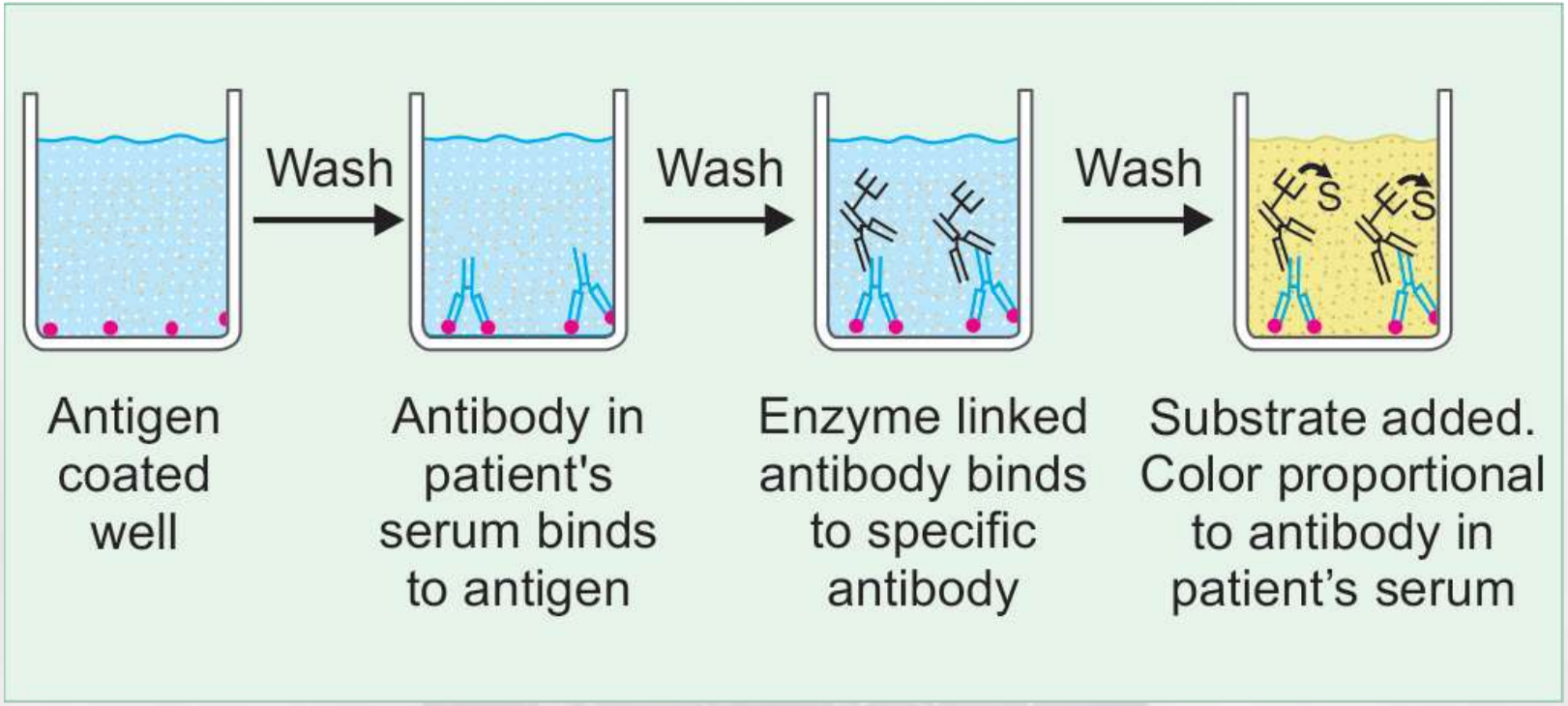
Disadvantage

Multi step procedure

Time consuming

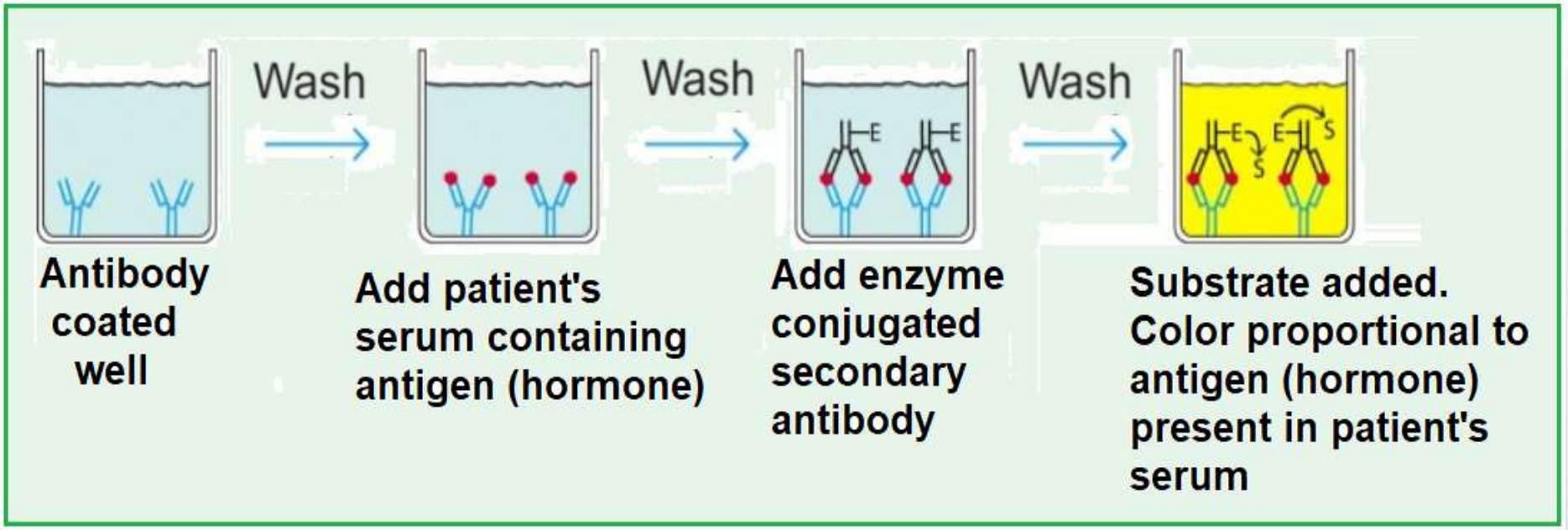


ELISA Test



Indirect enzyme-linked immunosorbent assay (ELISA) to detect antibody.

ELISA Test



Sandwich ELISA test to detect antigen (eg, hormone)

ELISA Test

Incubate antibody with specific antigen

To antigen-coated well, add Ag-Ab mixture prepared

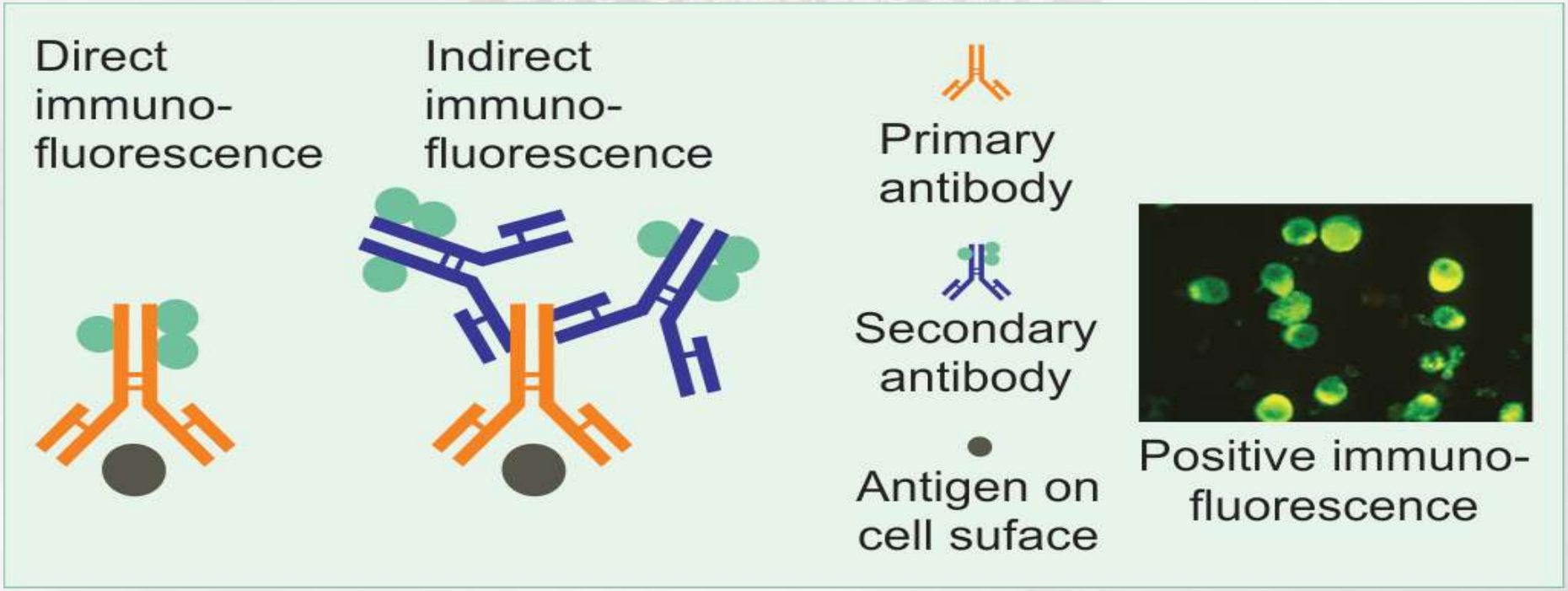
Add enzyme conjugated secondary antibody

Substrate added. Color proportional to antigen in the patient's serum

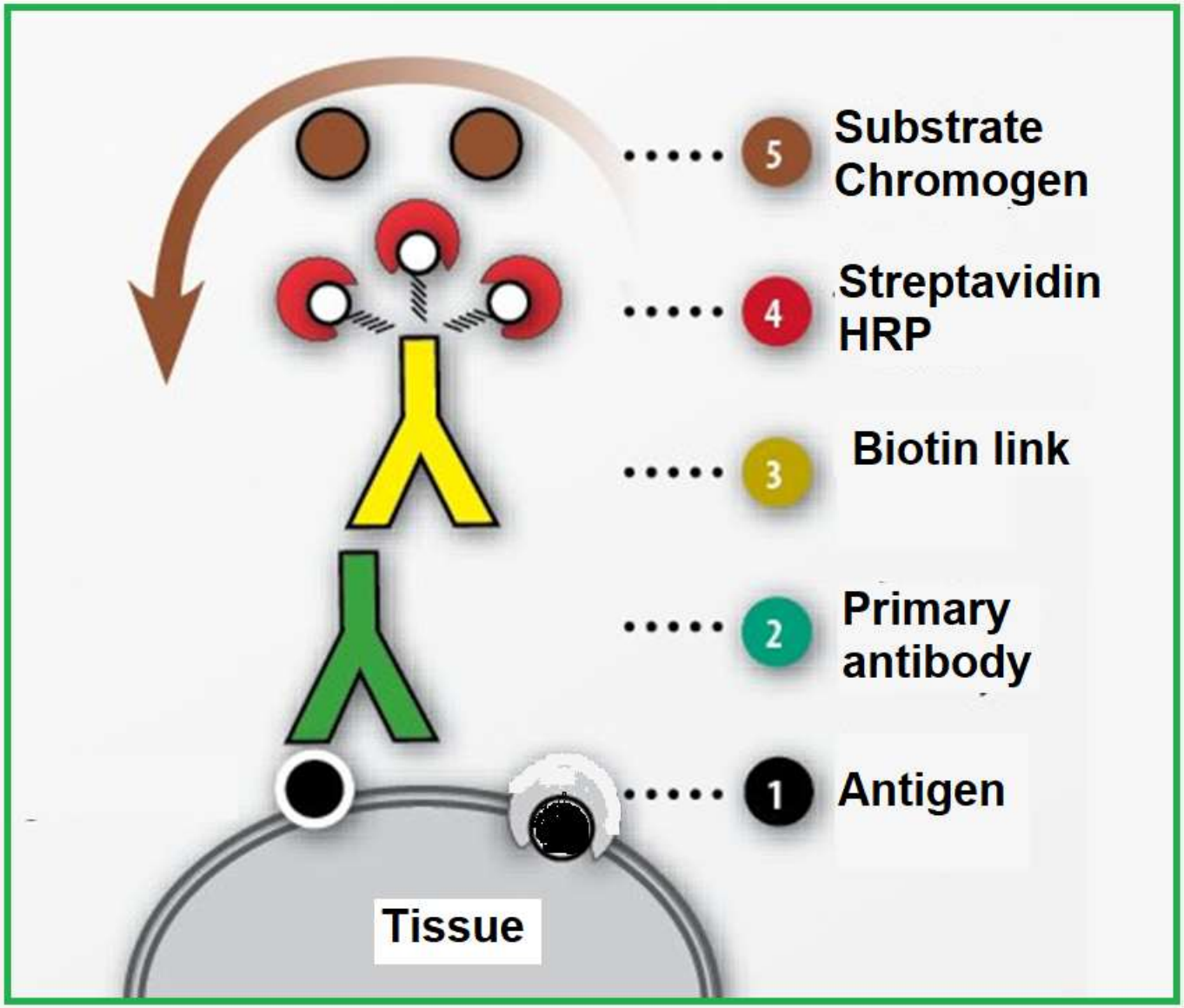
Competitive ELISA to detect antigen (eg, free testosterone)

Immunofluorescence

Antibody tagged with fluorescein isothiocyanate is incubated with the cells. Antibody fixes with cell surface antigens. Subpopulations of blood cells (e.g. helper T cells) are usually enumerated by this technique.



