



An Introduction to in vitro Diagnostics Technology

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In vitro Diagnostics: From Uroscopy to PCR





Mission of the Department

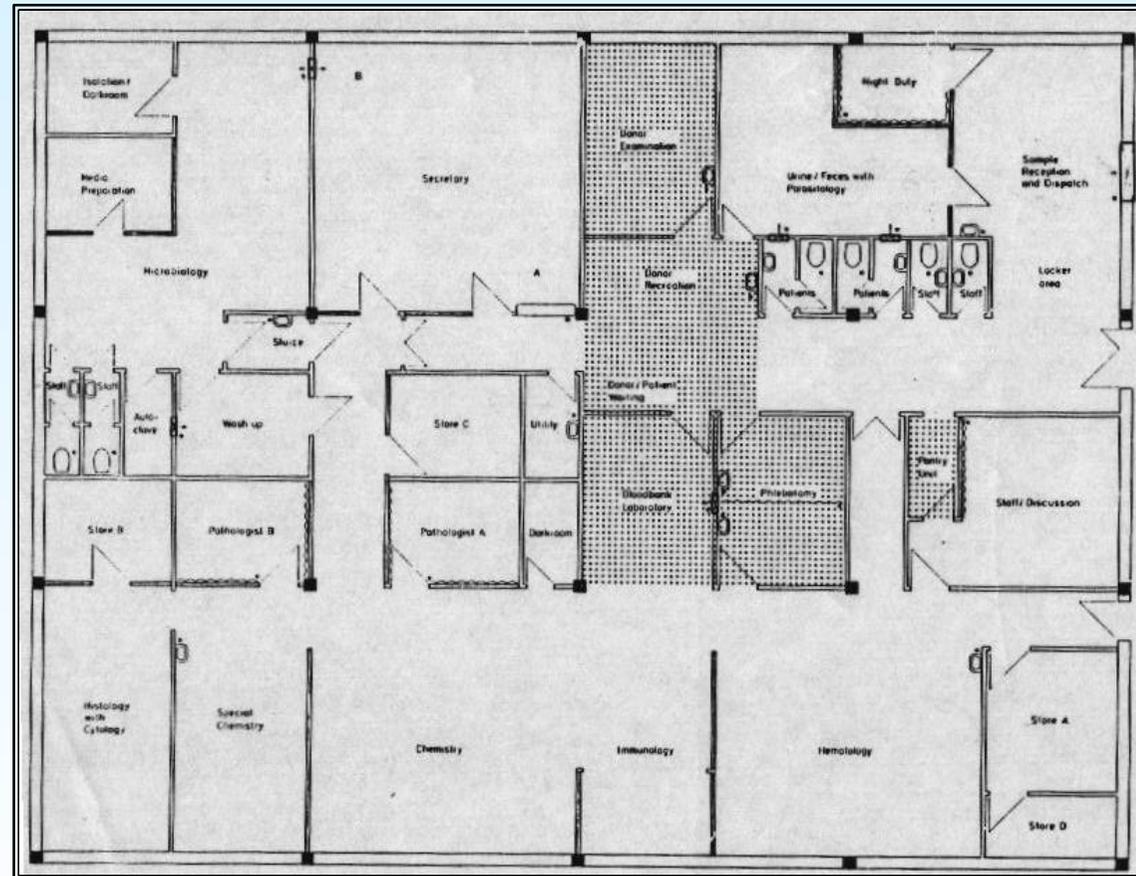
- The primary function of clinical laboratories is to provide accurate and timely information, based on in vitro probes examinations, for the diagnosis, monitoring and treatment of the patient.
- Qualitative and quantitative tests in body fluids, tissues and products and drug-monitoring provide the ground for medical reasoning.
- The Laboratories and Units that constitute the in vitro Diagnostics Department of a general Hospital, as well as, the major methods and corresponding equipment used in these laboratories are presented.

Internal Structure



- Clinical Biochemistry.
- Serology.
- Immunology.
- Hematology.
- Microbiology.
- Histopathology.
- Cytology.
- Blood Bank.

Typical Layout





Clinical Biochemistry

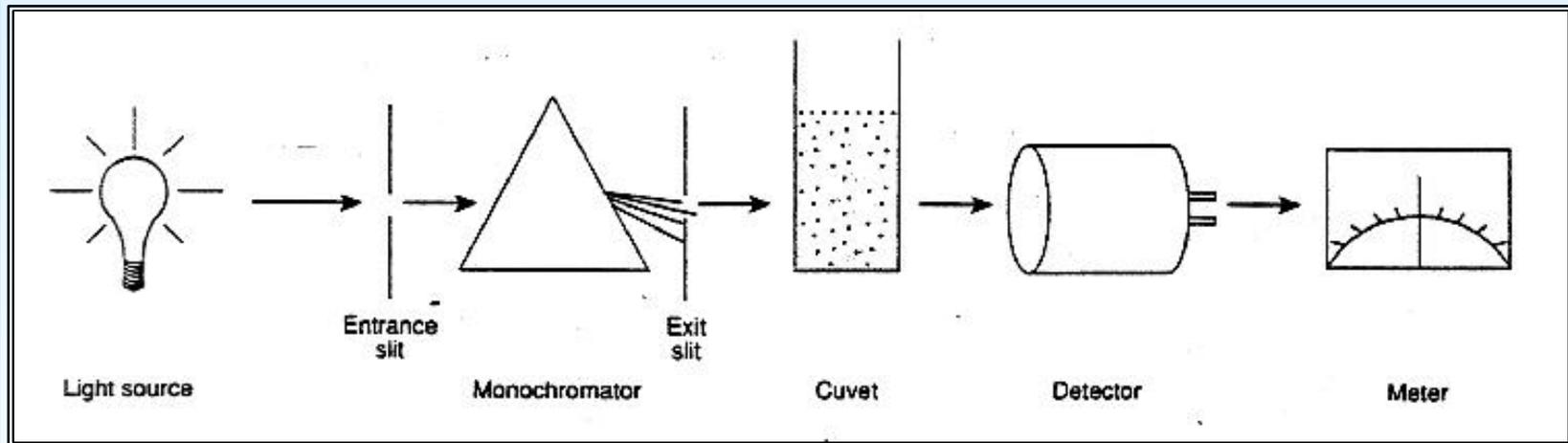
- *Clinical Biochemistry* (also *Serology* & *Immunology*), are concerned with the analysis of biochemical composition of body fluids, functions of tissues and the monitoring of administered drugs, mainly in blood (*Toxicology*).
- The methods used comprise of:
 - ◆ *Spectrophotometry (IR, VIS & UV)*.
 - ◆ *Electrochemical methods*.
 - ◆ *Separation techniques LC, GC, HPLC, TLC, etc.*
 - ◆ *Immunoassays RIA, ELISA, LIA, IFA, PIF, etc.*



Spectrophotometry

- *Spectrophotometry* is the major analytical method and includes also *Fluorometry*, *Flamephotometry*, *Plasma Emission Spectrophotometry*, and *Atomic Absorption Spectrophotometry* in the corresponding Autoanalyzers.
- These methods are based upon the physical principles of concentration determination through:
 - ◆ *The measurement of the light spectral lines (IR, VIS & UV) absorption in liquid or atomized gas samples.*
 - ◆ *The measurement of the intensity of the characteristic light spectral lines, emitted by excited atomized gas or vapour samples.*

