RINCIPLE OF COLORIMETER AND SPECTOPHOTOMETER AND VARIOUS TYPE OF ANALYSER USED IN CLINICAL BIOCHEMISTRY

COLORIMETER

- What is colorimeter ?
- Colorimetry.
- Principle of colorimeter.
- Beer's and Lambert's law.
- Components of colorimeter.
- Functions of components.
- Advantages and Disadvantages of single cell photometry.

What is colorimeter ?

meter is a instrument used for the measurement of
d substance in solution.
strument is operative in the visible range of the
magnetic spectrum.

COLORIMETRY

- It is a most common analytical **technique** used in biochemical estimation in clinical laboratory.
- It involves the quantitative estimation of colour.
- A substrate must be estimated colorimetrically, must be coloured or it should be capable of forming **chromogens** (coloured complexes) through the addition of reagents.

- Coloured substance absorb light in relation to their colour **density.**

- The colour density will be proportional to the concentration of coloured substance.

- The instruments used in this method are called colorimeter or photometer.

PRINCIPLE OF COLORIMETER

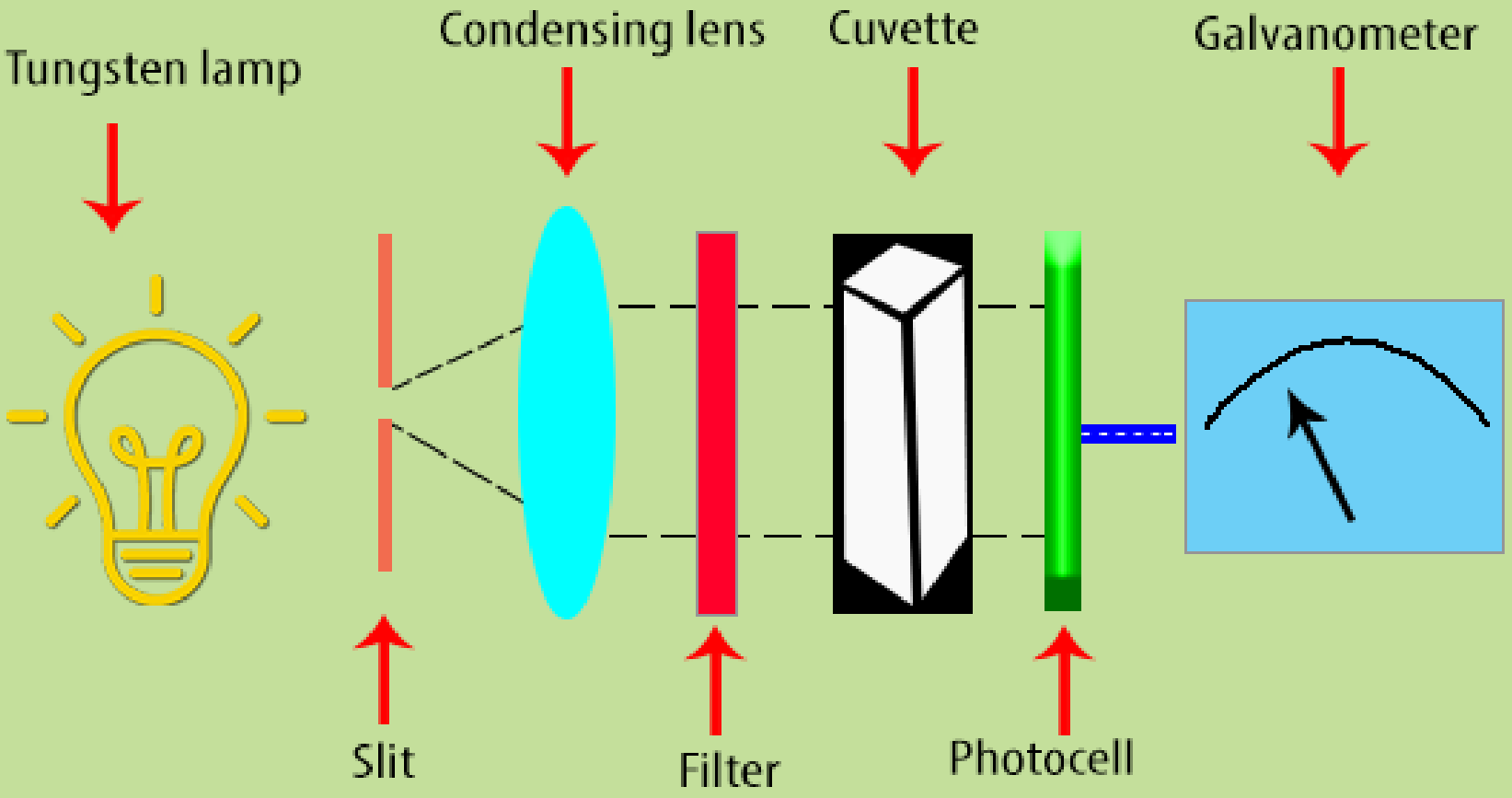
- When a monochromatic light passes through a coloured solution, some specific wavelength of light is absorbed which is related to colour density.