Biotin-Avidin Immunofluorescence



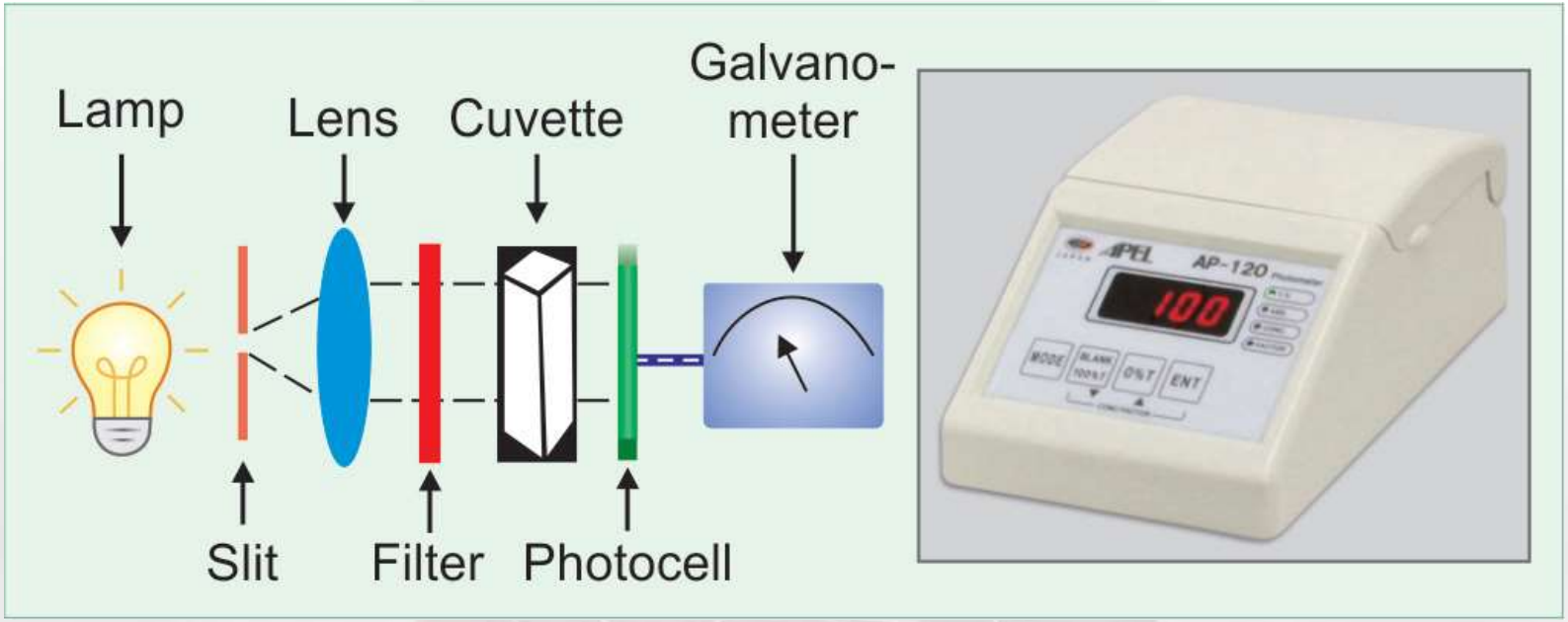
Colorimeter

Colored solutions have the property of absorbing light of definite wavelengths. The amount of light absorbed or transmitted by a colored solution is in accordance with the Beer-Lambert law.

As per the **Beer's law**, the intensity of the color is directly proportional to the concentration of the colored particles in the solution.

The **Lambert's law** states that the amount of light absorbed by a colored solution depends on the length of the column or the depth of the liquid through which light passes.

The **Beer-Lambert law** combines these two laws.



Photoelectric colorimeter. The components are shown diagrammatically in left side. The instrument is shown in the right side.

The ratio of intensity of emergent light to intensity of incident light (E / i) is termed as transmittance (T).

The absorbance is expressed as –log T.

The Optical Density is calculated as –log T.

The plot of the concentration versus transmittance is not linear, but a graph of the concentration against absorbance (OD) is linear.

Most of the clinical chemistry estimations are done by colorimetric methods.

A colored derivative of the compound to be measured is prepared and its OD is measured using a photo electric colorimeter.

This value is compared with that of a standard of known concentration.

Filter, used for selecting the monochromatic light (*mono* = single; *chrome* = color).

Filters will absorb light of unwanted wavelength and allow only monochromatic light to pass through. This light will have maximum absorbance when passed through a particular colored solution.

The color of filter should be complementary to the color of the solution.

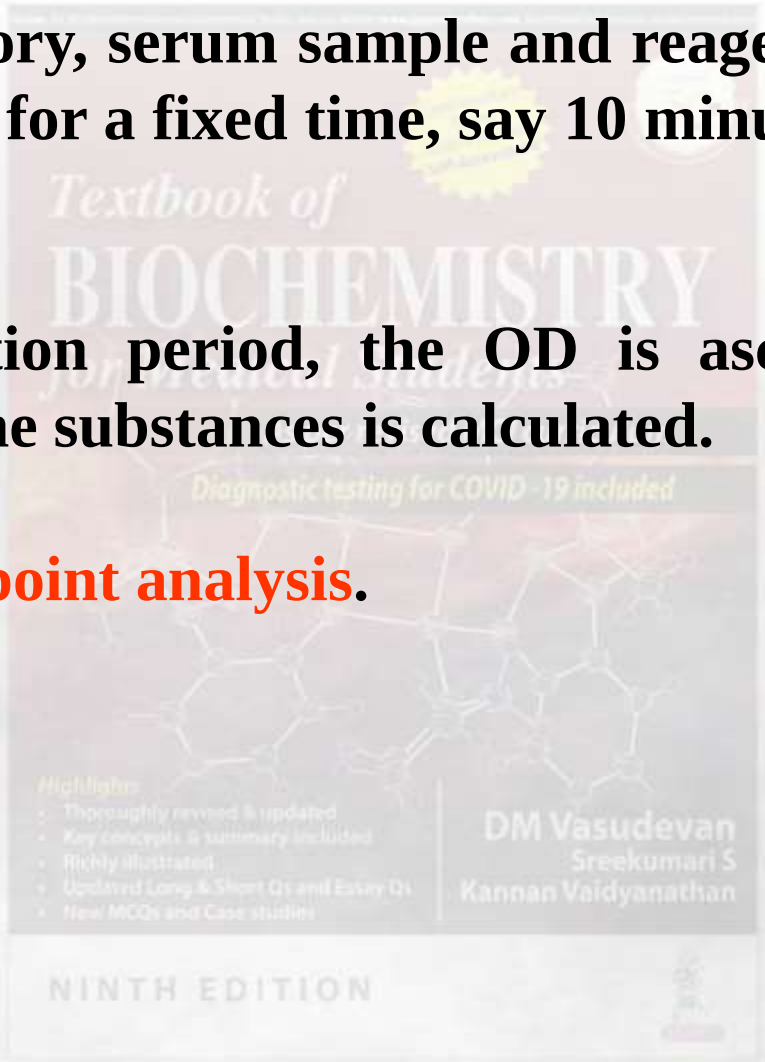
Color of filter and color of solution are complementary

Color of filter	Wavelength	Color of solution
Violet	420	Brown
Blue	470	Yellowish brown
Green	520	Pink
Yellow	580	Purple
Red	680	Green/blue

In clinical laboratory, serum sample and reagents are mixed and incubated at 37oC for a fixed time, say 10 minutes, to develop the colour optimally.

After the incubation period, the OD is ascertained and the concentration of the substances is calculated.

This is called end point analysis.



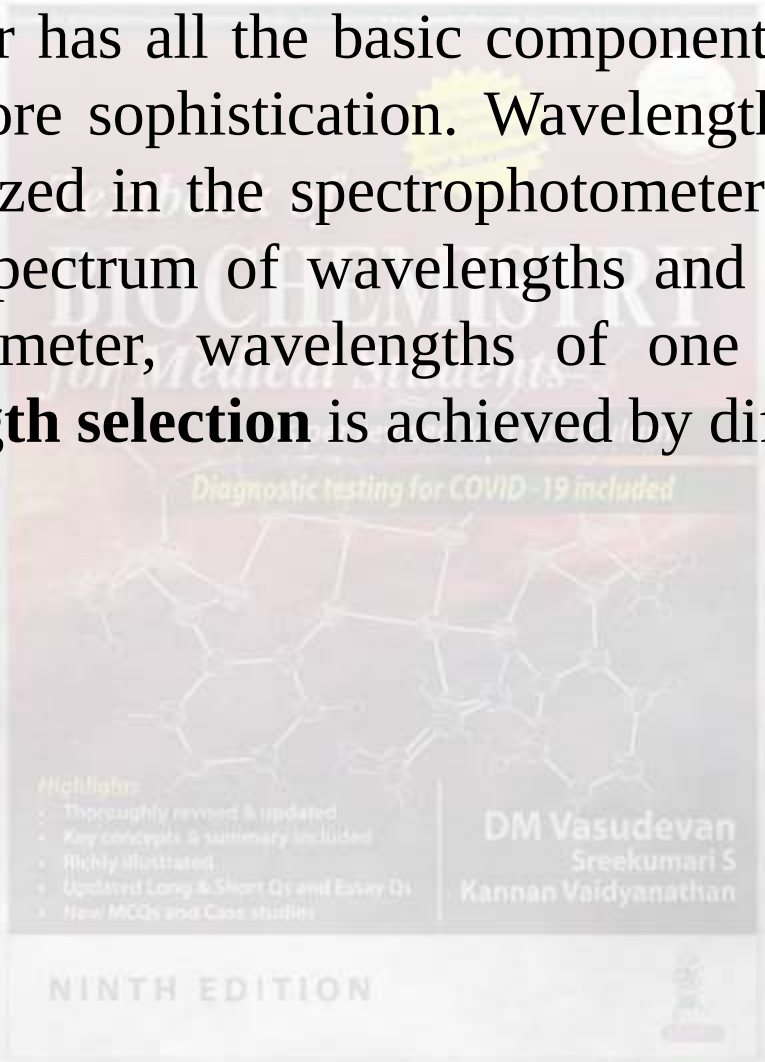
On the other hand, the serum and reagents are incubated, and readings are taken at 2 and 3 minutes exactly;

from the difference in OD between the two values, the concentration is calculated. This is the kinetic analysis.