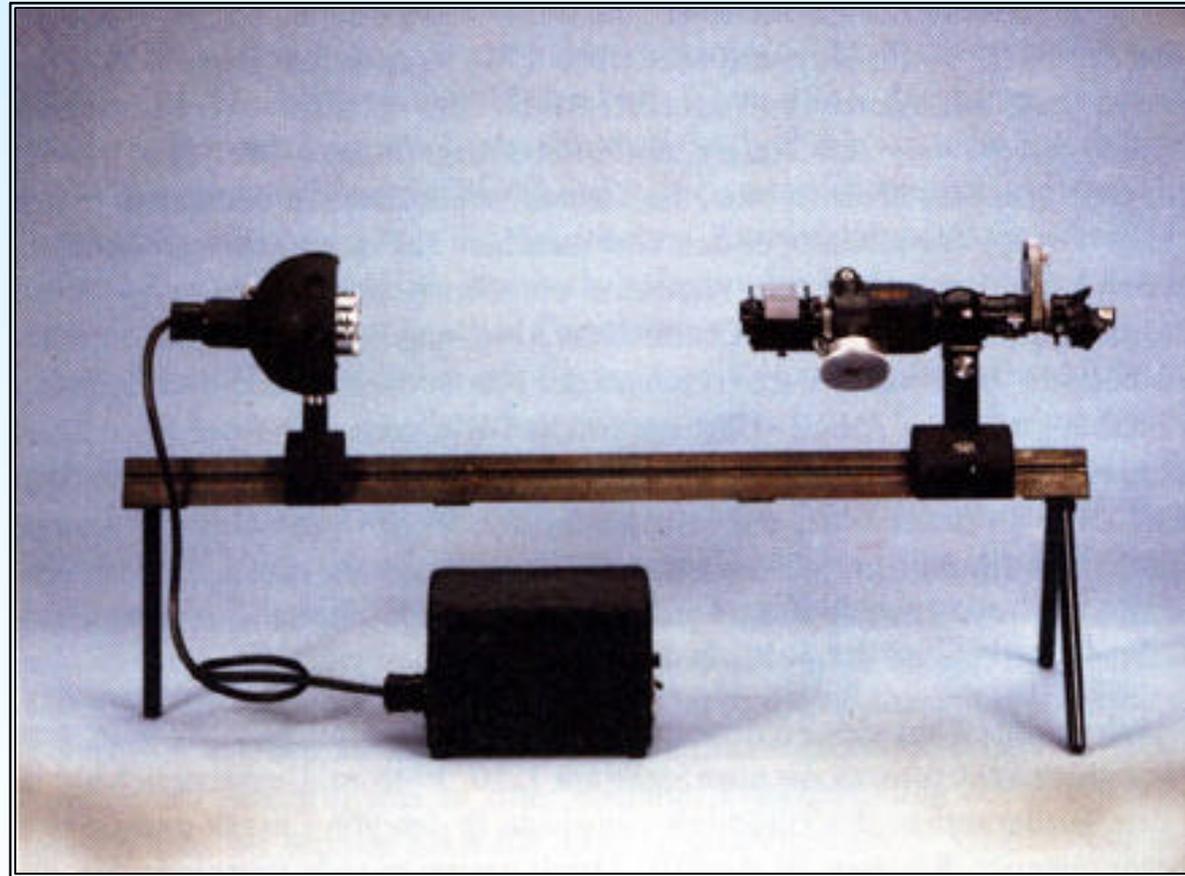
Spectrophotometry VIS, UV, IR



Clinical Chemistry Laboratory Charite, Berlin, around 1900



Spectrophotometer Carl Zeiss Jena, 1935



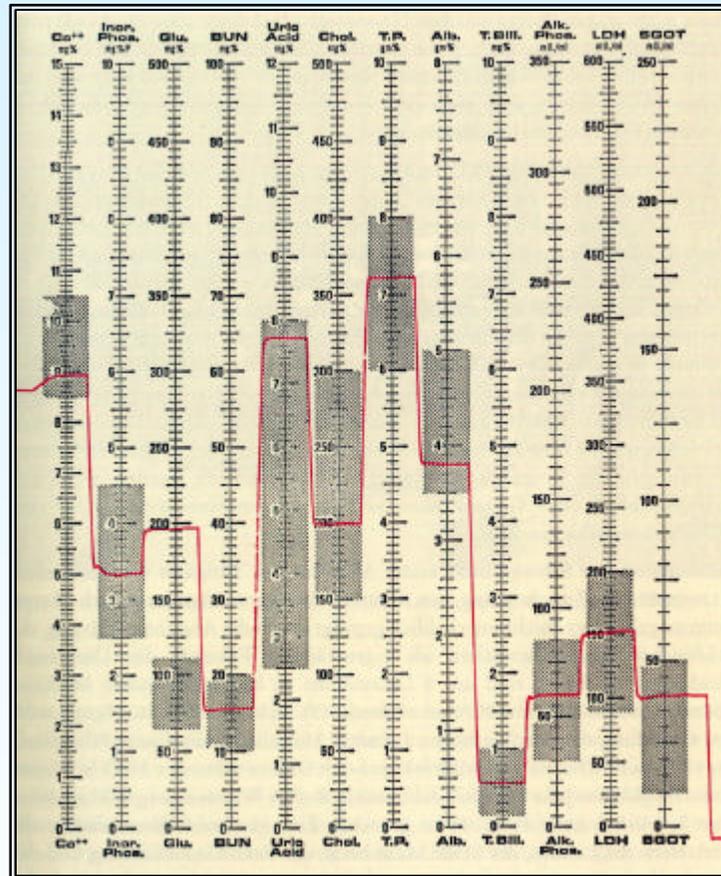
Spectrophotometer Eppendorf, 1950



Contemporary semi-automatic Spectrophotometer



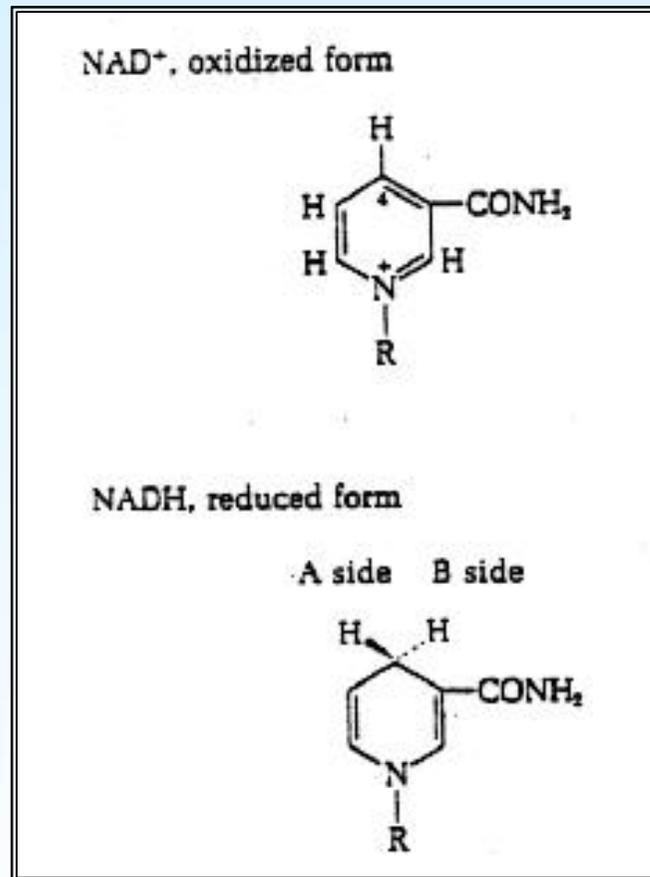
Biochemical profile of one of the first Autoanalyzers (Technicon SMA-12)



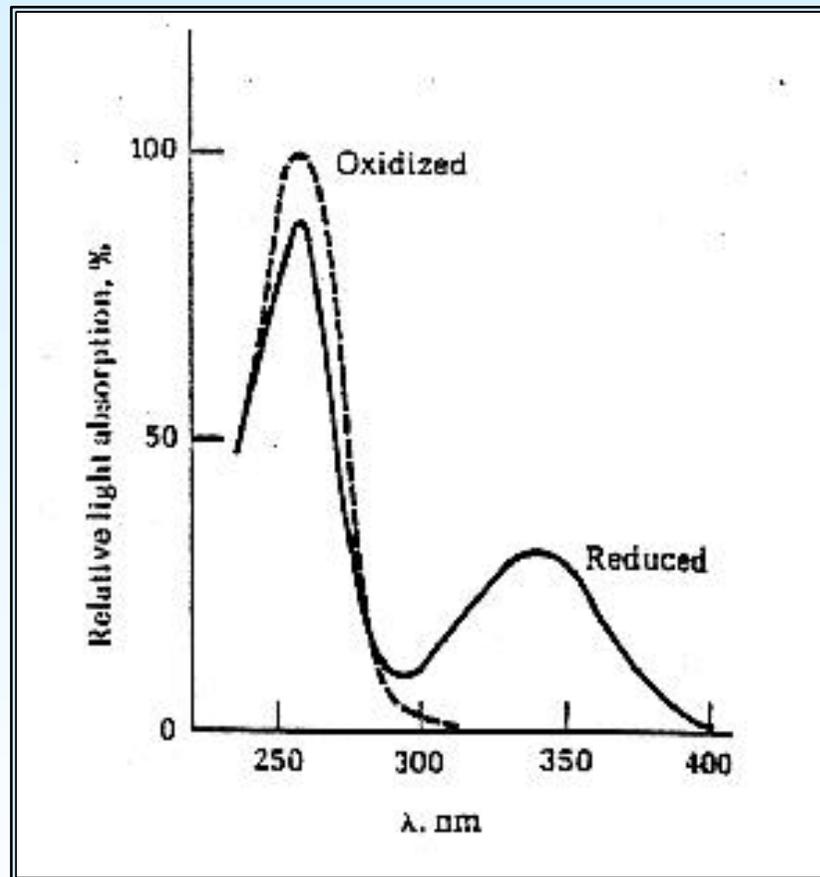
Sample introduction and measurement in a modern Autoanalyzer



The meaning of the Reagent: Oxidized and Reduced form of the NAD^+/NADH



Spectral Absorption Curves of NAD^+/NADH in aquatic solution





Glucose determination procedure (GLU)

HK

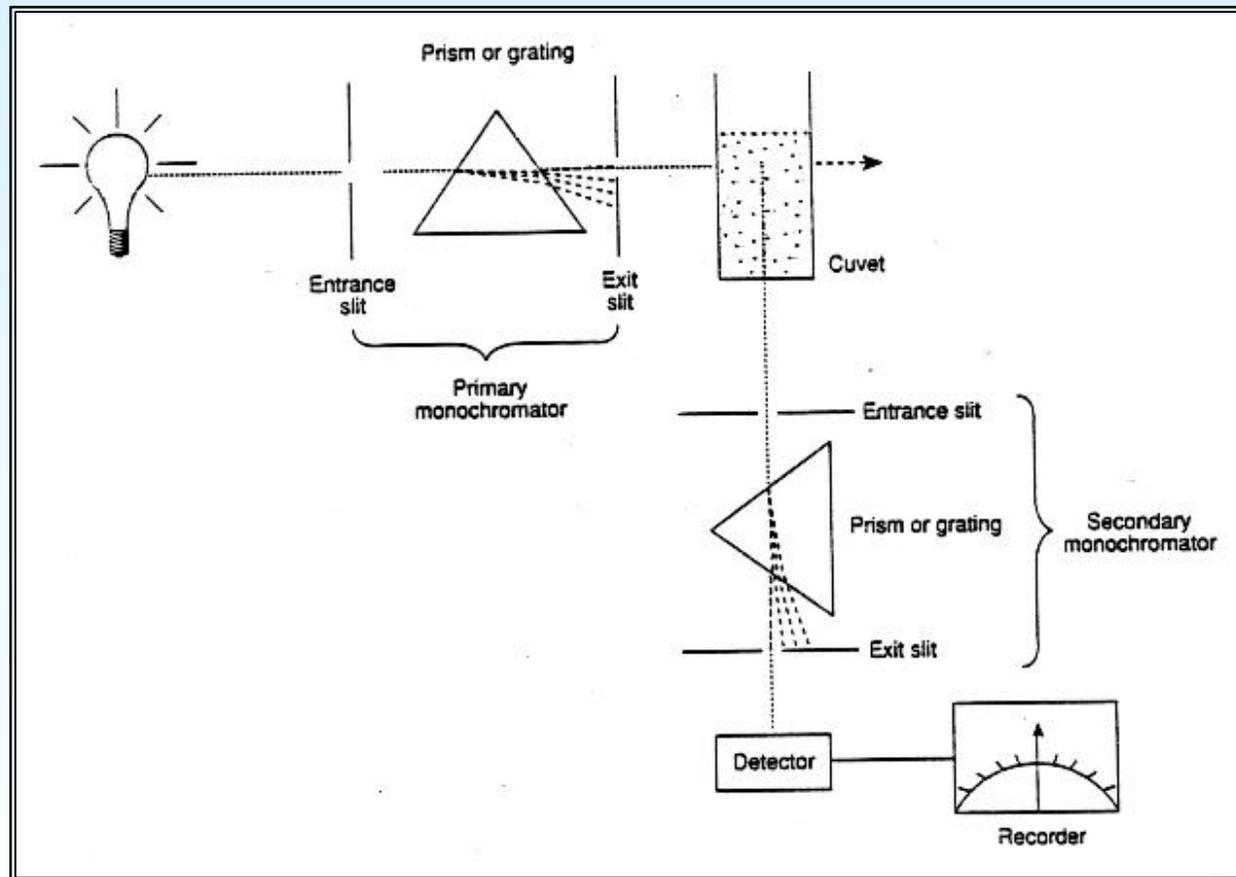


G6-P-DH



- The Molecular Correspondance NADPH / GLU is 1:1.
- The determination of the NADPH concentration is equal to the corresponding one of GLU.