- The amount of light absorbed or transmitted by a colour solution is accordance with two law, **i.e. Beer's law and Lambert's law.**

COMPONENT OF COLORIMETER

- Light source
- Slit
- Monochromator(filter)
- Cuvette
- Photocell
- Galvanometer

Components of the Colorimeter




DIGITAL
PHOTO COLORIMETER
.35

FUNCTION OF EACH COMPONANT

Light source

Two kinds of lamp:-

1. Halogen Deuterium :- for measurement in the ultraviolet range 200 – 900 nm.
2. Tungsten lamp:- for measurement in the visible 400 – 760 nm and near-infrared ranges.

MONOCHROMATOR(FILTER) :

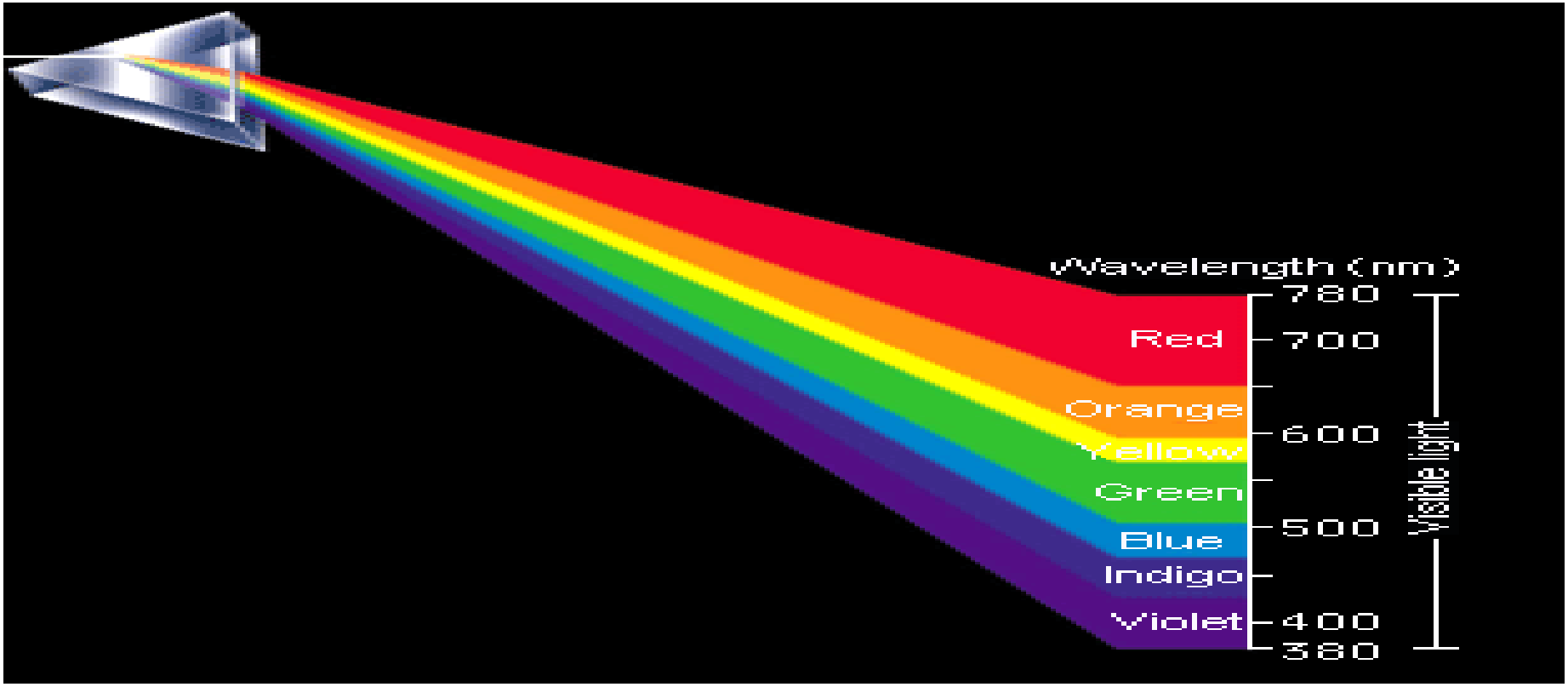
FILTER:

•Used for selecting the monochromatic light.
•Filters will absorb light of unwanted wavelength and allow only monochromatic light to pass through.

Three Types:
1.Prism
2.Grating
3. Glass

PRISM

•When light travels from one medium to another medium , it is refracted and enters in the new medium at a different angle.



Prism wavelength spectrum

Wavelength (nm)	Spectrum region		Colour absorbed	Colour transmitted
400–420	Visible		Violet	Green–yellow
420–500	Visible		Blue	yellow
500–570	Visible		Green	Red
570–600	Visible		yellow	Blue
600–630	Visible		orange	Green–blue
630–700	Visible		Red	Green

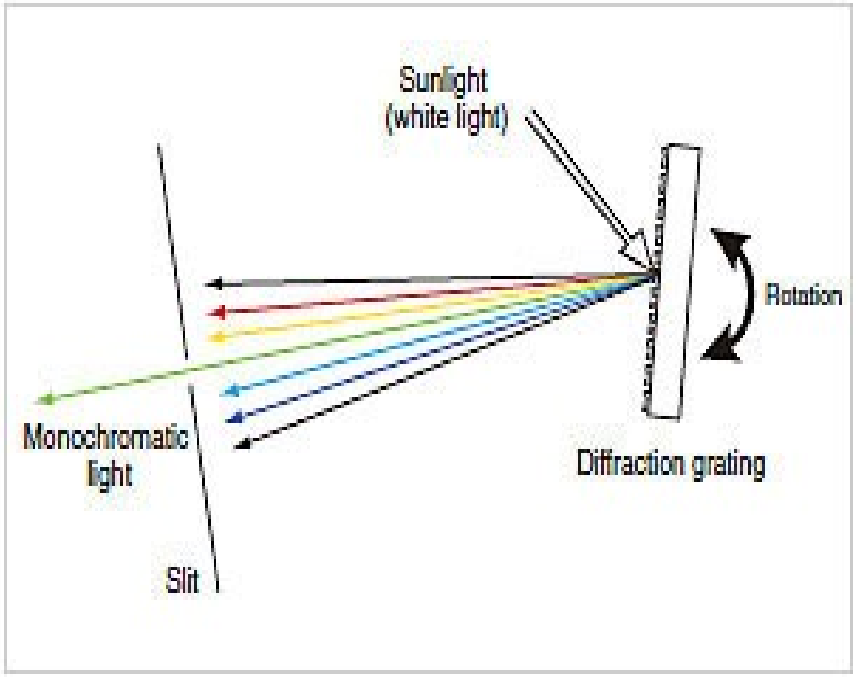
GLASS FILTER:-

- Glass filters are selectively transmit light in particular range of wavelength.