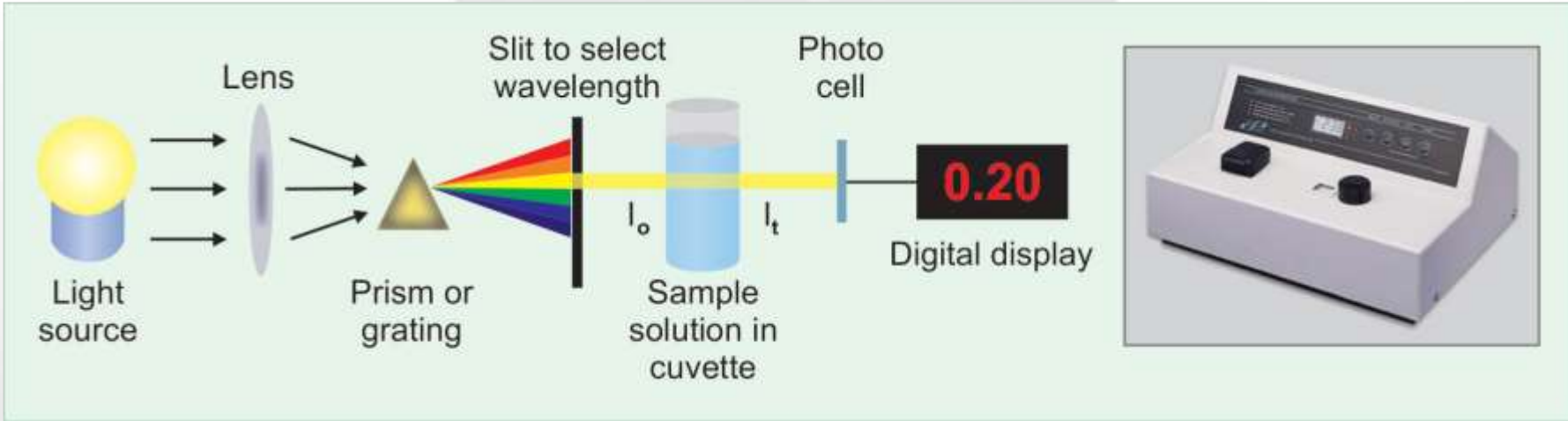
Here the optimum colour is not developed; but is quicker and hence is often used in autoanalysers.

Spectrophotometer

A spectrophotometer has all the basic components of a photoelectric colorimeter with more sophistication. Wavelengths in the ultraviolet region are also utilized in the spectrophotometer. Light is separated into a continuous spectrum of wavelengths and passed through the solution. (In colorimeter, wavelengths of one color are grouped together). **Wavelength selection** is achieved by diffraction gratings.



Spectrophotometer



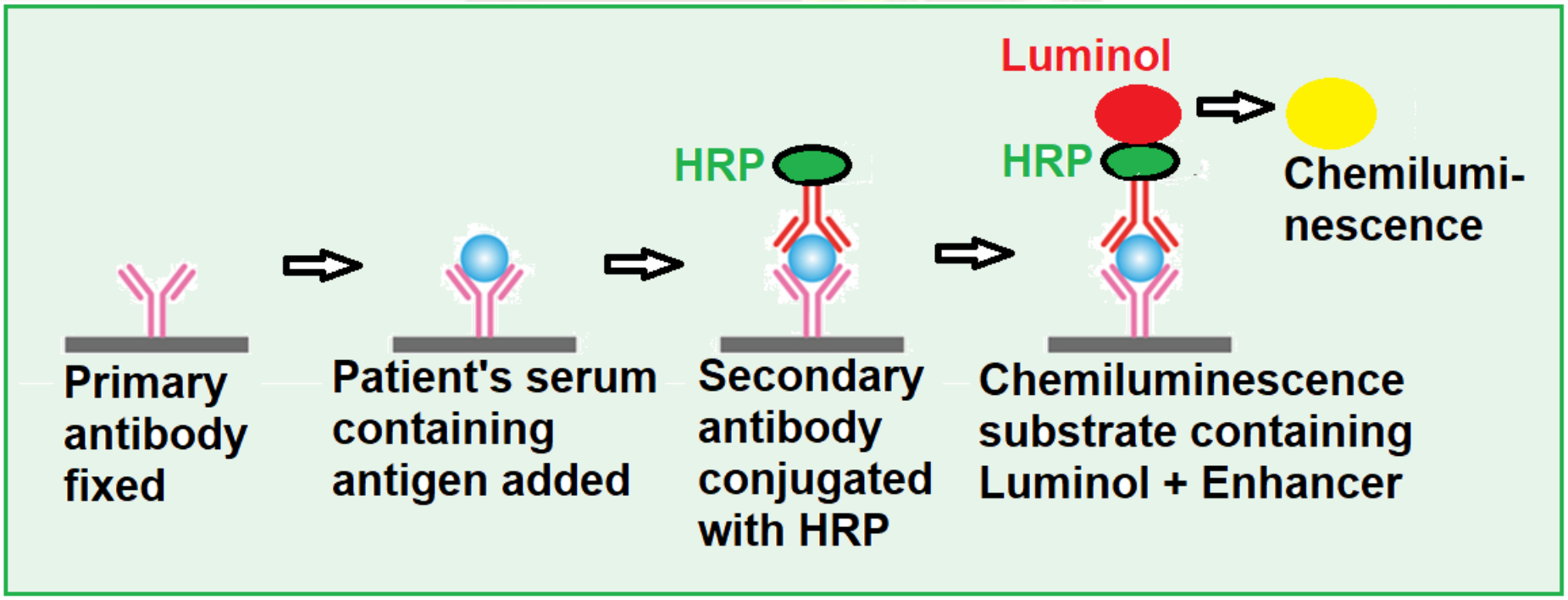
Spectrophotometer. The components are shown diagrammatically in left side. The instrument is shown in the right side. Io = incident light. It = transmitted light or emergent light.

Spectrophotometer

The **advantage** of the spectrophotometer over the colorimeter, is that the former is 1,000 times more **sensitive**. Therefore even minute quantities of the substance (very dilute solution) can be assessed in the spectrophotometer.

To take an example, protein solutions with high concentration (mg/mL) may be measured by colorimeter. If the protein concentration is only microgram/dL, then colorimeter is ineffective, where spectrophotometer can be used.

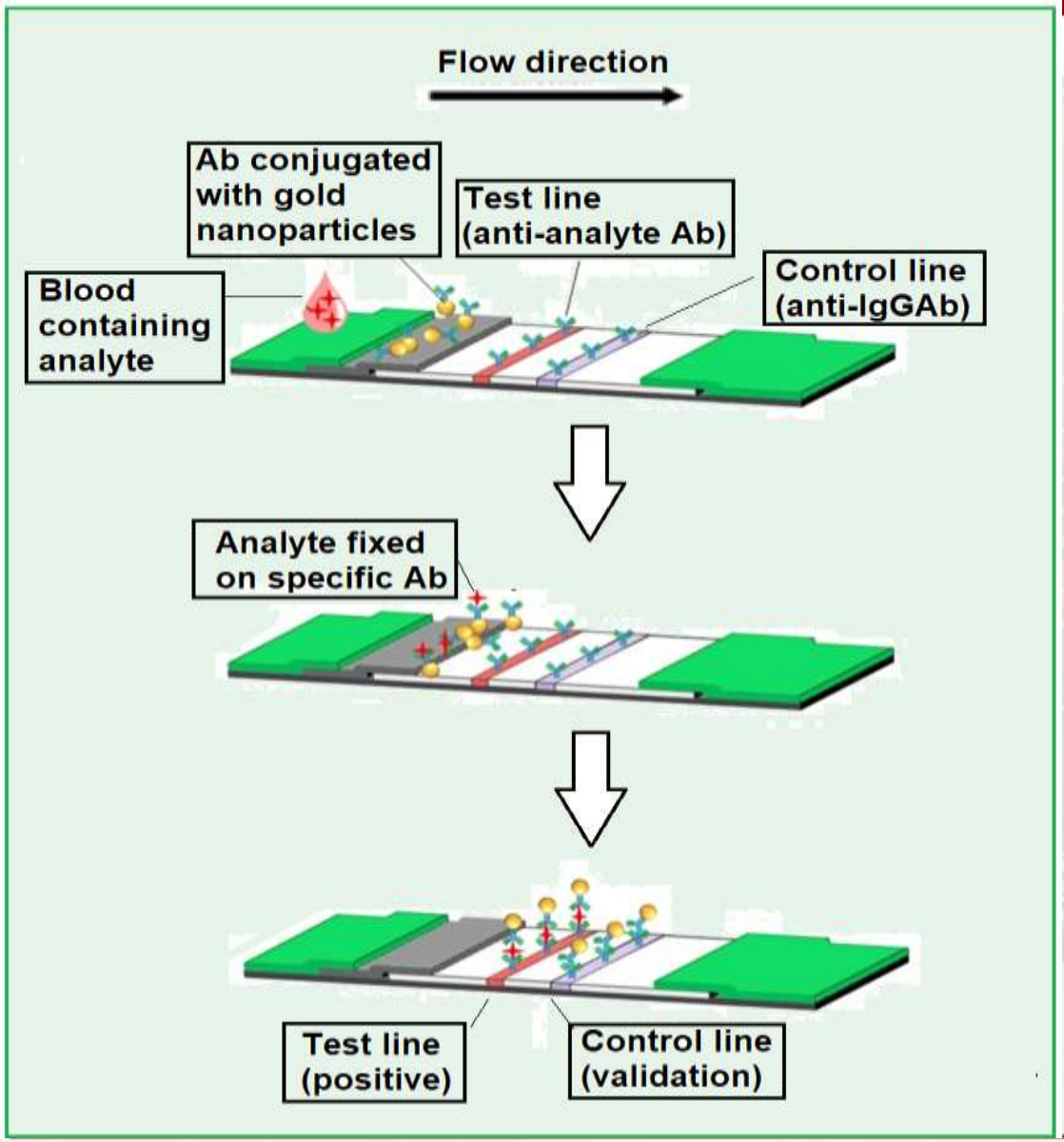
Chemiluminescence Immunoassay (CLIA)



Point of Care Testing

Point of Care Testing (POCT) (bedside testing) denotes any test done outside the proper laboratory area. It is estimated that about 40% of all biochemical tests belong to this category. POCT includes blood glucose test, blood gases and electrolytes analysis, pregnancy test, urine strip examination, drug of abuse screening, infectious diseases screening etc. The major purpose of POCT is to provide rapid patient information to the treating physician. Sample transport is avoided and many preanalytical and postanalytical errors may also be avoided with POCT. The major challenge of POCT is the establishment of appropriate quality control procedures.

Lateral Flow Immunoassay (LFIA)