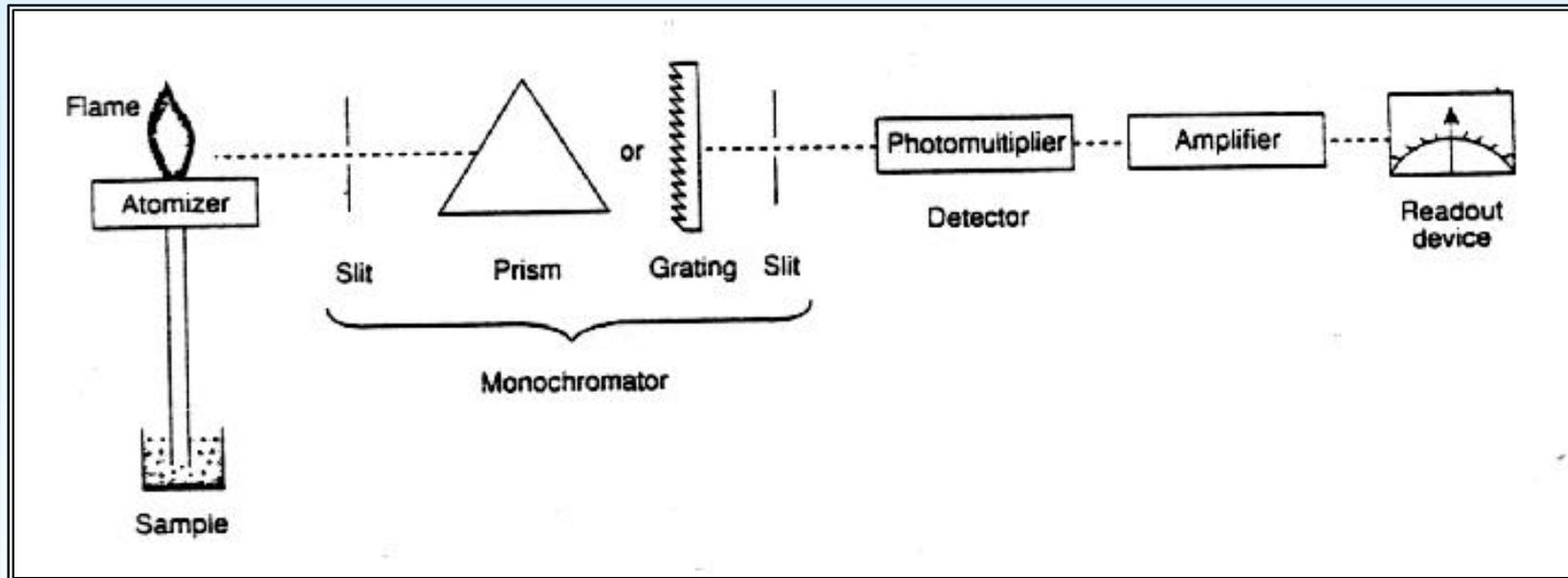
The principle of Fluorometry



Automatic Fluorimeter



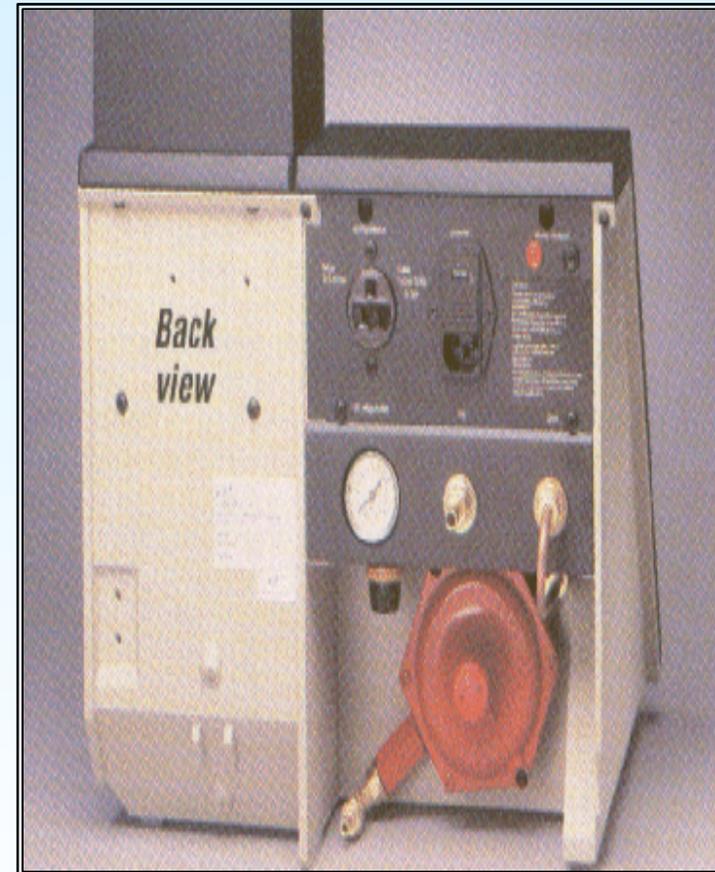
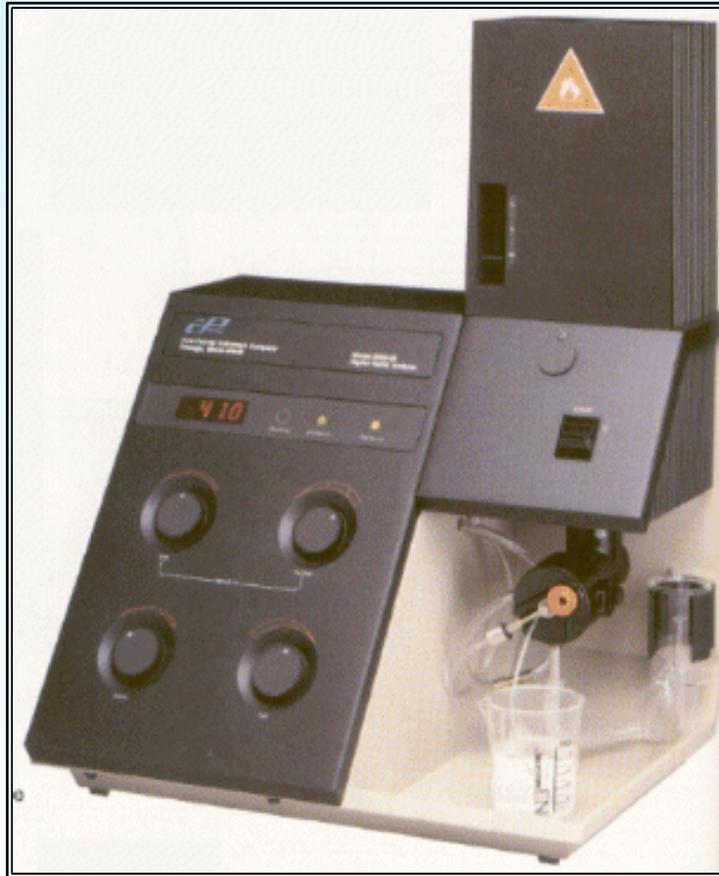
The principle of Flame-photometry



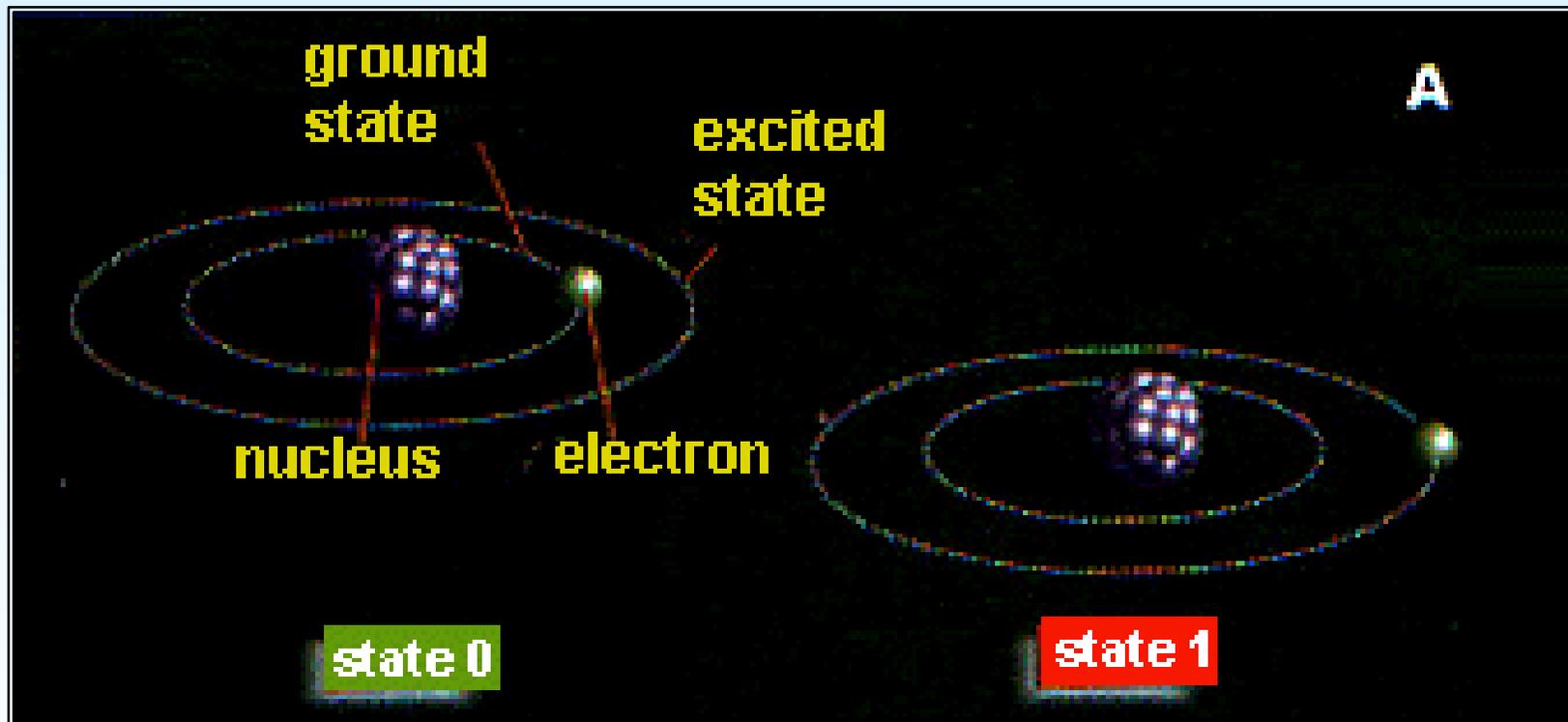
One of the first Flame-photometers



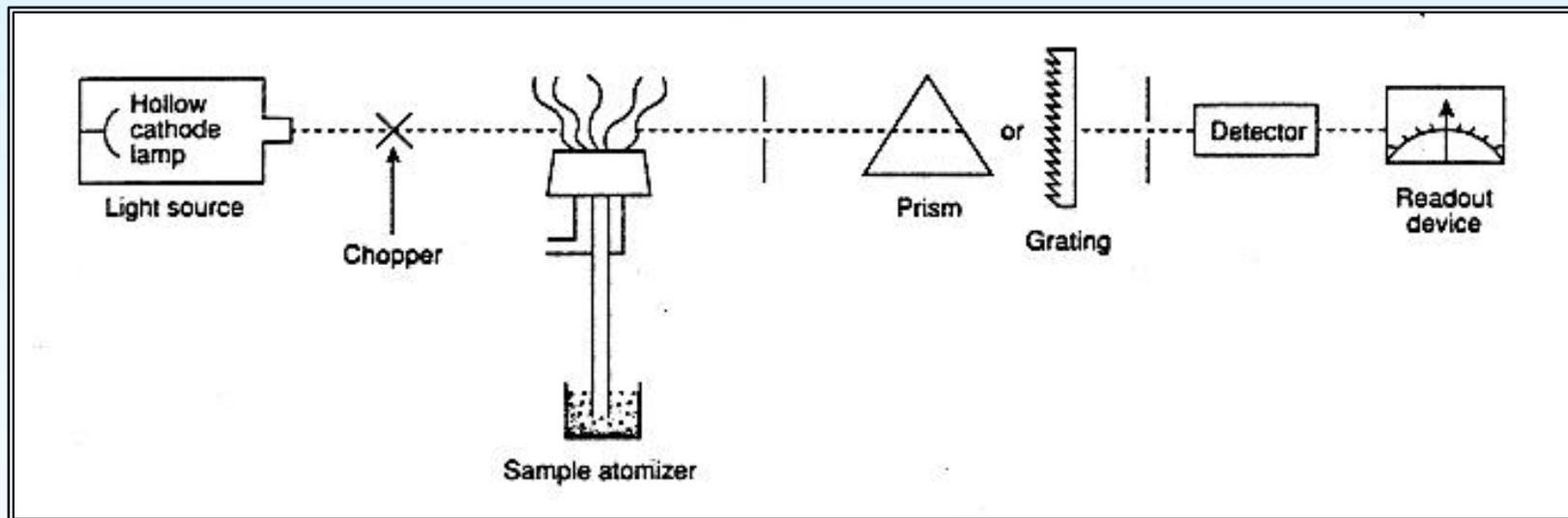
Simple general use Flame-photometer



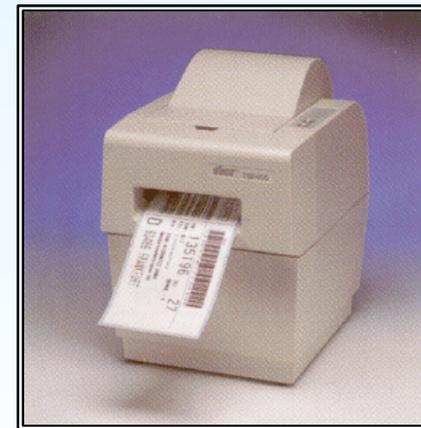
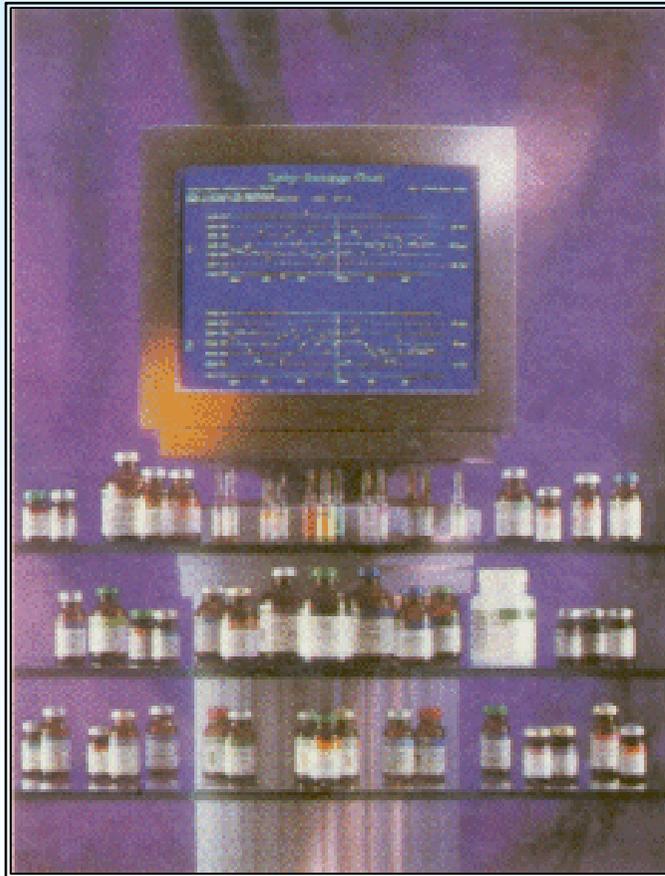
Atomic Absorption