GRATINGS :

- **GRAPHITE**

- Light (Tungsten light) is reflected on graphite. This graft separate light in different wave length . By rotation of slit, desirable wave length of light come out from slit. And Beam of that wave length is generated.
- Desired wavelength selected by the adjustment of an exit slit.



CUVETTE (Sample cell):

- As per lambert – beer's law path length is fixed to 1 cm.
- Sample cell has 1 cm diameter.
- A container that contains a sample is usually called cell.

THREE TYPES OF CELL:-

1.Glass

- 340nm wavelength of light absorbed in glass cell.
- cheap

2. Quartz

- It allows passage both type of light, ultraviolet & visible ranges.
- So used for measurement of both ranges. costly.

3.Plastic cuvette

- Shorter Life Span
- Easily get Scratches
- Low Cost

PHOTOCELL (PHOTODETECTOR)

●These are the devices to measure the intensity of light by converting light energy in to electric energy.

●They are made up of light sensitive material such as selenium.

GALVANOMETER

- Readout device.
- A galvanometer is used to detect and measure electrical current produced by the photodetector.

ADVANTAGE:-

- It is very easy to operate.

DISADVANTAGES:-

- Less sensitive.
- Limited range of filters are available.
- If the light source is not stable ,there is a possibility of errors due to a change from the initial light intensity during a measurement.
- The manual operation are limited.

Spectrophotometer


E
A
B
SPECTRONIC 20D

Principle

The working of colorimeter & Spectrometers is based on Beer's & Lambert's law.

Beer's Law:-It states that the optical density of a solution is directly proportional to the concentration of the solution .

Lambert's law:-It states that the optical density of a coloured solution is directly proportional to the path of light.

According to Beer's & Lambert's law where,

T=10-kcL,

T=transmittance

K=Constant characteristic of the solution

C=concentration of the coloured solution

L=Path of light through the coloured solution

O.D.=2 – log T%

Differences: Colorimeter & Spectrophotometer

Colorimeter

- Limited for the visible portion of spectrum (visible light)
- Cheap
- Two digit reading after desimal point.
- Less sensitive
- Glass are used.