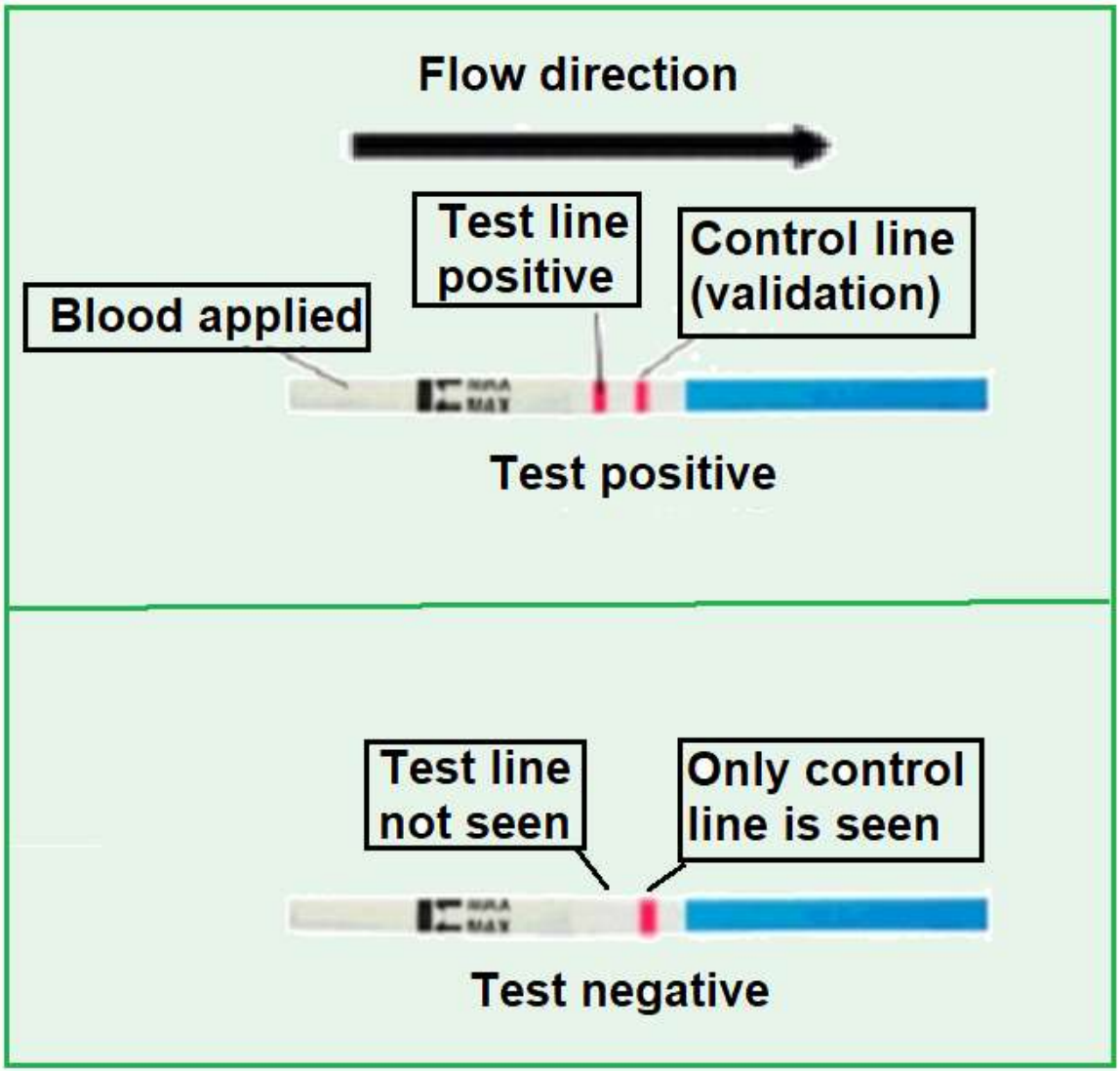
Top: the sample is deposited on the sample pad and migrates towards the conjugate.

Middle: the conjugated antibodies bind the target analyte.

Bottom: conjugated antibodies migrate to the test line, where the bound target analyte is captured.

Ab = antibody;
IgG = immunoglobulin G.

Lateral Flow Immunoassay (LFIA)



LFIA reading.

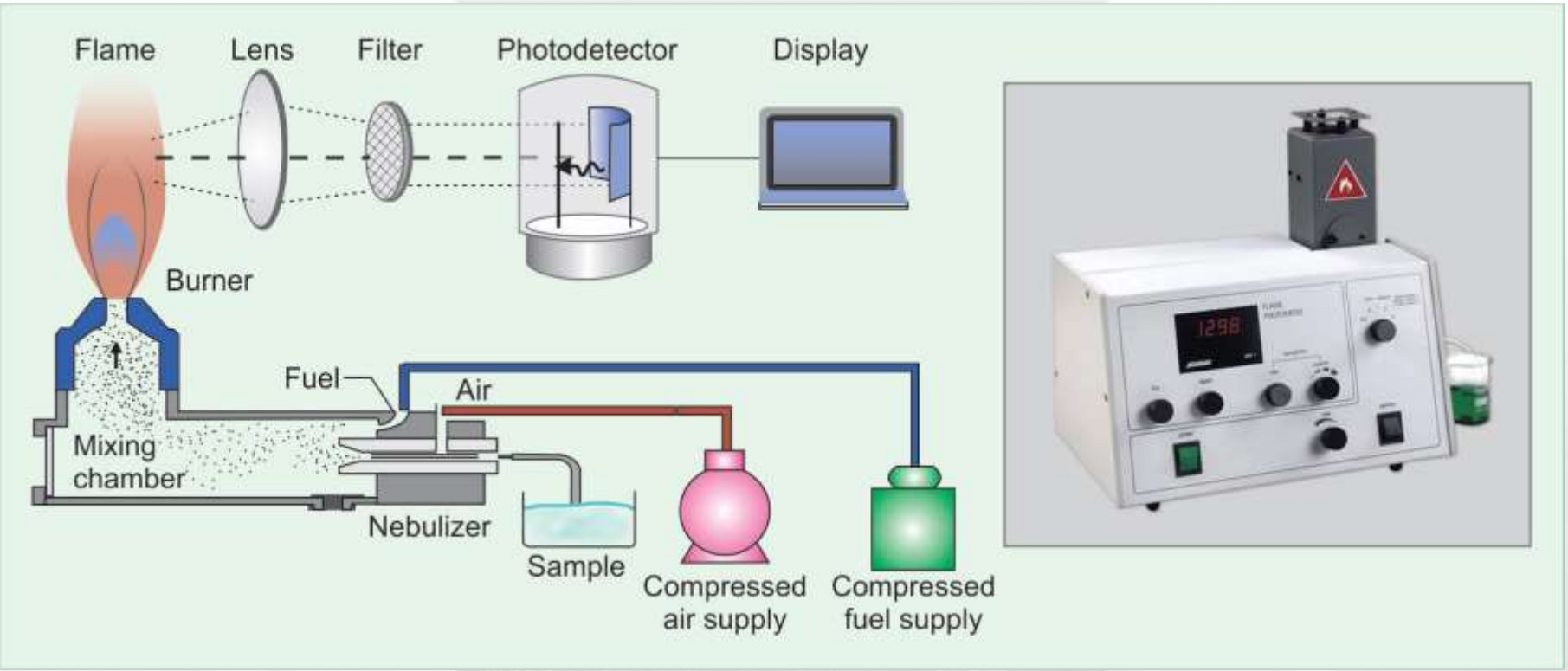
As an example, the pregnancy test is illustrated, which uses strips containing anti-hCG antibodies.

Upper portion shows a positive test

Lower portion indicates a negative test.

Flame Photometer

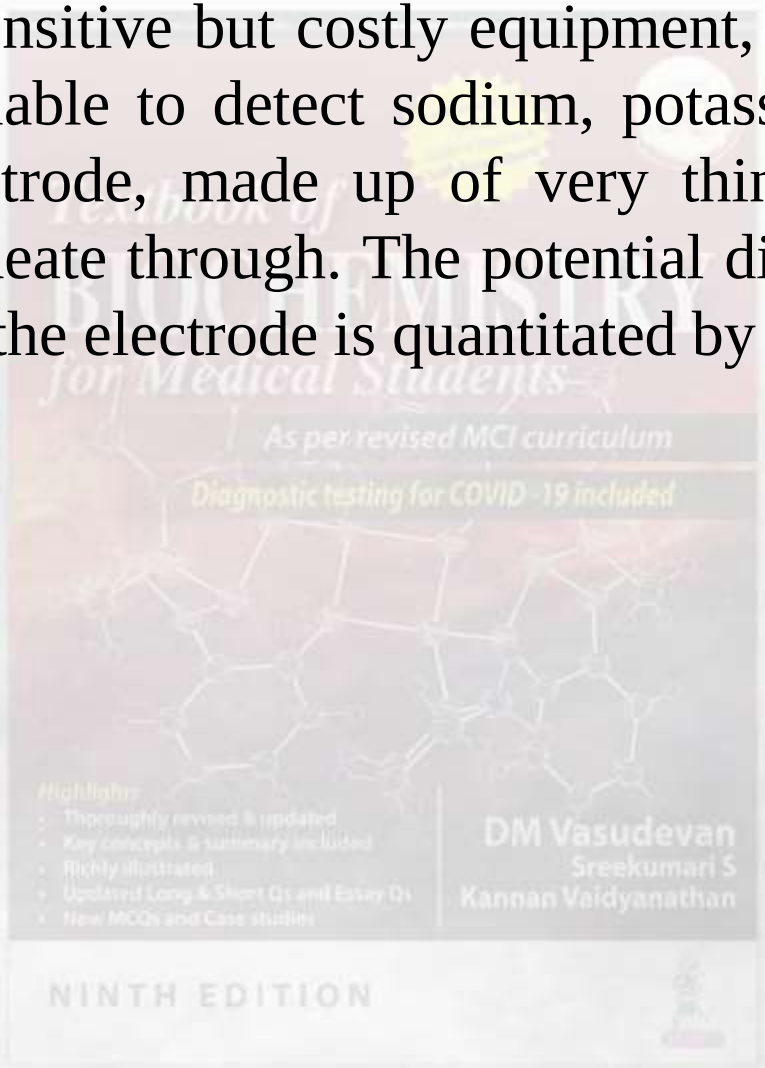
This is an analytical instrument used for quantitative analysis of **sodium, potassium, calcium, and lithium** in biological fluids. In a flame photometer the property of **emission spectroscopy** is utilized. Sodium, potassium, calcium, and lithium have the property of emitting a light of the characteristic wavelength of that particular element, when sprayed into a flame (incandescence). The equipment consists of an atomizer (nebulizer), which draws sample solution; and a compressor which pumps air at high pressure. It is fed into a flame. The flame will be blue, if the sample contains only distilled water. When the serum sample is introduced, the flame acquires a particular color.



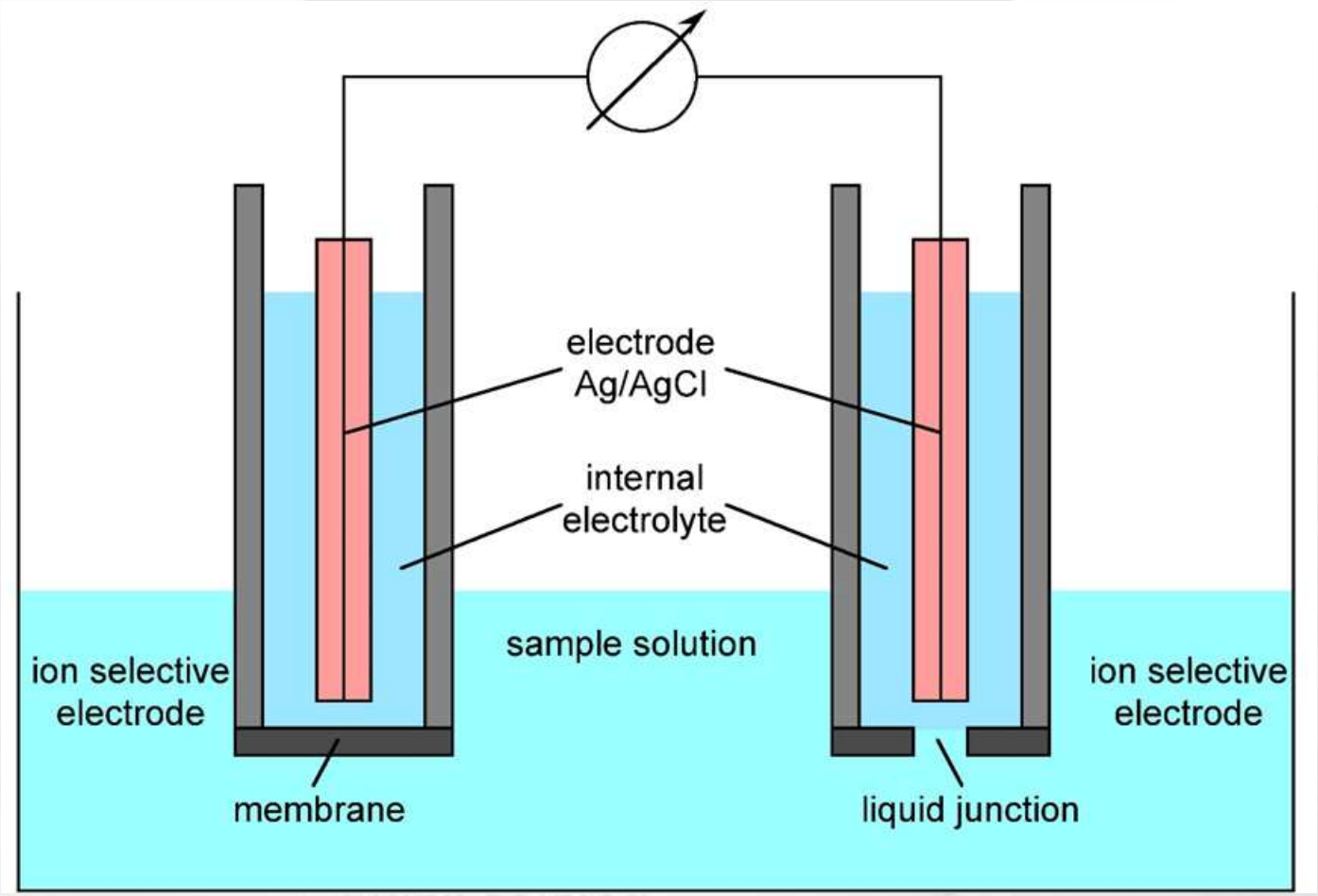
Flame photometer. The components are shown diagrammatically in left side. The instrument is shown in the right side.