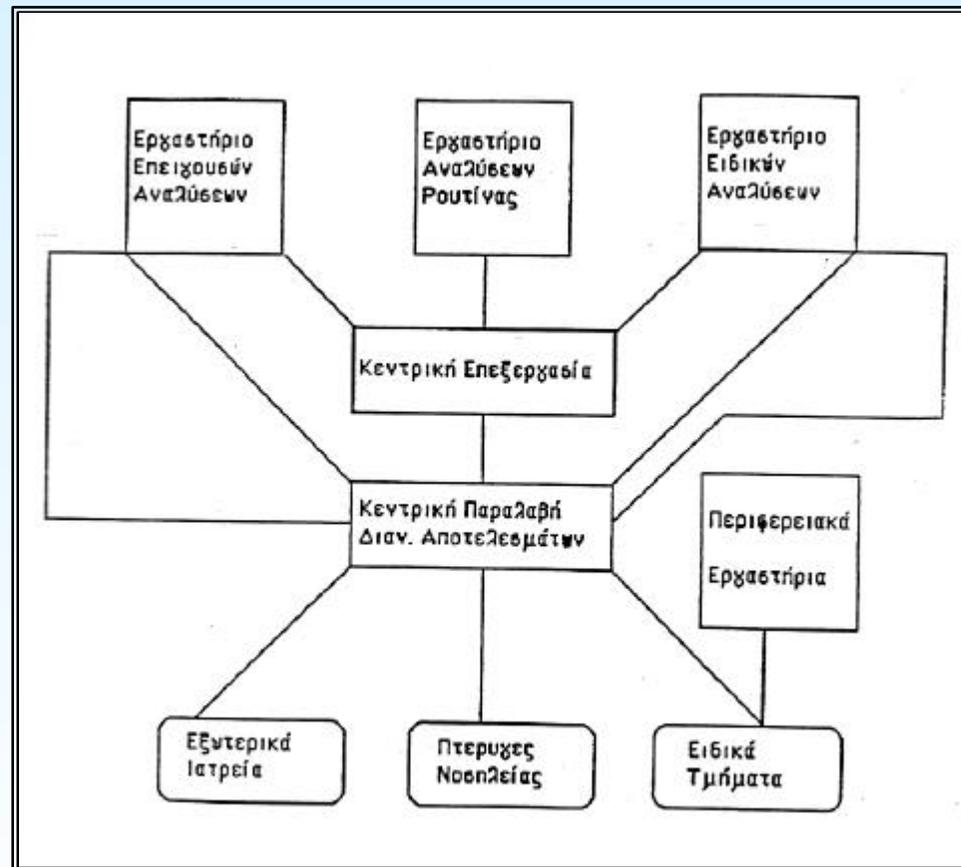
Atomic Absorption Photometer: Block Diagram



Laboratory Load Management Techniques



Structure of a Clinical Chemistry Laboratory

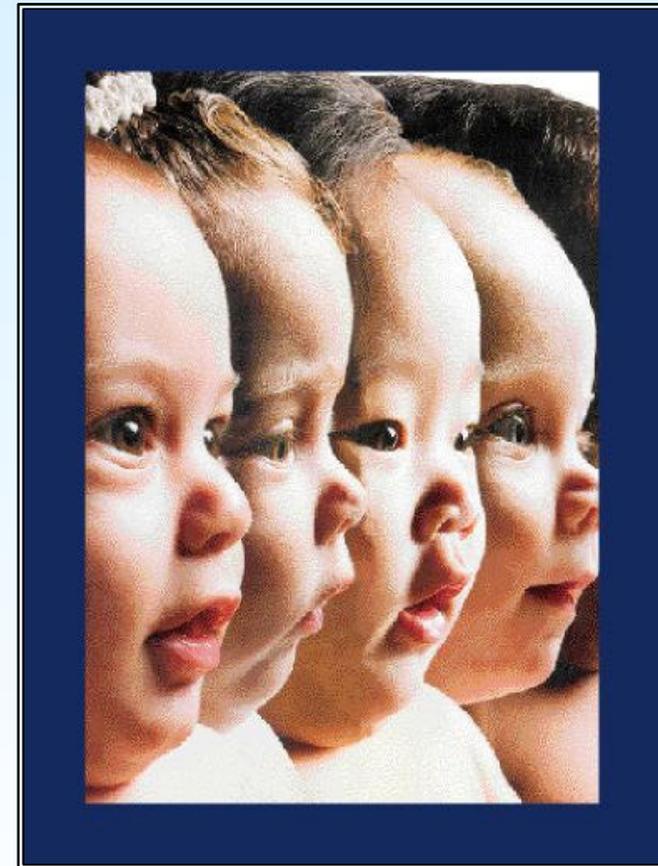
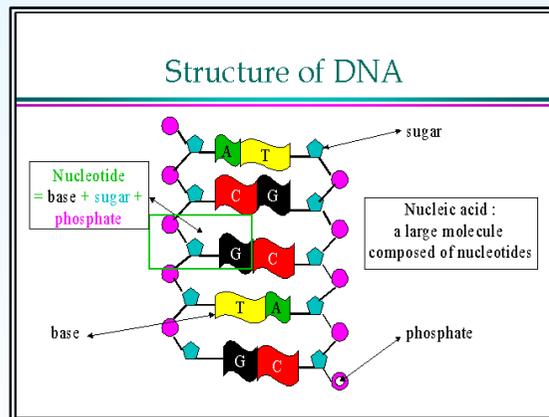
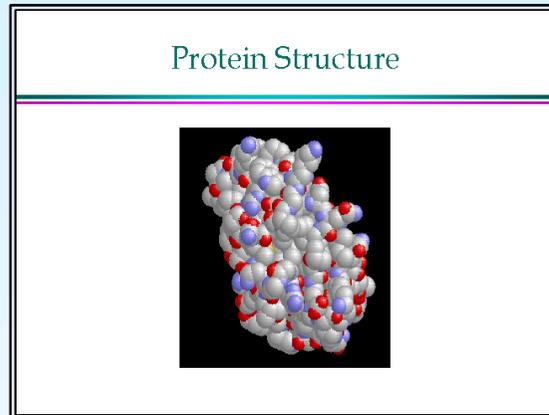


Typical menu of a highly automated Emergency Clinical Laboratory

| | | | |
|--|---|---|--|
| Αριθ. ασθενούς..... Ονομασία..... Διαγνώση..... Ημερ. γεννηθείας..... Ημερ. εισαγωγής..... | | ΑΣΦΑΛΙΣΤ. ΟΡΓΑΝΙΣΜΟΣ ΙΚΑ <input type="checkbox"/> ΟΓΑ <input type="checkbox"/> ΤΕΒΕ <input type="checkbox"/> <input type="checkbox"/> Κωδικός Ασθμ..... Παραπομπή..... | |
| ΚΛΙΝΙΚΗ / ΙΑΤΡΟΣ Αρθμ. Τηλ..... | ΥΛΙΚΟ ΔΕΙΓΜΑΤΟΣ Αίμα <input type="checkbox"/> Πύρεμα <input type="checkbox"/> Ούρα <input type="checkbox"/> Ερώδες <input type="checkbox"/> | ΕΡΩΤΗΣΗ/ΔΙΑΓΝΩΣΗ Ημ/γ.μ.ε. | |
| ΑΠΘΗΝ ΕΞΕΤΑΣΙΣ / ΕΡΓΑΣΤΗΡΙΟ ΕΠΕΛΑΤΗΣ ΔΙΑΓΝΩΣΗΣ | | | |
| 101 Γλυκόζη..... | | 121 Νο..... | |
| 102 Ουρία..... | | 122 Κ..... | |
| 103 Κρεατιν..... | | 123 Σε..... | |
| 104 Χοληστερ..... | | 124 Cl..... | |
| 105 Χοληρυθρ..... | | 131 Quick..... | |
| 106 Ο2.Πρωτ..... | | 132 ΡIT..... | |
| 107 Αιζονορ..... | | | |
| 111 CK..... | | 141 Λευκά..... | |
| 112 LDH..... | | 142 Ερυθρά..... | |
| 113 GOT..... | | 143 Hb..... | |
| 114 GPT..... | | 144 Ht..... | |
| 115 γ-GT..... | | 145 Αιμάθροτ..... | |
| 116 AP..... | | | |
| 117 Αμυλάση..... | | 151 Αέρια Αίμ..... | |