Spectrophotometer

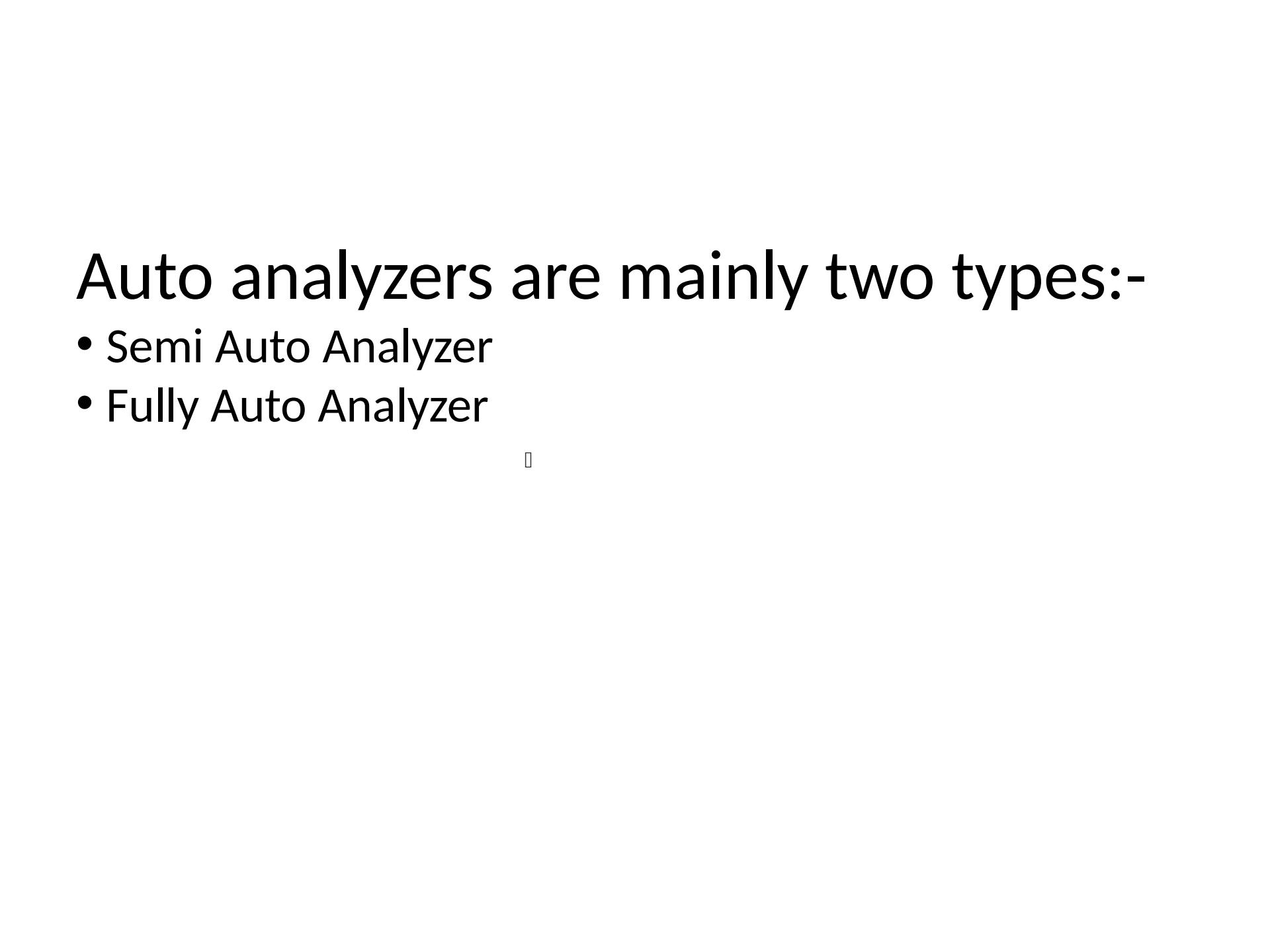
- Ultra violet & infrared region also visible e.g.340nm
- Very costly
- Four digits reading after desimal point .
- More sensitive
- Prism are used.

- Glass cuvette or test tube is used for reading which absorb 340nm light.
- Tungsten lamps are used.
- Can’t use specific filter.
- Can’t do kinetic method.

- Quartz cuvette is used which does not absorb 340nm light.
- Halogen lamps are used.
- Can use specific filter.
- Can do kinetic method.