Ion Selective Electrodes

Nowadays, more sensitive but costly equipment, using ion selective electrodes are available to detect sodium, potassium, calcium, and lithium. Glass electrode, made up of very thin glass membrane, allows ions to permeate through. The potential difference across the glass membrane of the electrode is quantitated by the instrument.

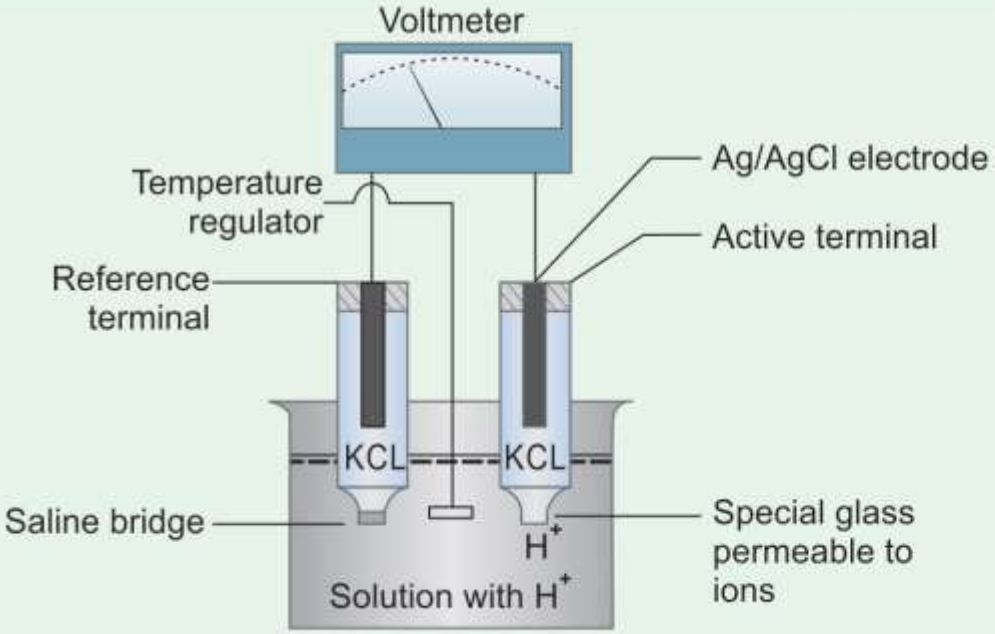


Ion Selective Electrodes



The pH Meter

The pH is measured accurately by potentiometric methods. The **glass electrode** allows passage of hydrogen ions. Inside the bulb of the glass electrode, a solution of known hydrogen ion concentration is kept and a silver-silver chloride electrode connects the solution to the input part of the instrument. The reference electrode or **calomel electrode** (mercury with mercurous chloride) is filled with KCl solution. In the equipment, the test and the reference electrodes are made physically together for easy handling.



The pH meter. The components are shown diagrammatically in left side. The instrument is shown in the right side.

In **semi-automated photometric analyzers** pipetting, reagent mixing and incubation are manually done, while aspiration of the colored solution into the photometer as well as measurement, calculation and display or printing of the results are automatically done.

The instrument is meant for parameter oriented analysis. It can analyze only one parameter at a time (e.g. urea), but many samples can be measured at a rapid rate.

Autoanalyzer

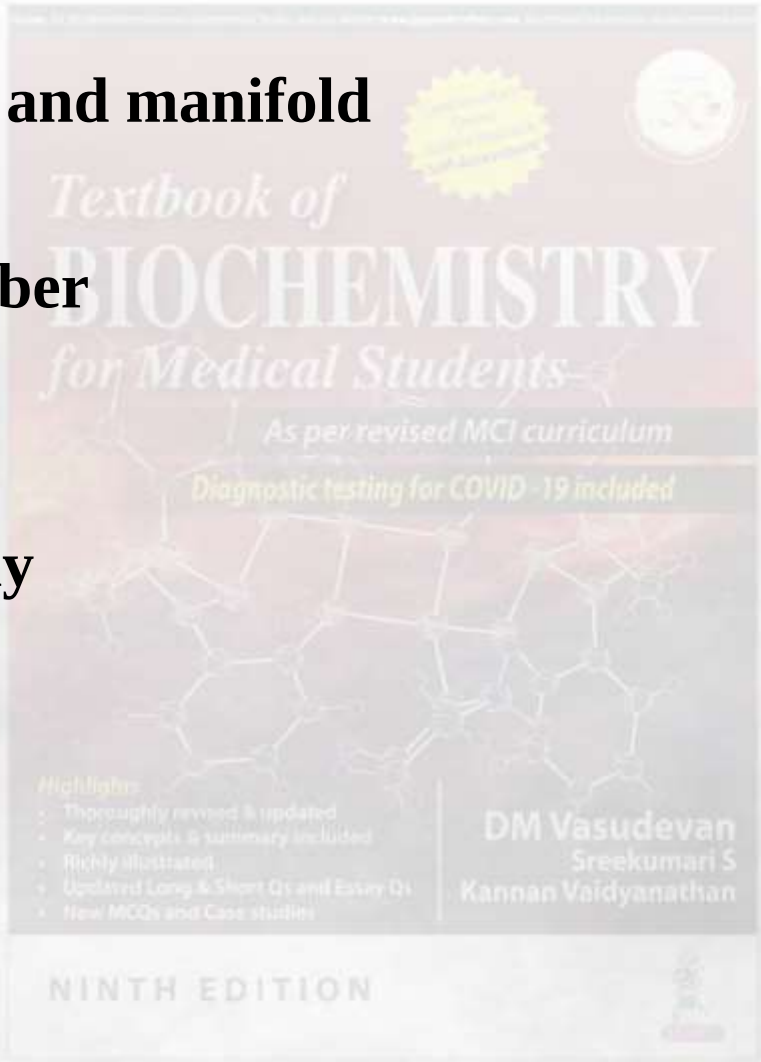
Different analytes in multiple blood samples may be analyzed within a short time.

In **batch analyzers** all samples are analyzed for one constituent only and analysis is parameter oriented.

The **discrete selective analyzers** have the capability of analyzing simultaneously 40 or even more parameters in a single sample. It is therefore called sample oriented. At the same time, it offers the freedom to the operator to select any number of analyses on a sample.

Components of Autoanalyzer

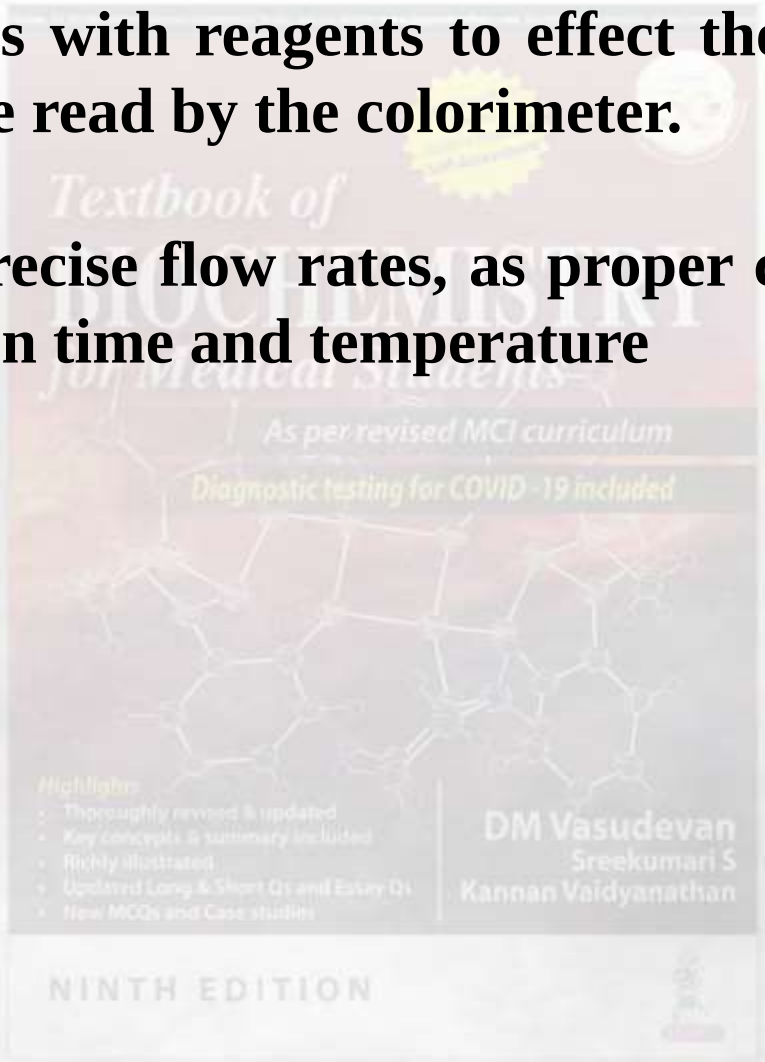
- Sampler
- Reagent pump and manifold
- Mixing
- Reaction chamber
- Heating bath
- Colorimeter
- Record / display



Proportioning pump

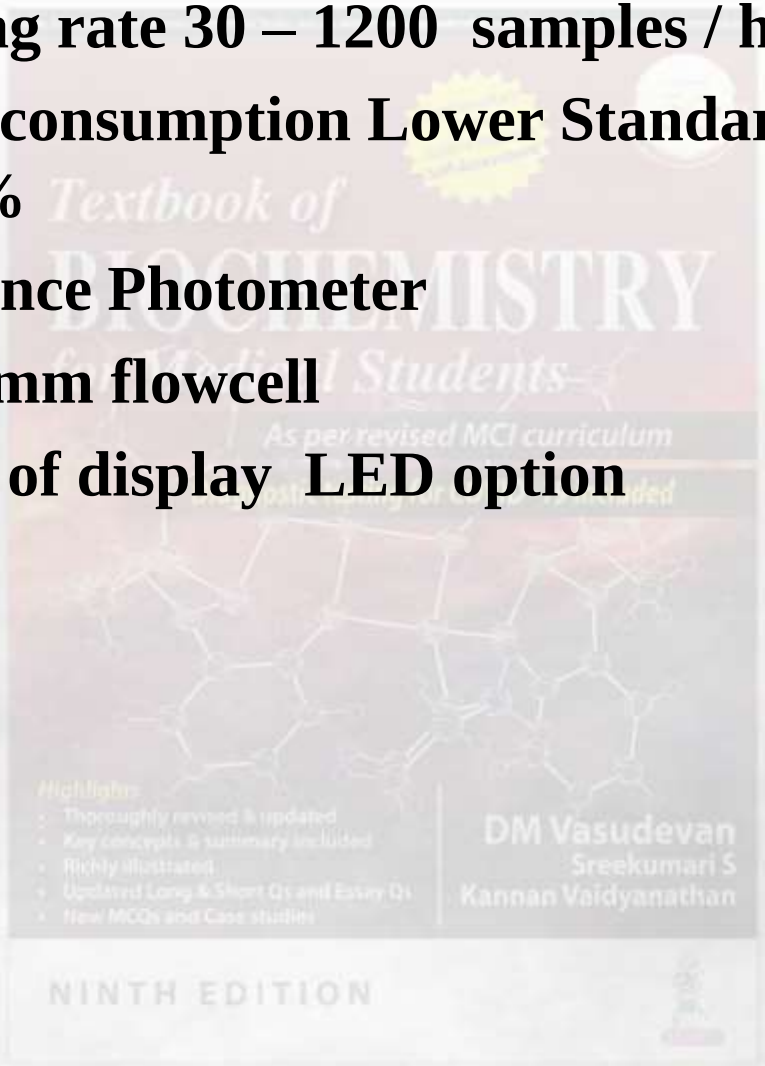
Introduces samples with reagents to effect the proper chemical color reaction to be read by the colorimeter.