Molecular Biology: From the Protein to the DÍ Á in vitro Diagnostics

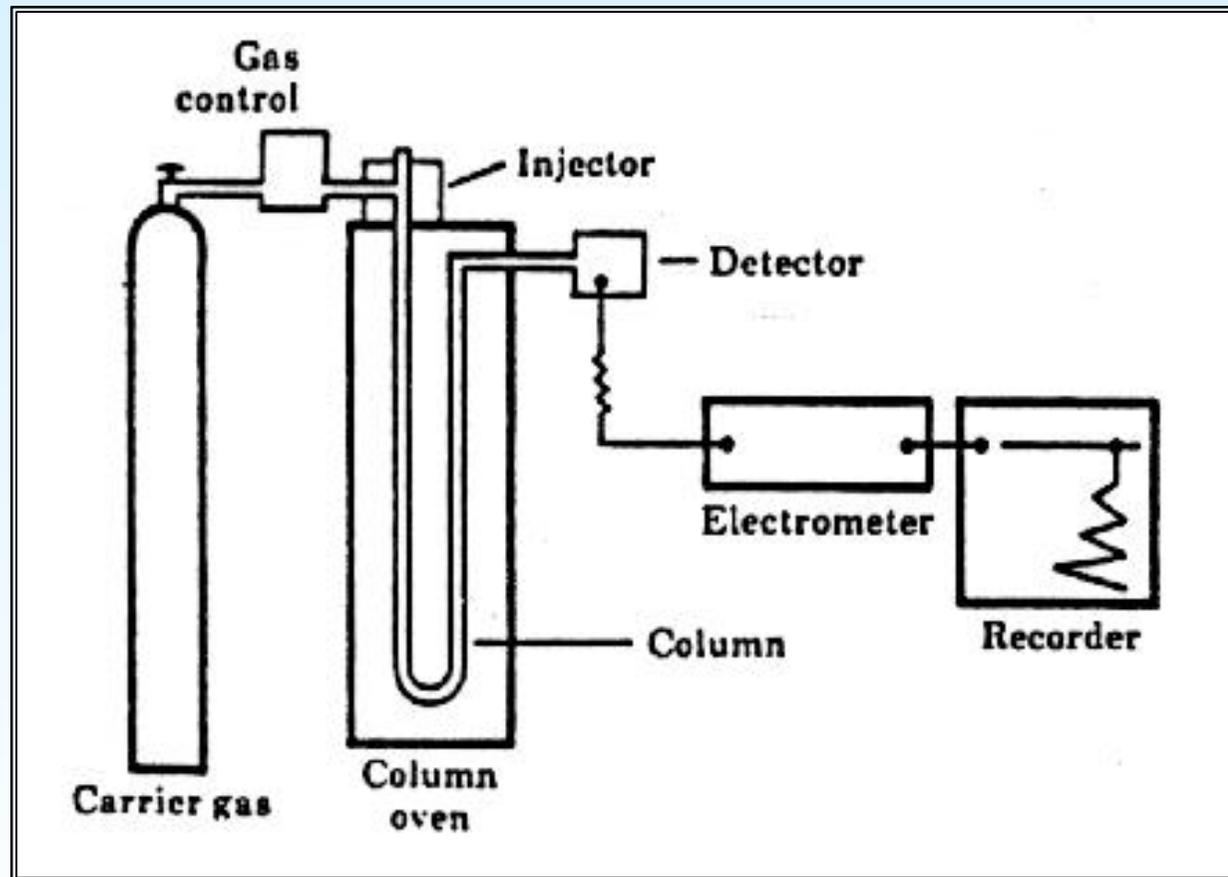




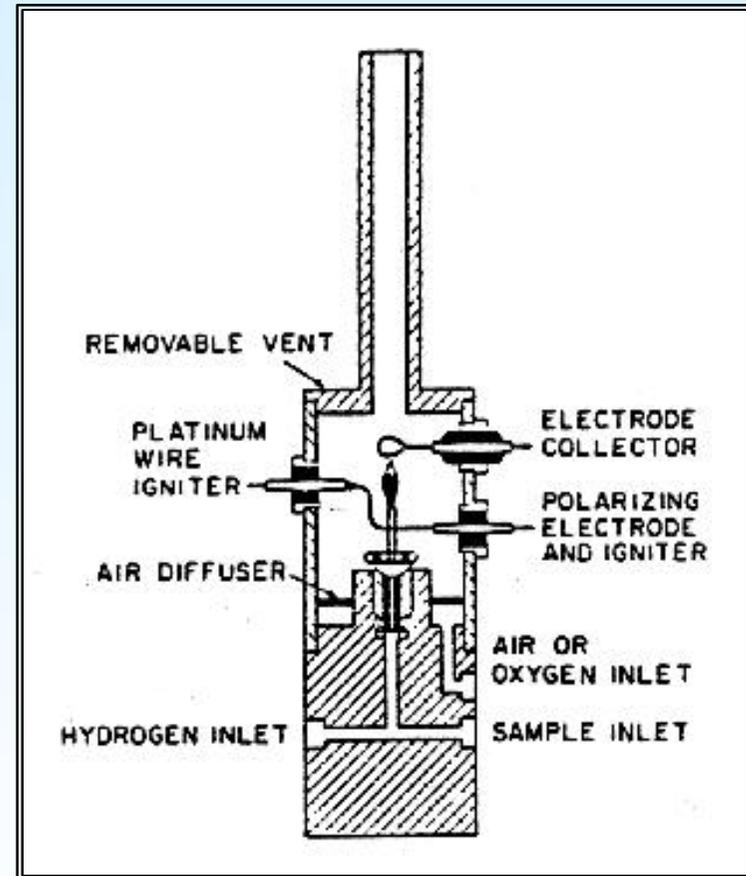
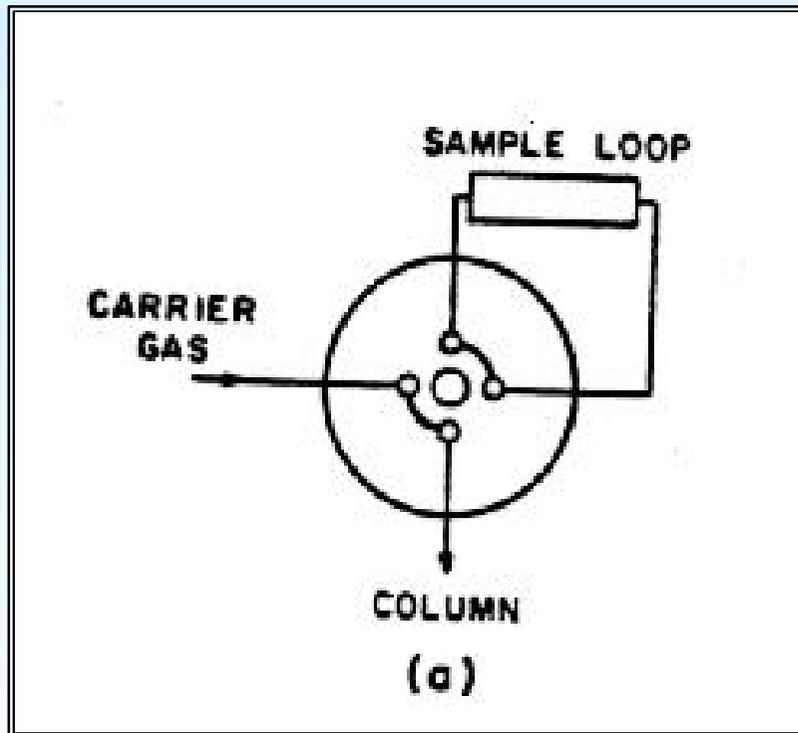
Separation techniques

- Separation techniques include several types of *Gas, Liquid and Thin Layer Chromatography*, and *Electrophoretic Techniques*.
- Main task is the separation of mixtures of proteins or other substances and the determination of the concentration of each component.
- Separation is achieved by forcing the mixture to flow through or run upon a solid or liquid phase and by taking advantage of the different speed each component develops during the procedure, because of the different size, ionization, chemical affinity, electrical motility etc. of the molecules or ions that constitute each component.

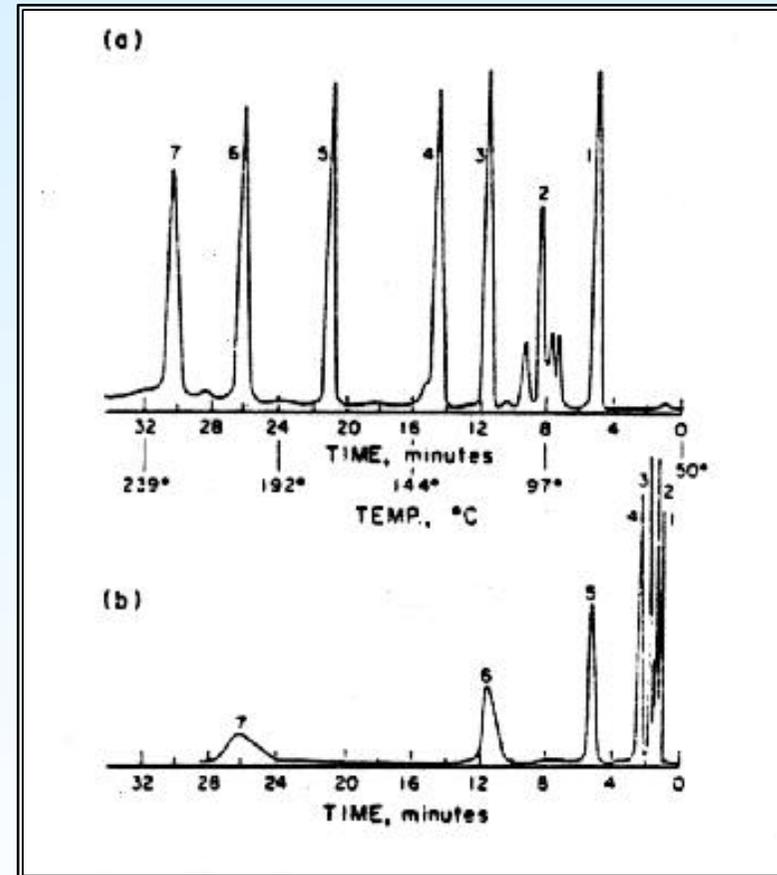
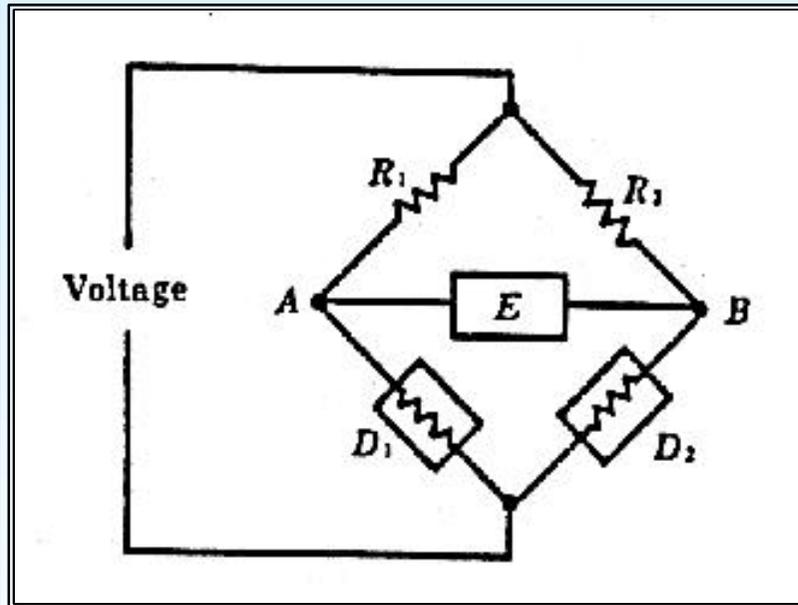
Gas Chromatography (GC)



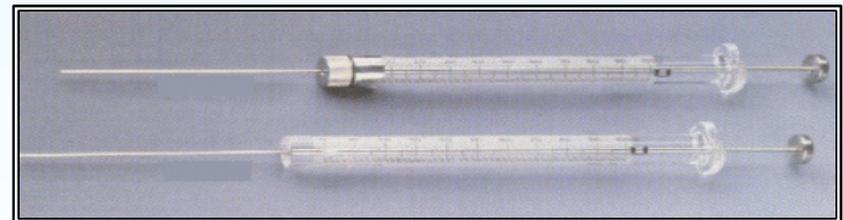
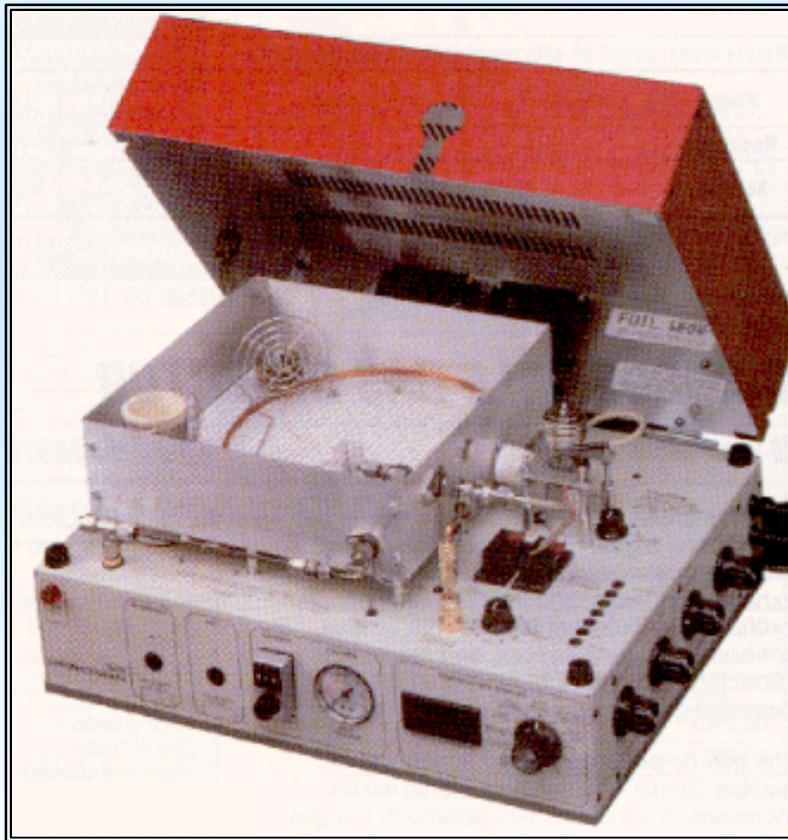
Gas Chromatography: Sample introduction into a Flame Ionization Detector (FID)



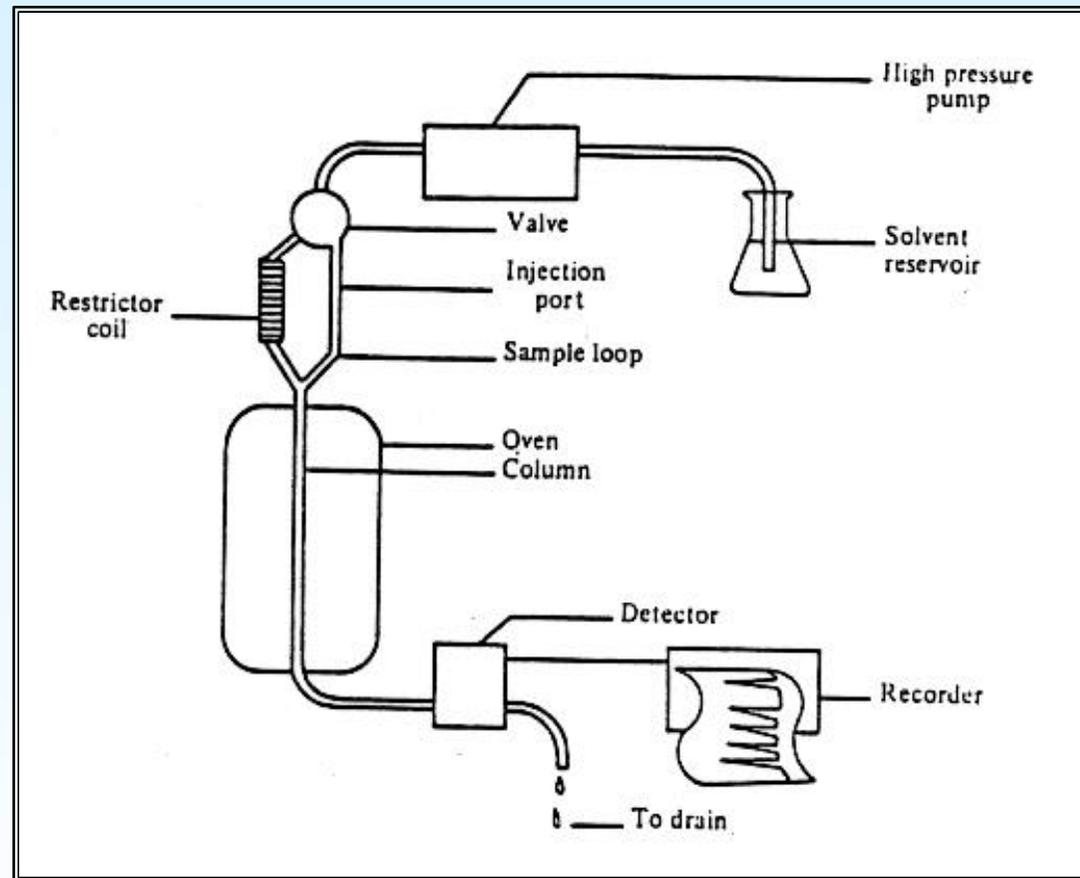
Gas Chromatography: Thermal Conductivity Detector (TCD)



