

Clinical Biochemistry

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Clinical Pathology 4th Class

Clinical Biochemistry: means study of the chemical composition and process in the body of living organisms, such as:

- 1) Total proteins**
- 2) Ketones**
- 3) Urea**
- 4) Enzymology**
- 5) Minerals**

Laboratory Devices used for Clinical Biochemistry

- 1) Refractometer**
- 2) Spectrophotometer**
- 3) Chemistry Auto analyzers**
- 4) Enzyme Linked Immunosorbent Assay (ELISA reader)**
- 5) Atomic absorption spectrophotometer**

Refractometer



RHB-32



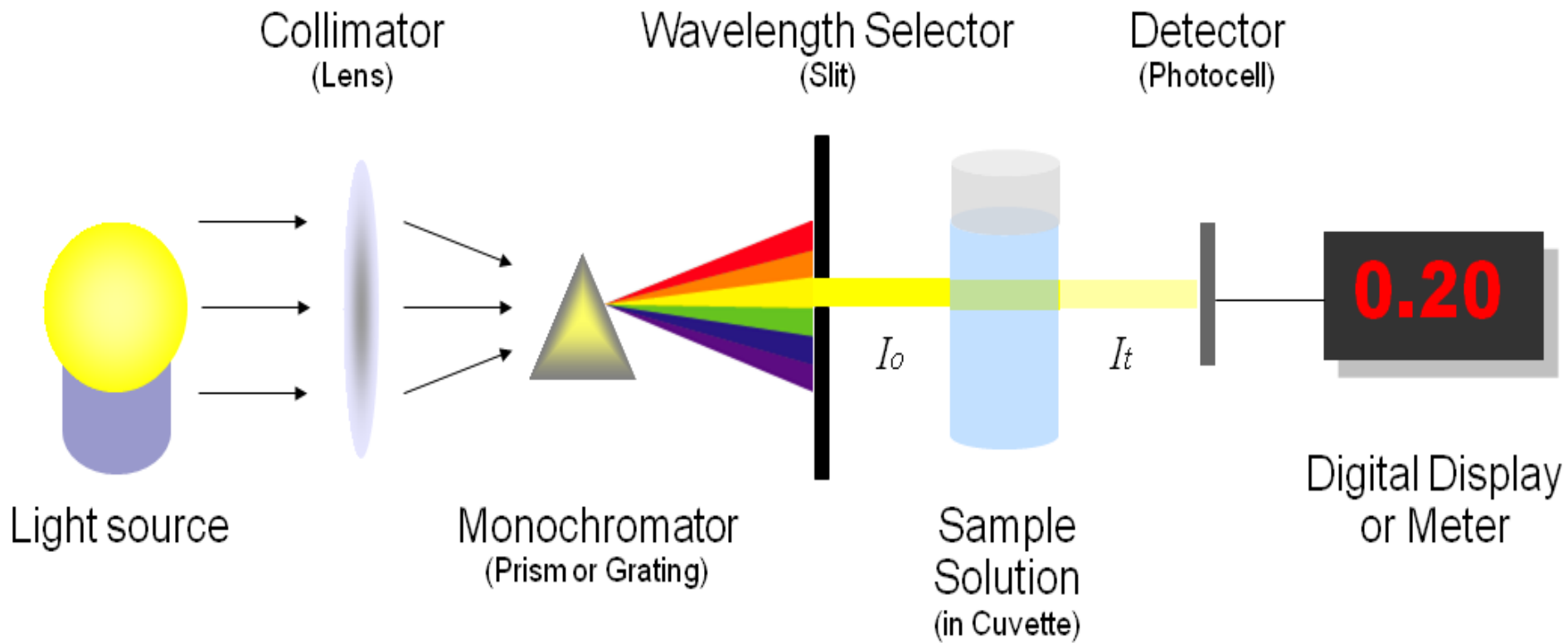
Spectrophotometer



Chemistry auto analyzer



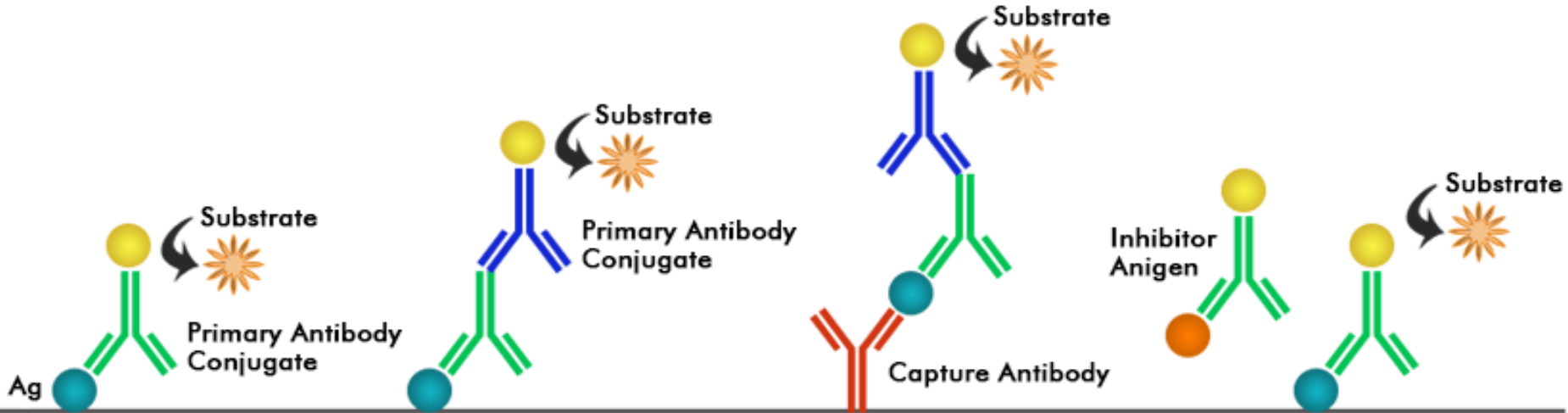
Principle of Spectrophotometer



ELISA Reader



Types of ELISA



DIRECT ELISA

INDIRECT ELISA

SANDWICH ELISA

COMPETITIVE ELISA

Atomic absorption spectrophotometer



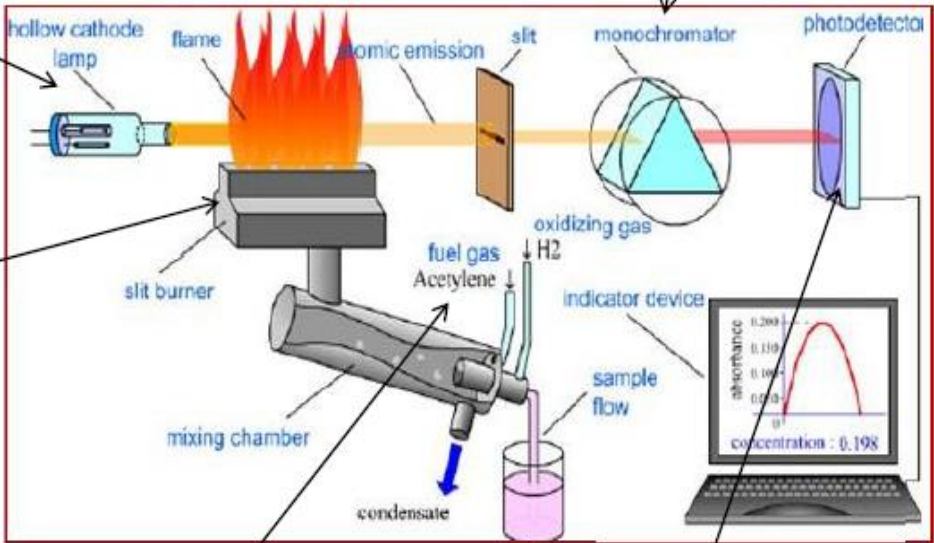
Principle of Atomic absorption spectrophotometer

Monochromator

- Select light with specific frequency/wavelength of interest
- Provide the specific light which will be absorb by metal

Hollow Cathode Lamp

- Emits light of specific wavelength to be absorbed by element



Atomiser

- Atomise sample to atomic state
- Atom vapour (gas phase) produced

Fuel

- Burning mixture of ethyne with O₂ (combustion mix)
- Sample dehydrated/solvent removed

Detector

- Measure the decrease in intensity of light absorb by analyte
- Convert the decrease in light intensity into electrical signal using photomultiplier

1) Total protein

Is usually measured by:

1. **Biuret method**: which is a highly specific spectrophotometric technique for protein measurement, but it is inaccurate in determining protein concentration <1 g/dl.
2. **Refractometer**: is useful if an emergency result is required.

The serum must be clear for accurate determination of protein concentration:

- (1) **Hemolysis** may cause a mild increase in protein concentration.
- (2) **Turbidity** from lipemia or cells may result in falsely high readings.
- (3) **Icterus** alters the color of the specimen but does not alter the reading.

2) Ketone bodies:

Ketone bodies are:

1. Acetoacetate,
2. β -hydroxybutyrate