Auto analyzers are mainly two types:-

- Semi Auto Analyzer
- Fully Auto Analyzer



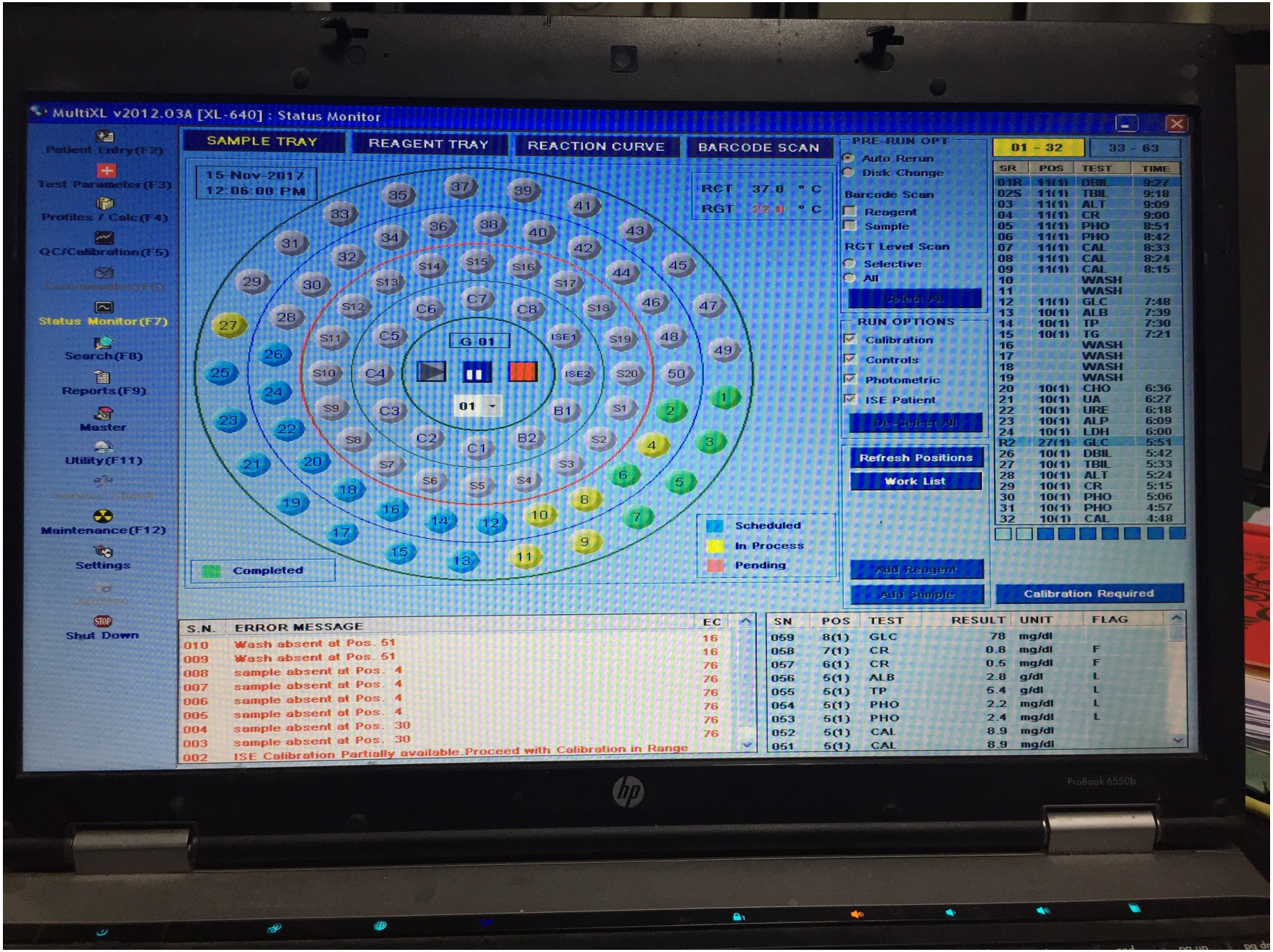
Semi Auto Analyzer

- Example : ERBA CHEM 5- PLUS

- **Advantage :**
  - Displaying the test results
  - Printing & memorizing these results
  - Graphs of all linear & nonlinear reactions.

- **Disadvantage :**
- initial stage of Analysis are performed manually
  - Pipetting of reagent
  - Pipetting of specimen
  - Mixing & incubation.
- This instrument require minimum 500 microliters of reagent for test.
- Manual L-J chart to draw