Pumps fluids at precise flow rates, as proper color development depends on reaction time and temperature



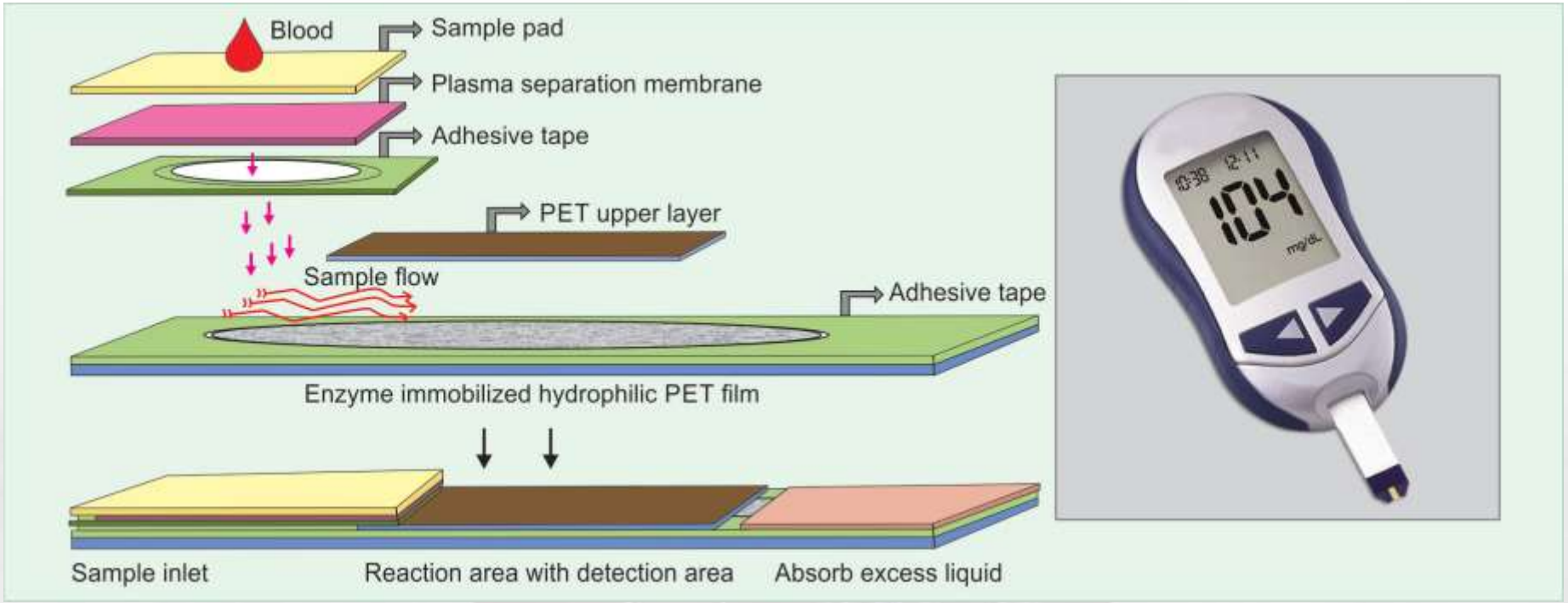
Advantages

- **Higher sampling rate 30 – 1200 samples / hour**
- **Lower reagent consumption Lower Standard Deviation**
  1% => 0.4%
- **High Performance Photometer**
- **30 mm and 50 mm flowcell**
- **Different types of display LED option**



Dry Chemistry Systems

Here all the reagents necessary for the reaction are embedded on a plastic matrix in their dry state, thus obviating the need to prepare reagent solutions. The reaction is initiated by the addition of the sample over the matrix and the color that is produced by the reaction is measured by **reflectance spectrophotometry**. This system is very useful in the emergency practice of critical care medicine. **Glucometers** are working on this dry chemistry principle. Glucometers are usually used by the diabetic patients for blood glucose analysis at their home.

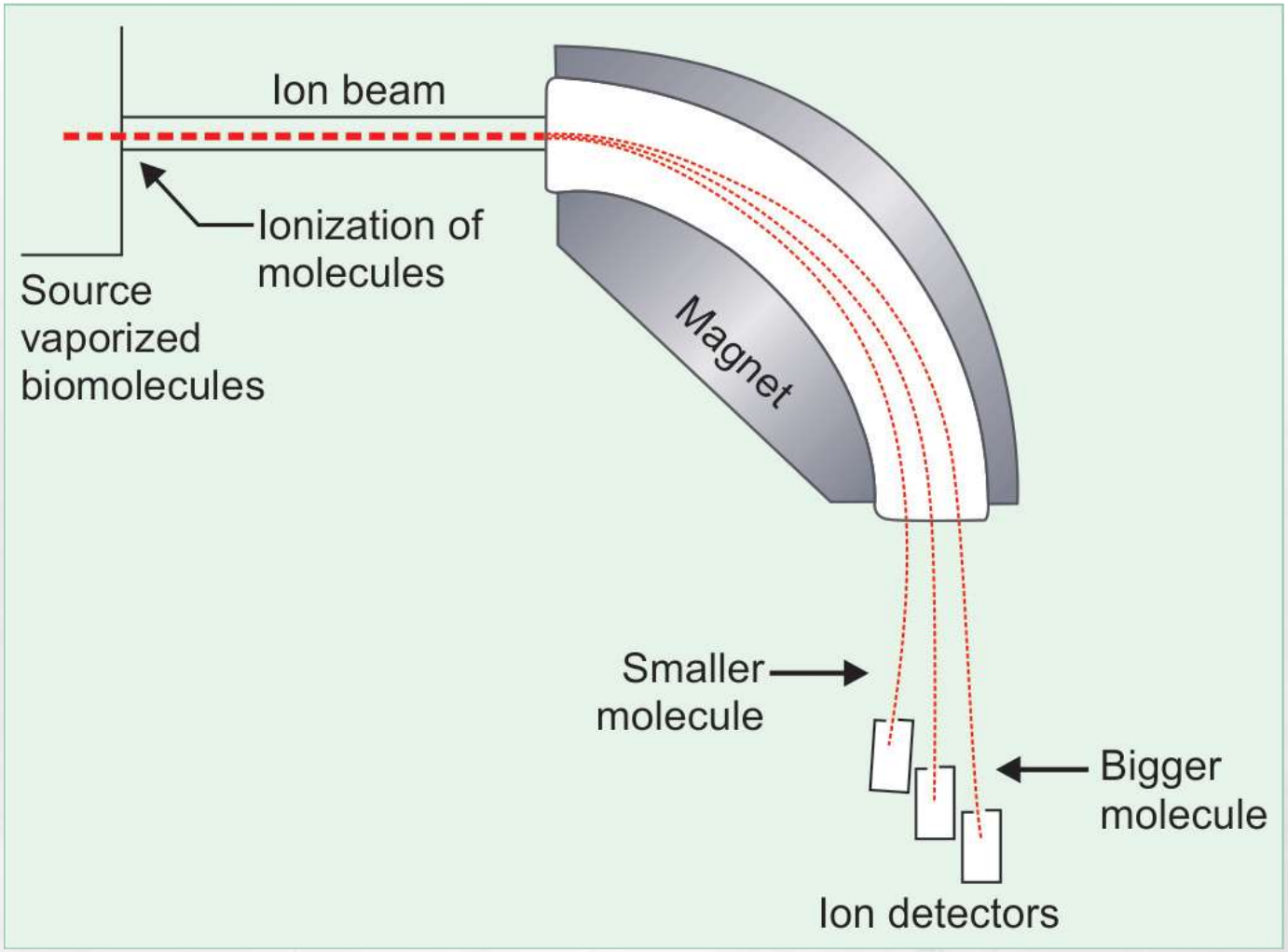


Glucometer. The principle of lateral flow is explained in the left side and the glucometer with the strip is shown in the right side.

Mass Spectrometry (MS)

The MS identifies molecules based on their mass or molecular size. The relative abundance of each ion is a function of its mass to charge ratio. When interfaced to the HPLC or gas–liquid chromatography, the MS functions as a powerful detector.

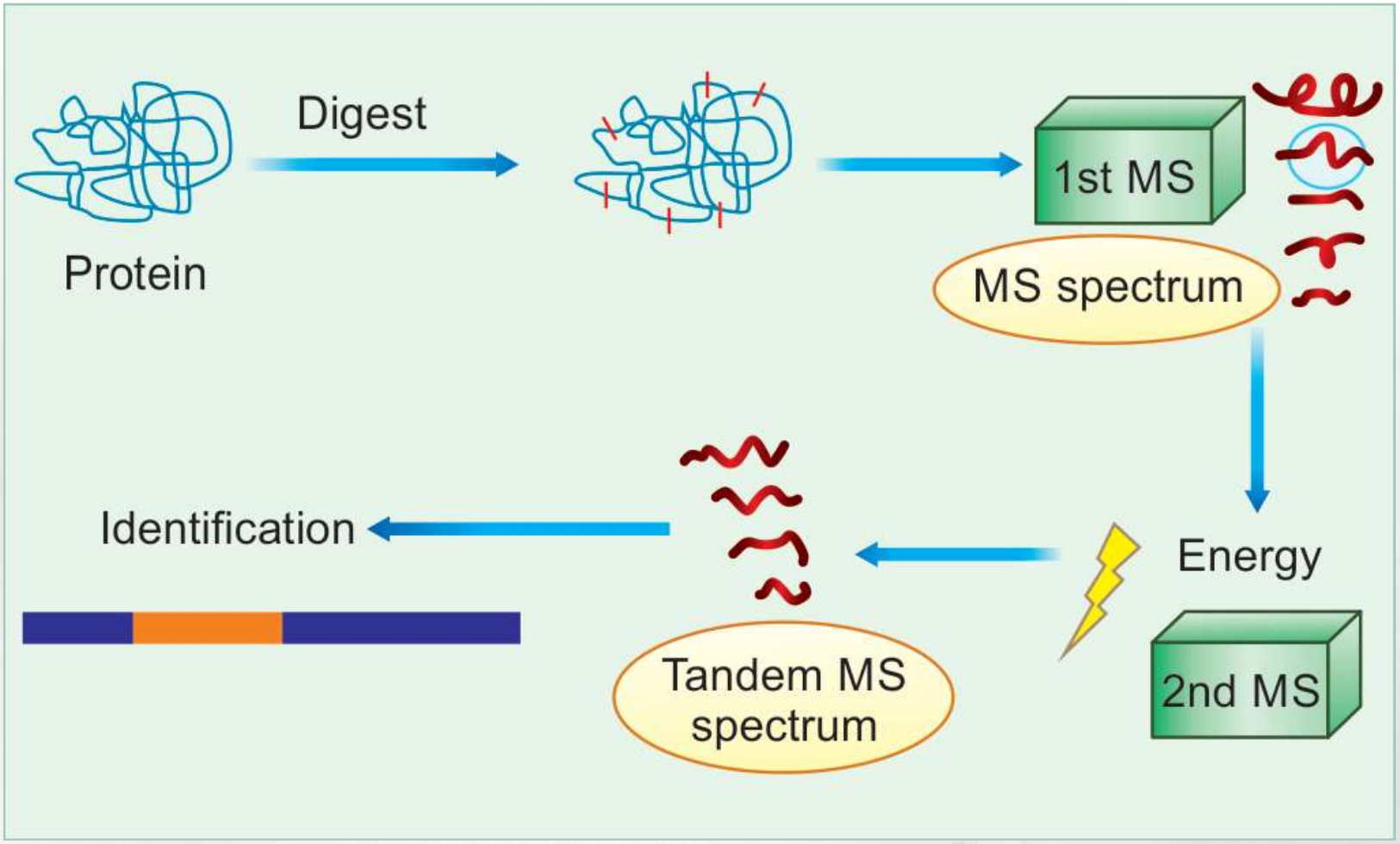
The sample is vaporized such that the molecules become positively charged. Electrical field is applied, so that the particles move. At the same time, a magnetic field is applied, so the cations are deflected at right angle to their original direction, and hit on the detector. For molecules of identical charge, the time required for the molecule to reach the detector is inversely proportional to the mass of the molecule.



Mass spectrometry.