3. Acetone

*The former two ketones are intermediary metabolites of lipid metabolism, while the latter is a waste product.

Ketone bodies measured by the **Nitroprusside reaction**, which is the most qualitative test detect ketones, and specific for acetoacetate and to a lesser extent acetone, beta hydroxybutyrate is not detected.

An enzymatic and colorimetric method has been developed for estimation of beta hydroxybutyrate

Nitroprusside method: Tablets containing sodium nitroprusside are available commercially. These tablets are placed on a clean surface, and a drop of sample is placed on the tablet. If ketones present, tablet color will vary after 30 second from lavender to deep purple, representing a trace, moderate, or strongly positive reaction.

3) Urea

A nitrogenous compound, is synthesized in the liver from carbon dioxide (CO₂) and the ammonia generated from deamination of amino acids.

*Urea results are usually expressed as **blood urea nitrogen (BUN)**, which their levels are used to evaluate kidney function based on the ability of the kidney to remove nitrogenous waste (urea) from the blood.

Urea can be estimated by:

1. Spectrophotometer
2. Chemistry Auto analyzers

4) Enzymology

The blood level of most enzymes is low in a healthy animal. It may be elevated:

1. if the enzyme has leaked out of damaged cells
2. if the cells have increased production of the enzyme and the excess amount has leaked out of the cells into the blood.

*Spectrophotometer and ELISA to measure the amount of product produced.

- Directly measuring enzymes concentration is difficult because of its low level.
- The tests performed to determine enzyme concentration in blood indirectly by enzymatic reaction, which is forms a product but no change in the enzyme as follows:

The substance on which an enzyme (E) work is called a substrate (S). Each of many enzymes has a specific substrate.

Each enzymatic reaction produces a specific product (P) from the interaction of substrate and enzyme:



5) Minerals

Serum minerals have been measured by a variety of techniques including:

- 1) Colorimetric analysis using spectrophotometer
- 2) Atomic absorption spectrometry