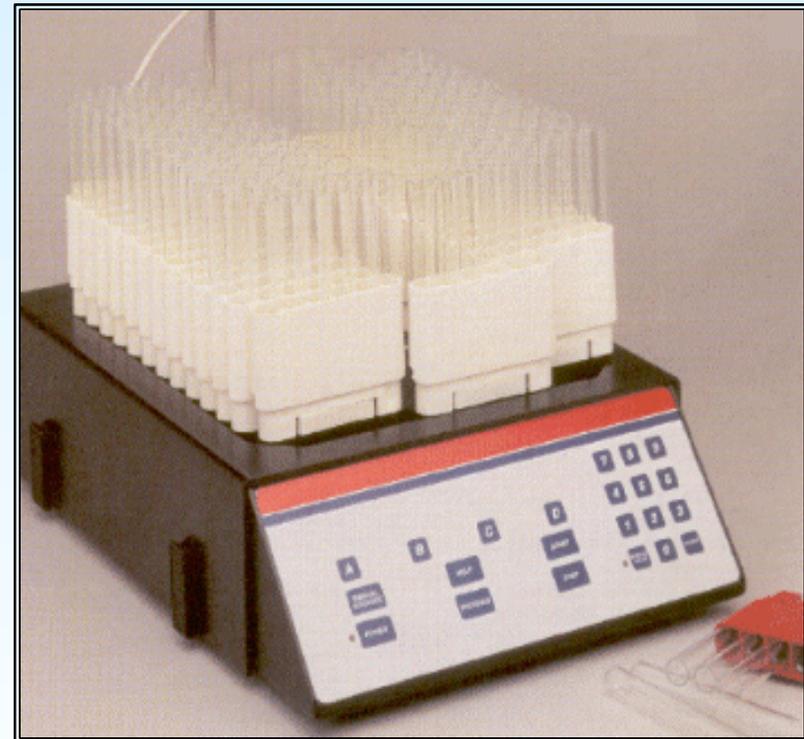
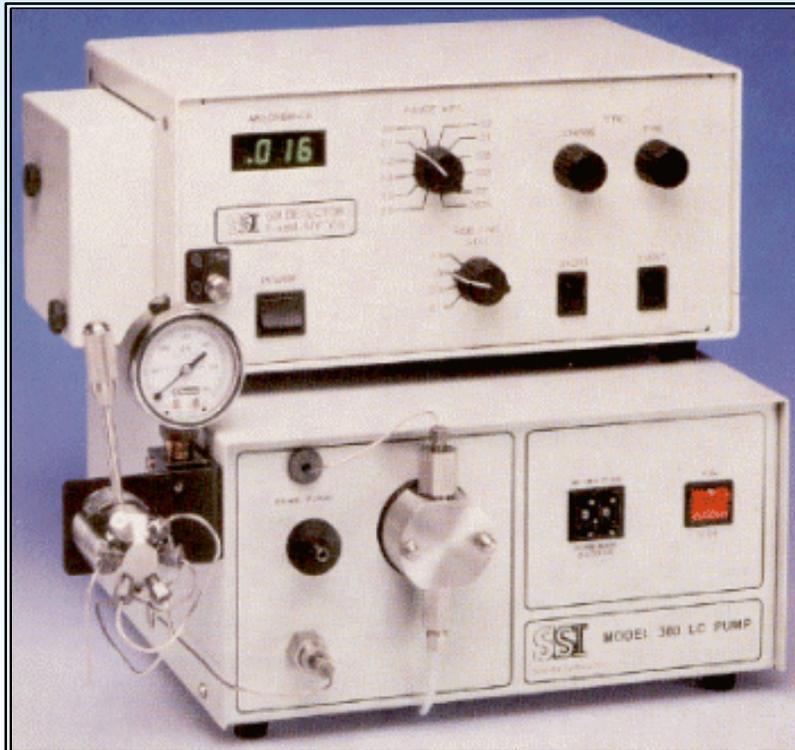
Typical Gas Chromatography System (GC)



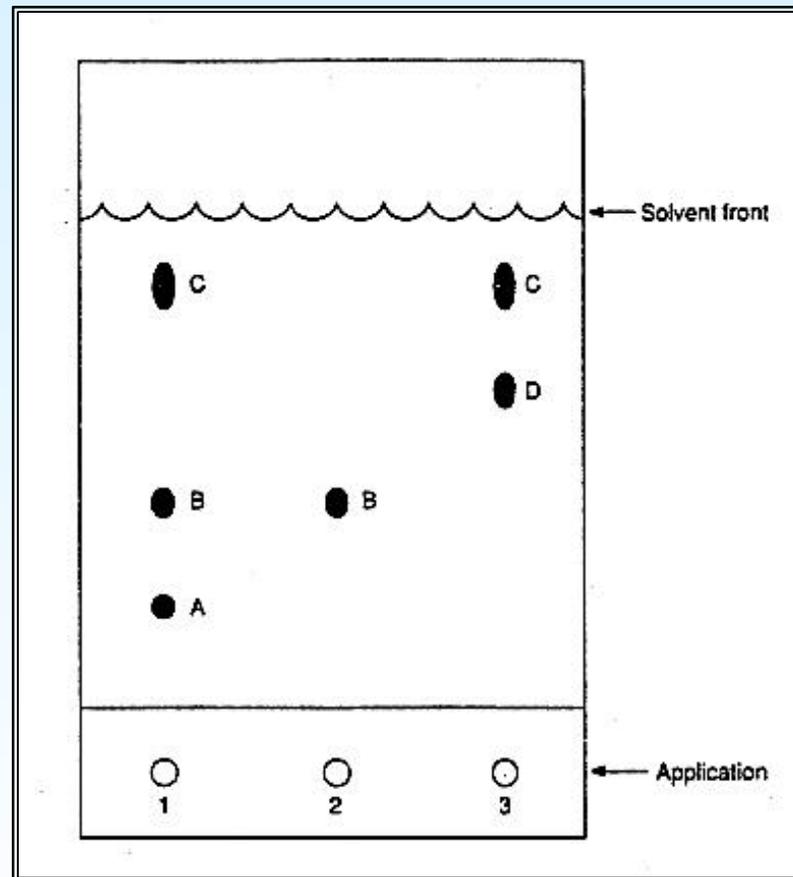
Principle of the High Performance Liquid Chromatography (HPLC)



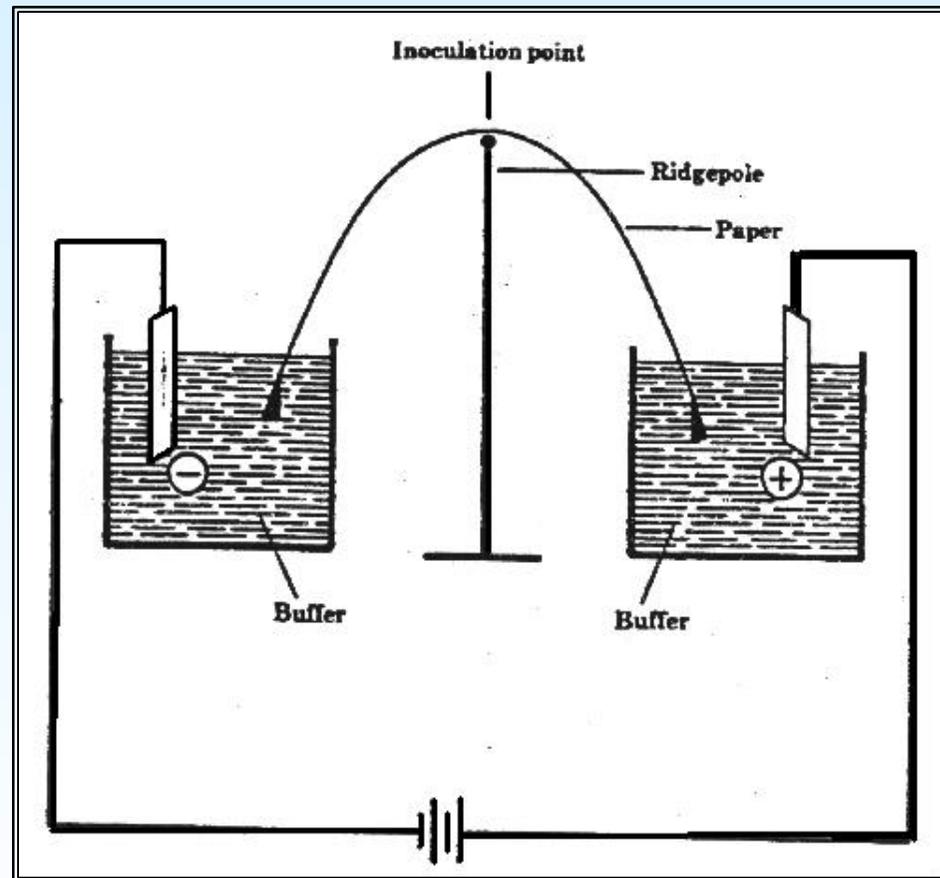
Typical High Performance Liquid Chromatography System (HPLC)



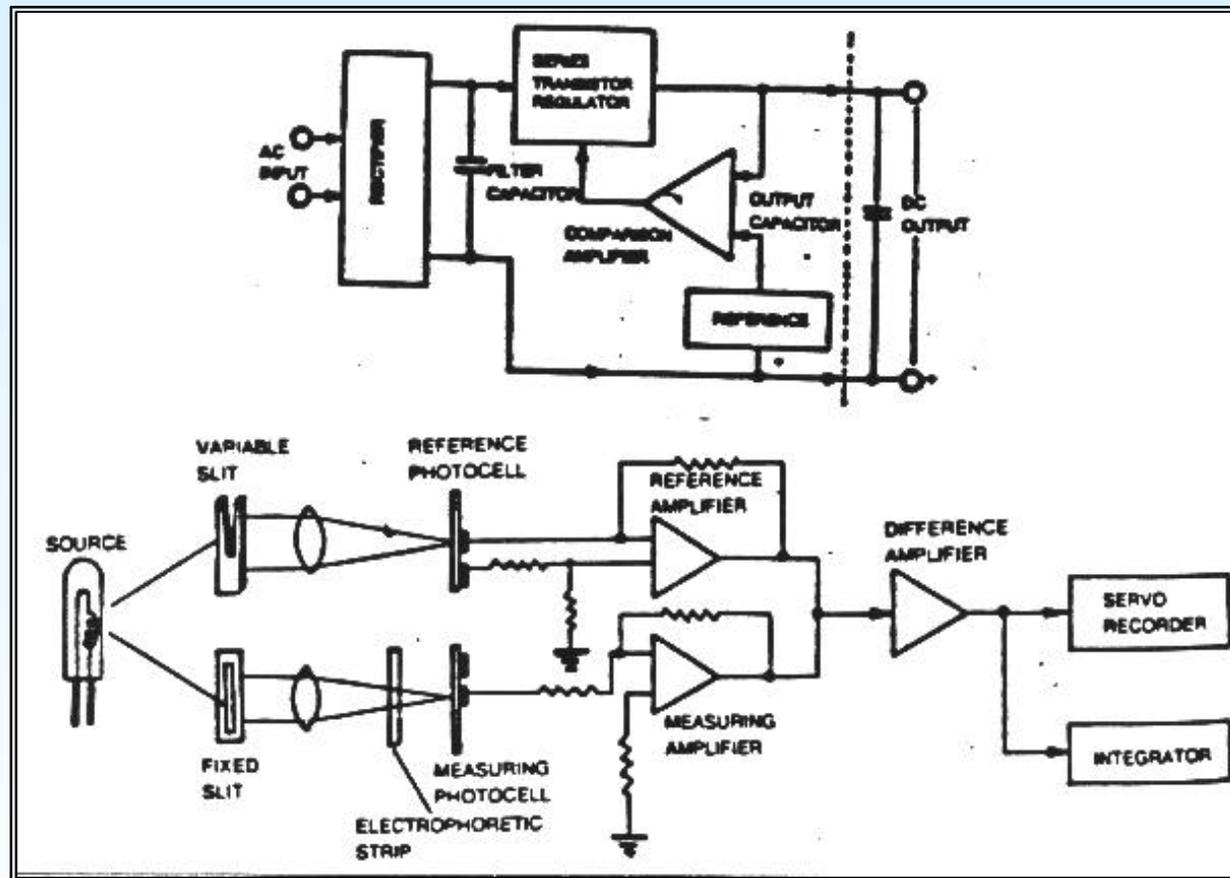
Principle of the Thin Layer Chromatography (TLC)