Tandem Mass Spectrometry (MS-MS)

Here two mass spectrometers are arranged sequentially. The first MS separates big peptides, based on their mass. From this observation, a single peptide can be directed into the second MS (called parent ion). The parent ion enters the collision cell of the second MS. Then the ions collide with argon gas molecules and are broken into smaller ions. These daughter ions (product ions) are detected and quantified by their mass spectrum in the second instrument. The high selectivity of MS-MS is that the parent ion mass and daughter ion mass are used for characterization. The applications of MS-MS include identification and quantification of proteins, drug screening, pesticides, and pollutants and **screening of inborn errors** of metabolism, especially organic acidurias.



Principle of tandem mass spectrometry (MS-MS).

Preanalytical Variables

Errors can often creep into the laboratory results. They may be preanalytical, or postanalytical, either instrument related or human errors.

Good laboratory practice aims at minimizing the errors and providing accurate results in the minimum possible time (turn around time or TAT) so that the clinician can make necessary changes in the treatment measures.

Preanalytical variability is defined as errors which occur when nonanalytical factors change the concentrations of analytes, so that the results do not reflect correctly the condition of the patient. Preanalytical variability may be due to (a) precollection causes, or (b) blood collection causes.

Types of preanalytical variables

Patient identification: The labeling of specimen may be improper. Corrected by bar coding.

Turnaround time (TAT): The time required from the specimen reaching the laboratory and the result being dispatched should be kept minimum.

Laboratory logs: Entry of test details in lab registers.

Transcription errors: Electronic identification and tracking of specimens.

Patient preparation: Improper standardization of the collection time and manner of collection.

Specimen collection: Container, anticoagulant.

Separation, aliquoting

Personnel: Variation from person to person.

Pre analytical variables

(A) Specimen Collection Variables

Age, gender, race, pregnancy

Fasting, diet

Caffeine, tobacco, alcohol, other drugs

Timing of sample

Duration of Tourniquet application

Site of sampling

Anticoagulants; ratio to blood sample

Transportation of samples

Storage, processing, centrifugation

Hemolysis, lipemia, hyperbilirubinemia

Exposure to UV light

Standing time before separation of cells

Exercise Increases

A L T	**40%**
A S T	**30%**
Creatinine	**20%**
A C P	**15%**
Myoglobin	**15%**
Phosphate	**12%**
Iron	**12%**
Total lipids	**12%**
Potassium	**8%**

CAFFEINE

F F A	↑	
Total lipids	↑	
Glucose	↑	

SMOKING

T G	↑	20%
G H	↑	15%
Cortisol	↑	15%
Glucose	↑	10%
Urea	↑	10%
Cholesterol	↑	5%
HDL Cholestero		-- 5%

HEMOLYSIS

VIGOROUS SUCTION OF SYRINGE FORCEFUL TRANSFER FROM SYRINGE SOAP IN TEST TUBE VIGOROUS SHAKING

INCREASE IN VALUES

POTASSIUM (RBC = 80 mEq; Serum= 4 mEq)

A C P

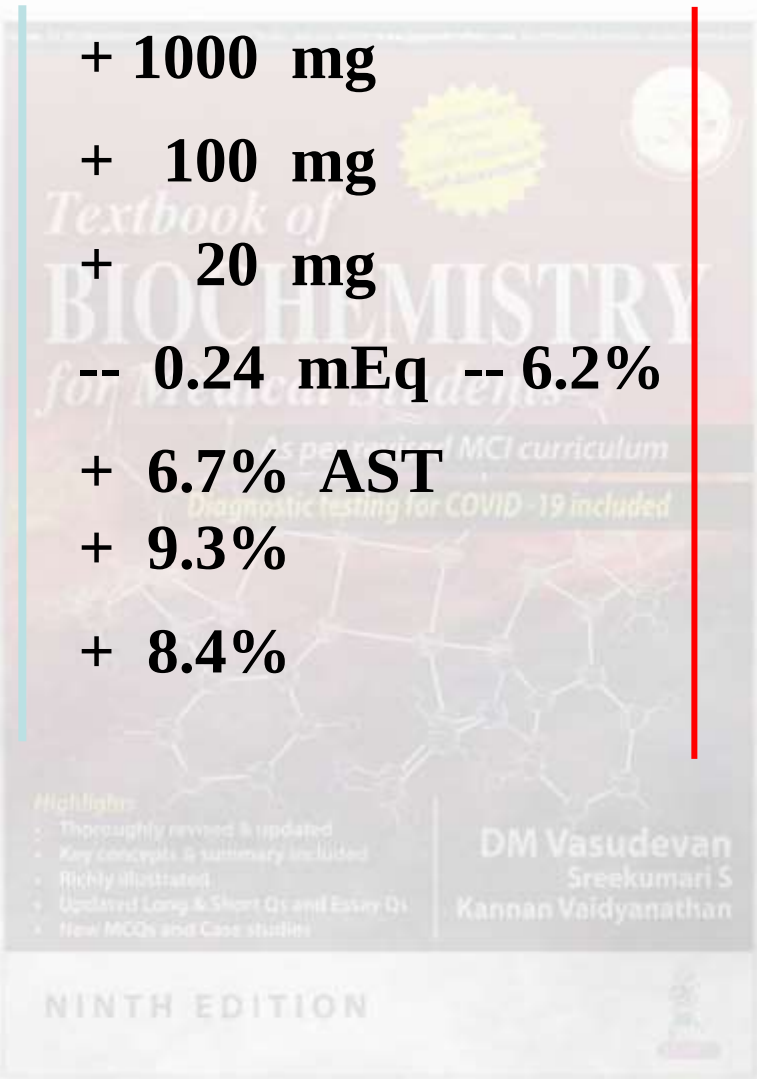
L D H

A S T

- **Tourniquet Application and Time:**
- **The tourniquet should be applied approximately three to four inches above the venipuncture site.**
- **The tourniquet should be on the arm no longer than one minute.**
- **remove the tourniquet when blood starts to flow into the tube**
- **Prolonged tourniquet time can lead to an increase in various chemistry analytes, due to hemoconcentration of blood at the puncture site.**

Tourniquet Should not be Kept for More Than 2 Minutes

Total Protein	**+ 1000 mg**
Total Lipids	**+ 100 mg**
Cholesterol	**+ 20 mg**
Potassium	**-- 0.24 mEq -- 6.2%**
Iron	**+ 6.7% AST** **+ 9.3%**
Bilirubin	**+ 8.4%**



SPECIMEN WITHOUT ANTICOGULANT IS CENTRIFUGED ONLY AFTER CLOT FORMATION IS COMPLETED

SPECIMEN WITH ANTICOGULANT IS CENTRIFUGED AS SOON AS POSSIBLE

IMMEDIATE SERUM SEPARATE

- **GLUCOSE**
- **CORTISOL**

PROTECT FROM LIGHT

- **BILIRUBIN**
- **VITAMIN A**

Types of Preanalytical Variations

Test conducted. The appropriate test should be requested.

Patient identification. The labeling of specimens may be improper. Corrected by bar coding.

Turn around time (TAT). The time required from when the specimen reaching the laboratory and the result being dispatched should be kept minimum. Time of arrival, completion of test and dispatch should be noted.

Laboratory logs. Entry of patient and test details in laboratory registers and computers.

Transcription errors.

Patient preparation. Improper standardization of the collection time and manner of collection.

Types of Preanalytical Variations, continued

Specimen collection. Corrected by using vacuatainer tubes and collection of samples by laboratory personnel**.**

Separation, aliquoting. Monitoring of the performance of the centrifuge as well as the container used for storage**.**

Personnel. Variation from person to person should be minimized**.**

Throughput time (time taken from arrival of specimen to completion of assay) to be monitored on a weekly or monthly basis**.**

Circadian variations in analytes

Analyte	Pattern of secretion
Cortisol	Peak, 6-8 am; minimum 10 pm. Secretion increases 3-5 fold from late evening to a maximum at waking. Earliest indication of abnormality is a loss of diurnal rhythm of secretion
ACTH	Same as above
Growth hormone	Peak 10 pm; low 8 am. Maximum during sleep and minimum at waking time. Increases during exercise .
Renin and Aldosterone	Peak 8 am, low at late evening. Changes with posture also. To be collected in the recumbent position
Catechol-amines	24 hour samples collected. Secretion is less during night. Night workers have a reversal of pattern

Anticoagulants

- Heparin is the most widely used anticoagulant. It interferes the least with test procedures.
- Ethylenediamine tetraacetic acid (EDTA) is a chelating agent, and is particularly useful for hematological examination.
- Sodium fluoride is used as a preservative for blood glucose by inhibiting the enzyme systems involved in glycolysis. Without an antiglycolytic agent, the blood glucose concentration decreases by about 10 mg/dl per hour at 25° C and false results may be obtained. However, such serum should not be used for enzymatic assays.
- Citrate is widely used for coagulation studies.
- Oxalate inhibits blood coagulation by forming insoluble complexes with calcium ions.

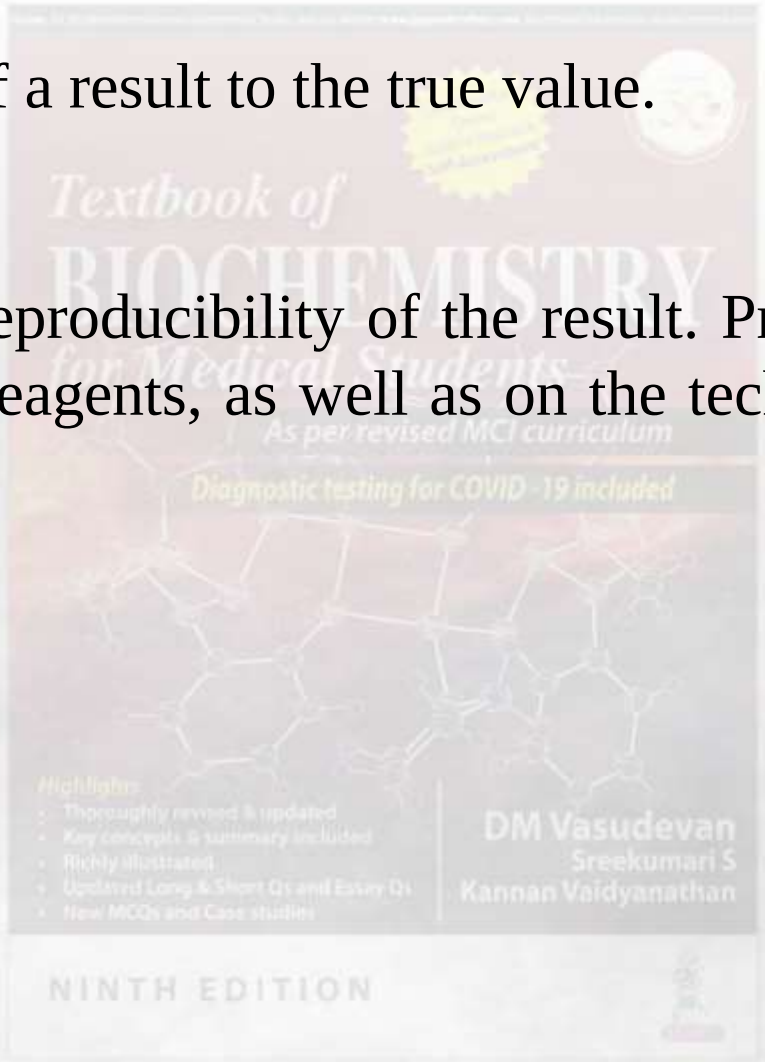
Quality Control (QC)