2 KVA UPS
Erba
XL-640


MultiXL v2012.03A [XL-640] : Status Monitor
Patient Entry(F2)
Test Parameter(F3)
Profiles / Calc(F4)
QC/Calibration(F5)
Consumables(F6)
Status Monitor(F7)
Search(F8)
Reports(F9)
Master
Utility(F11)
Service Check
Maintenance(F12)
Settings
Accessories
Shut Down
SAMPLE TRAY
REAGENT TRAY
REACTION CURVE
BARCODE SCAN
15-Nov-2017
12:06:00 PM
RCT 37.0 °C
RGT 22.0 °C
G 01
01
Completed
Scheduled
In Process
Pending
PRE-RUN OPT
Auto Rerun
Disk Change
Barcode Scan
Reagent
Sample
RGT Level Scan
Selective
All
Select All
RUN OPTIONS
Calibration
Controls
Photometric
ISE Patient
De-Select All
Refresh Positions
Work List
Add Reagent
Add Sample
01 - 32
33 - 63
SR POS TEST TIME
01R 11(1) DBIL 9:27
02S 11(1) TBIL 9:18
03 11(1) ALT 9:09
04 11(1) CR 9:00
05 11(1) PHO 8:51
06 11(1) PHO 8:42
07 11(1) CAL 8:33
08 11(1) CAL 8:24
09 11(1) CAL 8:15
10 WASH
11 WASH
12 11(1) GLC 7:48
13 10(1) ALB 7:39
14 10(1) TP 7:30
15 10(1) TG 7:21
16 WASH
17 WASH
18 WASH
19 WASH
20 10(1) CHO 6:36
21 10(1) UA 6:27
22 10(1) URE 6:18
23 10(1) ALP 6:09
24 10(1) LDH 6:00
R2 27(1) GLC 5:51
26 10(1) DBIL 5:42
27 10(1) TBIL 5:33
28 10(1) ALT 5:24
29 10(1) CR 5:15
30 10(1) PHO 5:06
31 10(1) PHO 4:57
32 10(1) CAL 4:48
Calibration Required
S.N. ERROR MESSAGE EC
010 Wash absent at Pos. 51 16
009 Wash absent at Pos. 51 16
008 sample absent at Pos. 4 76
007 sample absent at Pos. 4 76
006 sample absent at Pos. 4 76
005 sample absent at Pos. 4 76
004 sample absent at Pos. 30 76
003 sample absent at Pos. 30 76
002 ISE Calibration Partially available.Proceed with Calibration in Range
SN POS TEST RESULT UNIT FLAG
059 8(1) GLC 78 mg/dl
058 7(1) CR 0.8 mg/dl F
057 6(1) CR 0.5 mg/dl F
056 5(1) ALB 2.8 g/dl L
055 5(1) TP 5.4 g/dl L
054 5(1) PHO 2.2 mg/dl L
053 5(1) PHO 2.4 mg/dl L
052 5(1) CAL 8.9 mg/dl
051 5(1) CAL 8.9 mg/dl
ProBook 6550b


WARNING
CONTAINS HAZARDOUS MATERIALS
SERUM, PLASMA AND URINE
CAUTION
DO NOT OPERATE MACHINE WITH COVER OPEN.
DO NOT TOUCH MOVING PARTS WHEN IN OPERATION.PERSONNEL INJURY MAY RESULT DUE TO PROBE ARM MOVEMENT.
TO REDUCE DAMAGE TO THE INSTRUMENT, DO NOT SPILL SAMPLE OR REAGENT ON THE MACHINE.

Fully Auto Analyzer

The auto analyzer perform all the function of semi auto analyzer.

1.Automatic dispensing of reagent (by reagent probe).

2.Automatic dispensing of samples .

3.Automatic mixing of reaction mixtures.

4.Incubating of reacting mixture .

Advantages:-

- Many samples with different parameter can analyzed at time.
- Good precision
- Less reagent required.
- Less sample required.
- Less man power required .
- Maintain the temperature
- For Sample & For Reagent
- For incubation period.
- Can stored result in memory.
- It have facility to accommodate various samples, standards, calibrations & Q.C. Sera.
- Automated L-J Chart is visible
- Programmable wash cycles between samples & tests for minimum carry over.
- Auto dilution is also possible

Two types of fully auto analyzer:-

- Batch analyzer
- Random analyzer

Random Access analyzer

- Perform Any number of Parameter from any number of sample.
- More sample in the short period of time .
- Facility of continuous loading of sample
- Facility of “stat” analysis - Urgent sample.
- Facility of autodilution.
- Plotting of daily & monthly Q.C. Chart (L-Jchart).
- Capability to perform a test with 2 to 3 reagents.
- Some of the analyzer has separate assembly to wash cuvette so very less chance of contamination.

Example : ERBA XL 640