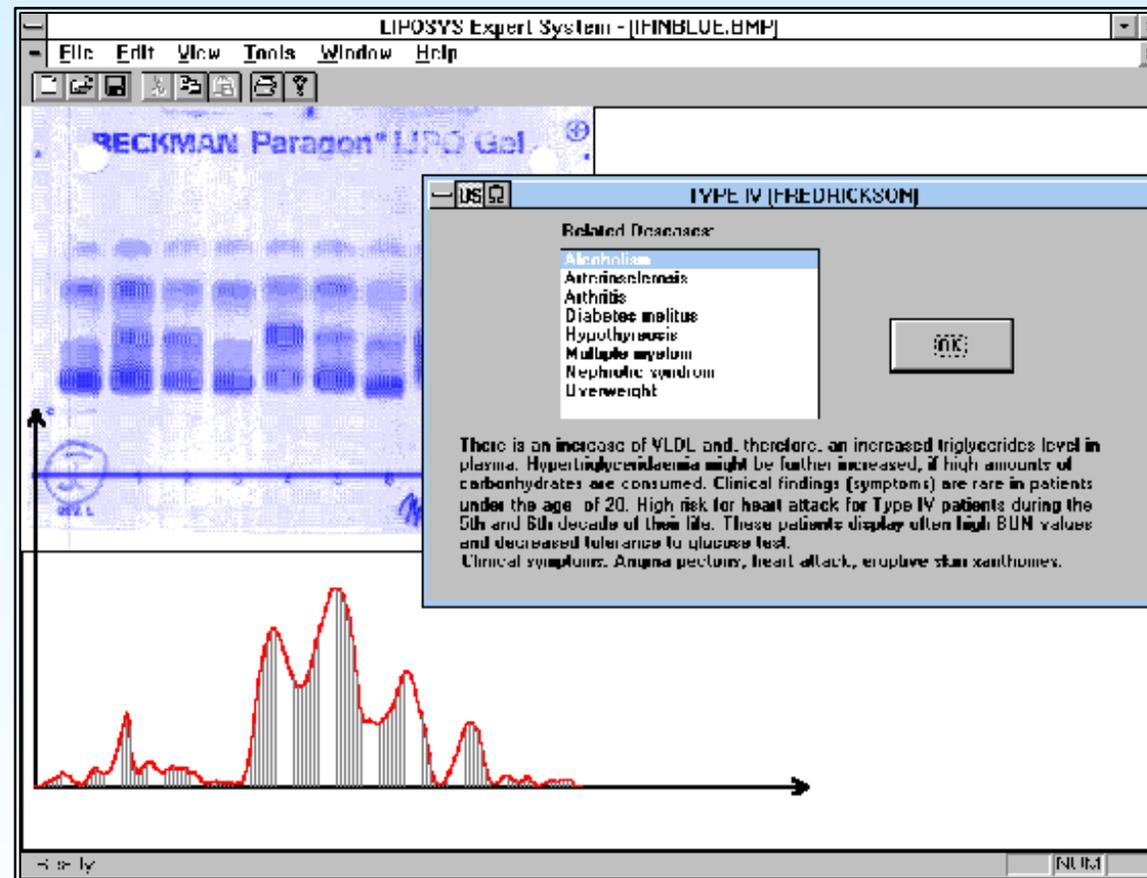
The principle of the Electrophoretic Techniques



Power Supply and Gel Evaluation System



Automatic Gel Evaluation and Decision Supporting System

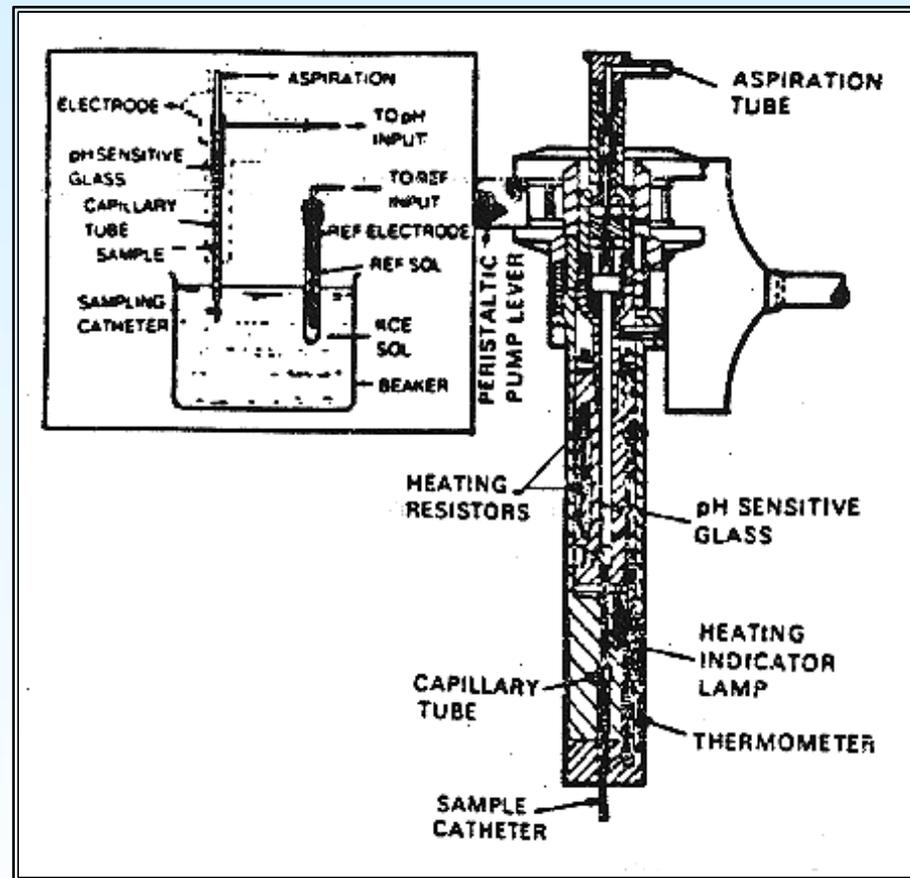




Electrochemical methods

- *Electrochemical methods include pH-meters, Ion Selective Electrodes, Conductivity Measuring Systems and Blood Gas Analyzers.*
- *Ion or dissolved gas concentration are determined, by measuring the variation of the corresponding output voltage or current, evoked each time on suitable electrochemical transducers, such as electrodes outfitted with appropriate membranes.*

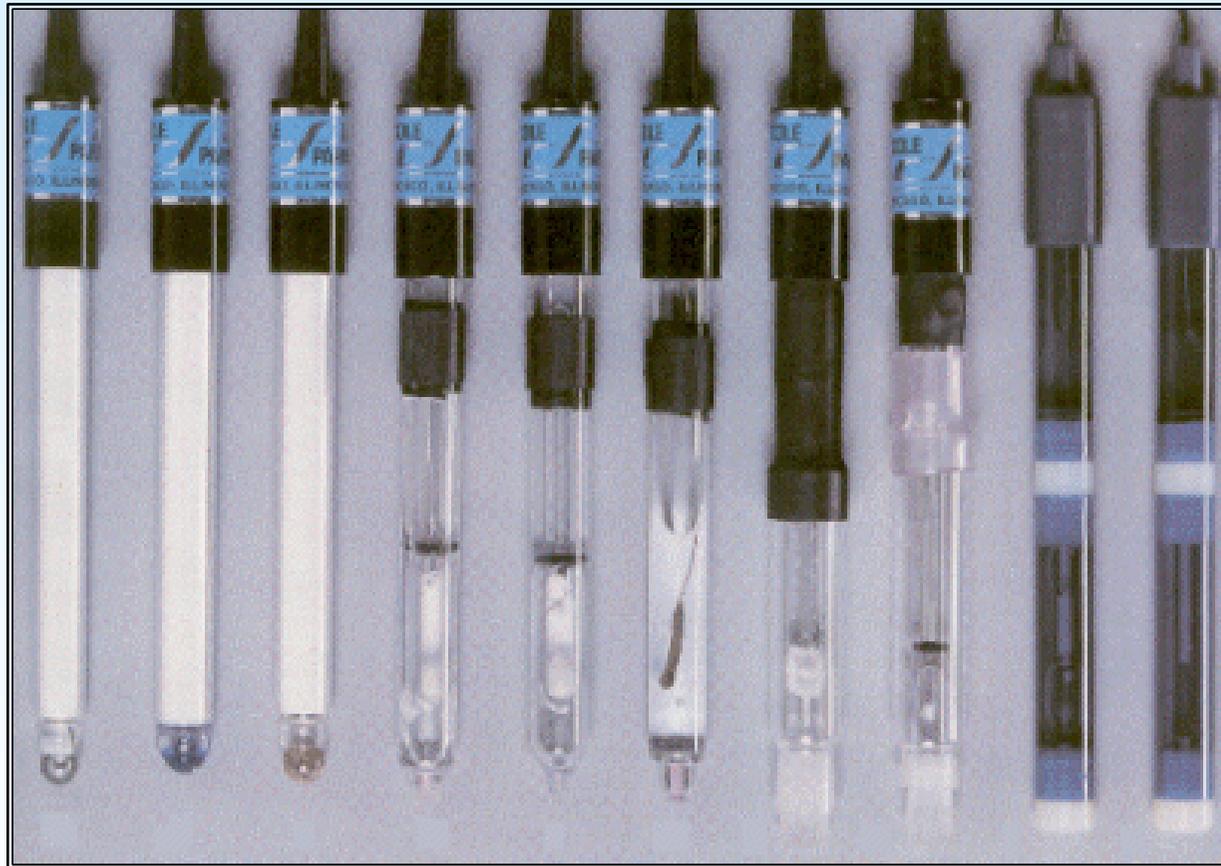
Operation principle of a pH-meter



An antique pH-meter



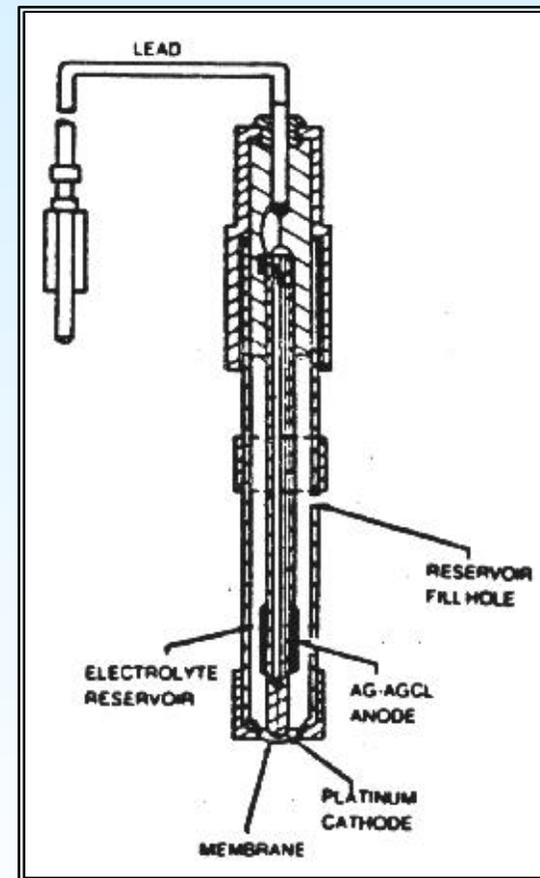
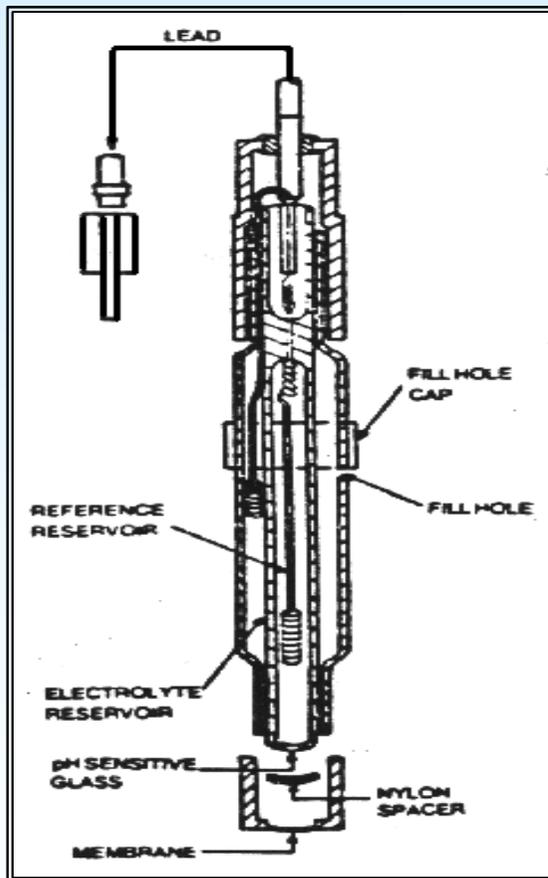
Modern pH-meters