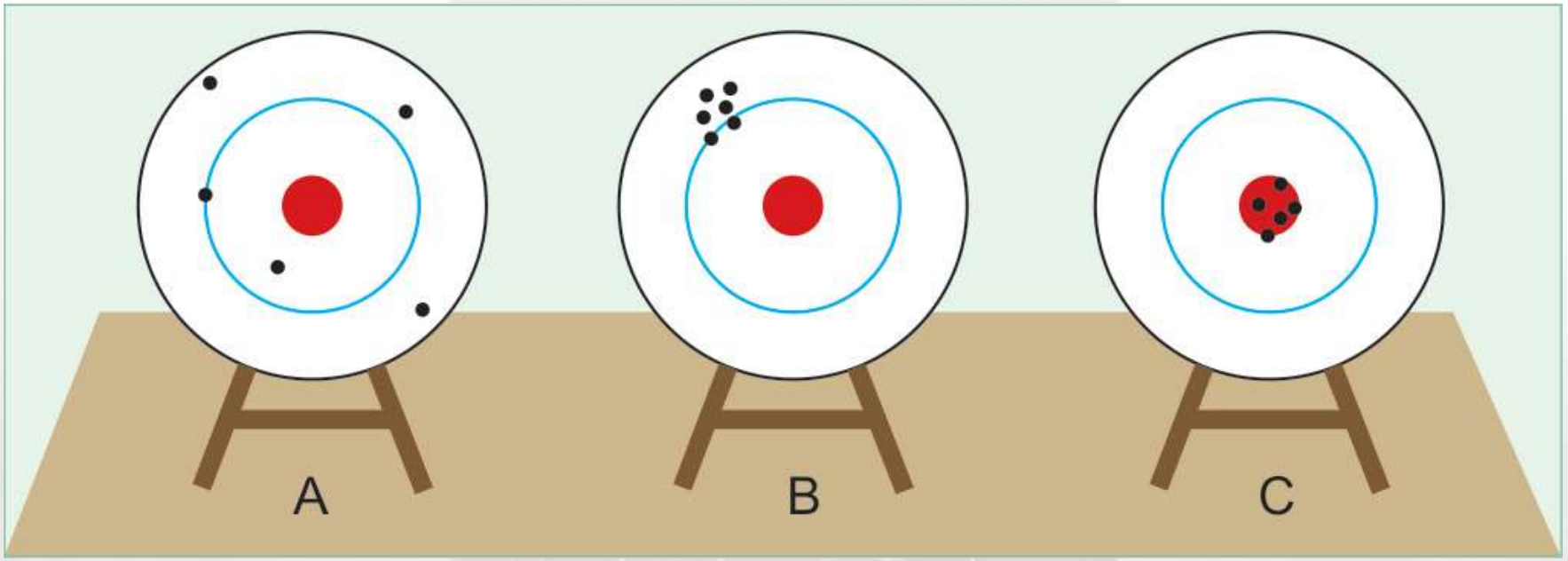
Accuracy

It is the closeness of a result to the true value.

Precision

This refers to the reproducibility of the result. Precision depends on the technique, the reagents, as well as on the technical ability of the technician.





(A) Imprecise;

(B) Precise but inaccurate;

(C) Precise and accurate.

Specificity

Specificity of a reaction denotes that only one substance will answer that particular test. Specificity is determined by the method of the analysis.

Sensitivity

It indicates that whether the method could be utilized to test a very dilute solution. The sensitivity of an assay is the fraction of those with a disease that the assay correctly predicts. Specificity is the fraction of those without the disease that the assay correctly predicts. A test should be both sensitive and specific.

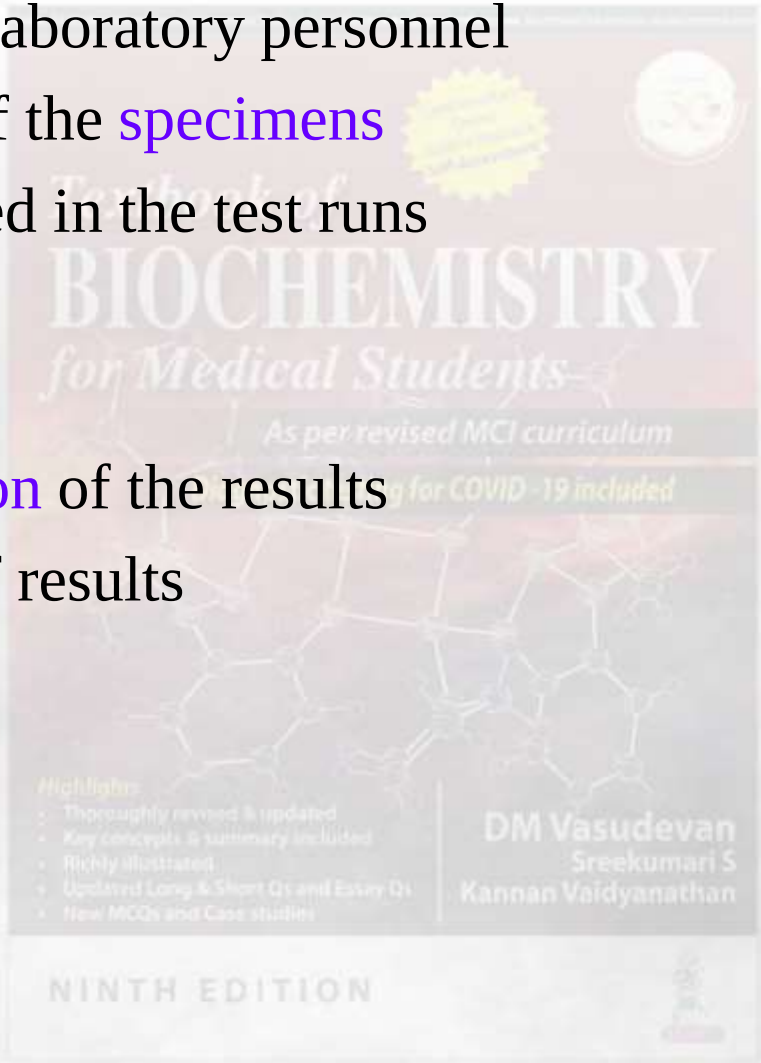
Quality Control - QC refers to the measures that must be included during each assay run to verify that the test is working properly.

Quality Assurance - **QA is defined as the overall program that ensures that the final results reported by the laboratory are correct.**

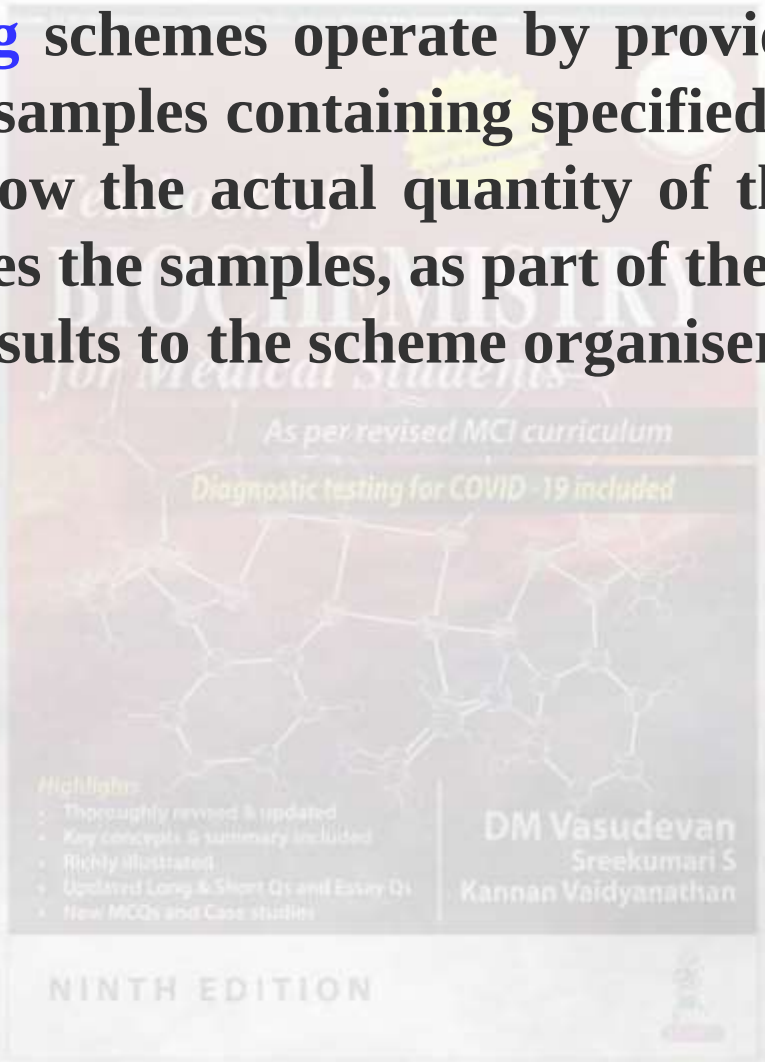
QC programs ensure that information generated by the laboratory is accurate, reliable, and reproducible.

Variables that affect the quality of results

- The training of laboratory personnel
- The condition of the specimens
- The controls used in the test runs
- Reagents
- Equipment
- The interpretation of the results
- The reporting of results



Proficiency testing schemes operate by providing participating laboratories with samples containing specified material but only the organisers know the actual quantity of the substance. The laboratory analyses the samples, as part of their normal routine, and reports the results to the scheme organisers.



Accreditation is a progressive and time-proven way of helping institutions evaluate and improve their overall performance.

Benefits accredititation

Building Trust

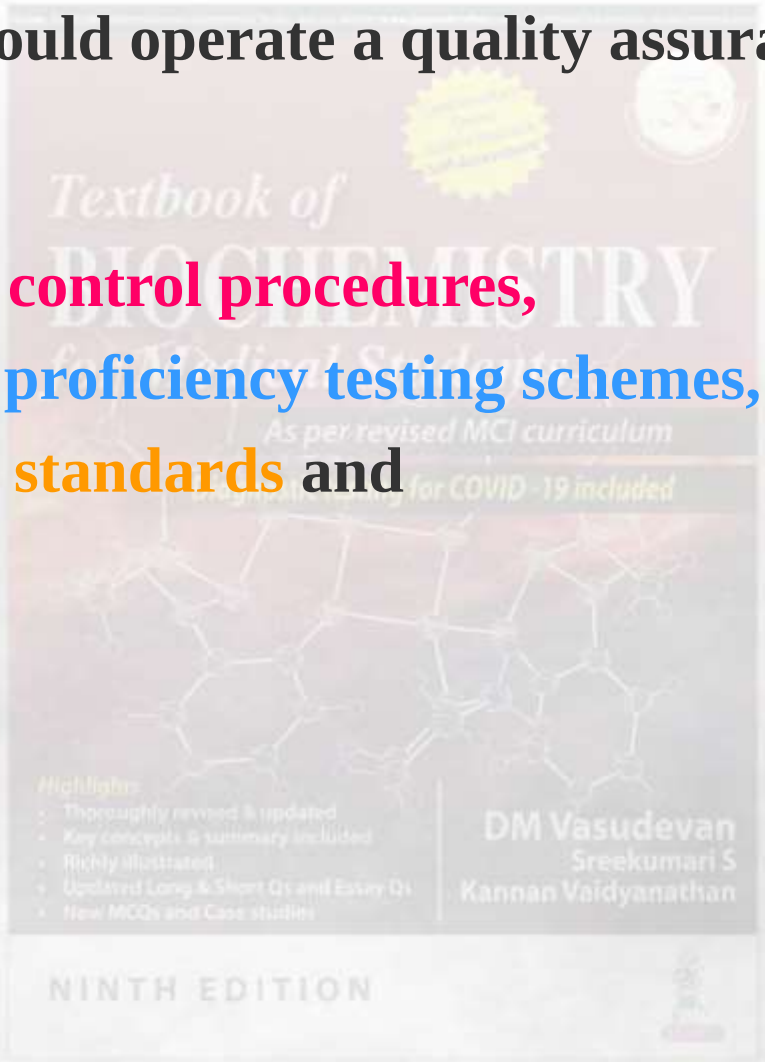
Customers will have the confidence in the quality of products and services that you provide.

Minimises Risk

Accredited organisations reduce the risk of providing unreliable test results, services and products

All laboratories should operate a quality assurance system, which includes

1. **Internal quality control procedures,**
2. **Participation in proficiency testing schemes,**
3. **Use of reference standards and**
4. **Accreditation**



Laboratory Waste Management

All institutions should effectively implement the basic practices of proper drainage of waste water to the sewer, incineration, landfall burial, and recycling.

Medical waste is formed from the healthcare facilities and poses potential hazard to mankind unless properly handled and treated. Approved methods like incineration, steam sterilization, thermal inactivation, and disinfection are to be carried out. The collection of the laboratory waste at the site of production is by using separate color-coded waste dispensers. Segregation of medical waste should happen at the point of origin, from where they are transported to the spot for final disposal, observing safety precautions.