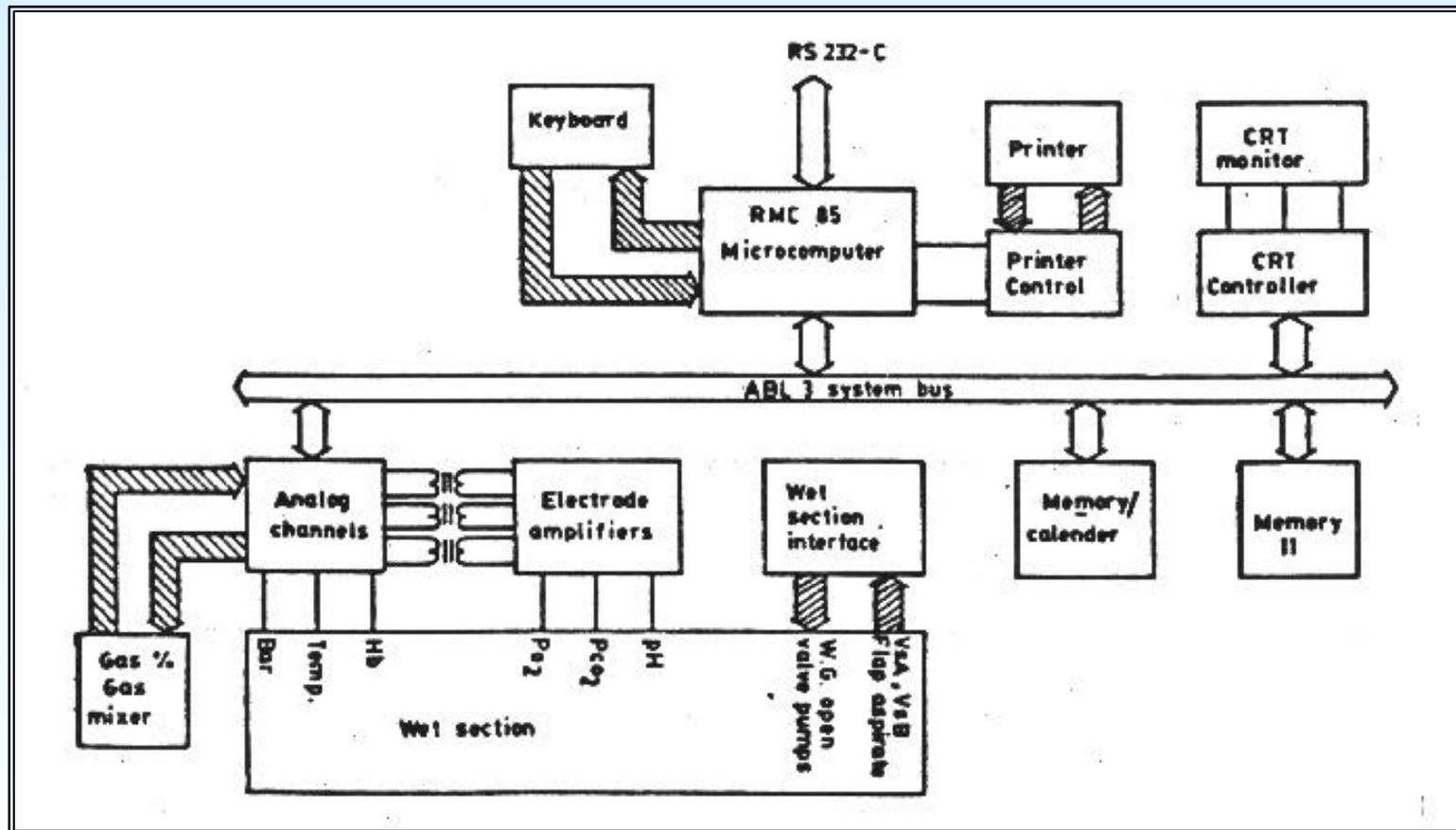
Ion Selective Electrodes



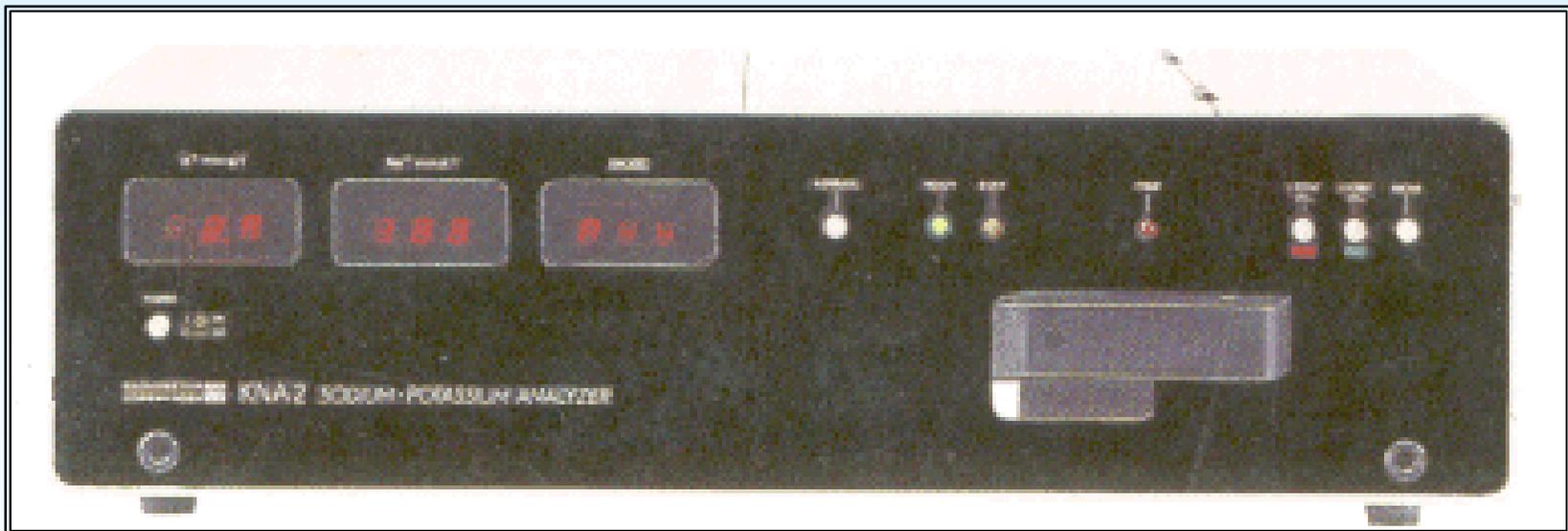
CO_2 and O_2 (Clark) Electrodes



Block Diagram of a Blood Gas Analyzer



Blood Gas Analyzer





Immunoassays

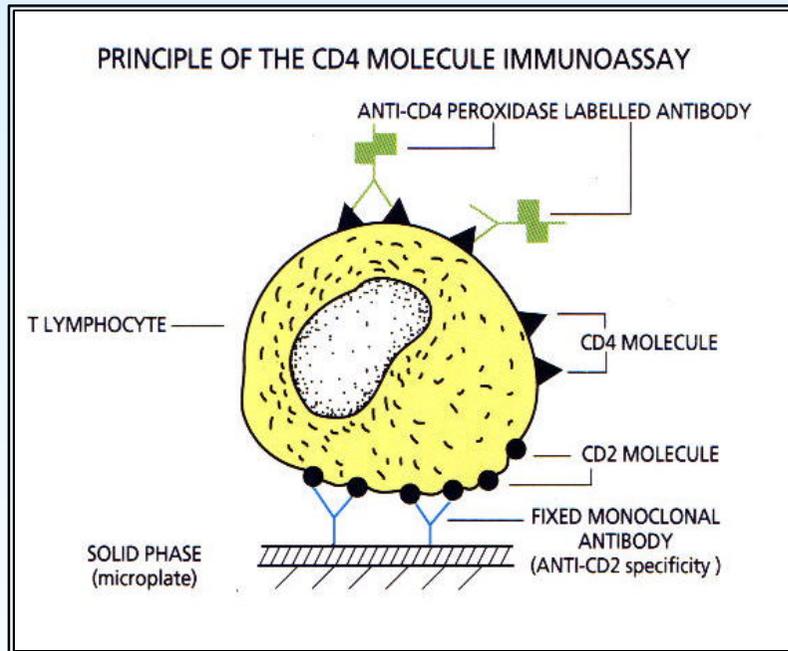
- *Immunoassays are procedures that rely on the use of antibodies as “specific” binding reagents.*
- *These assays are used to quantitate or determine the presence of therapeutic drugs, numerous biologic substances, infectious agents etc.*
- *The principle of those assays is that a specific reversible binding between an antigen and its corresponding antibody will take place and that this interaction will form a complex that can be differentiated from bound or “free” ligand.*



Labels coupled to ligands

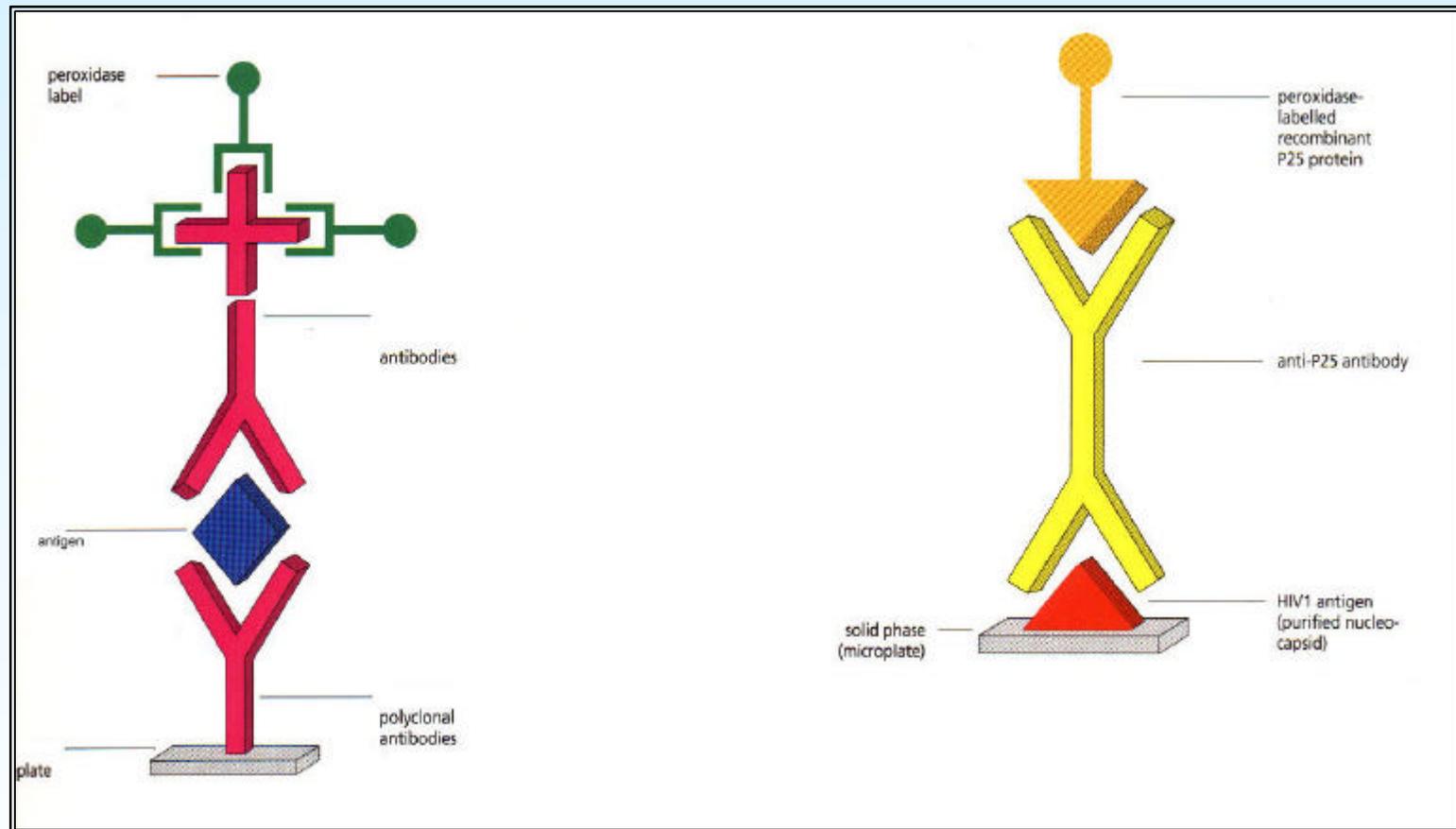
- *To measure this interaction or complex formation, various labels have been covalently coupled to ligands, allowing for the detection and quantitation of the molecule of interest.*
- *As labels are used **Radionuclides (RIA, IRMA), Enzymes (ELISA, IEMA), Luminescent (LIA) or Fluorescent (FPIA) compounds.***
- *Several equipment types are used to detect the tagged compounds.*

Immunochemistry Methods

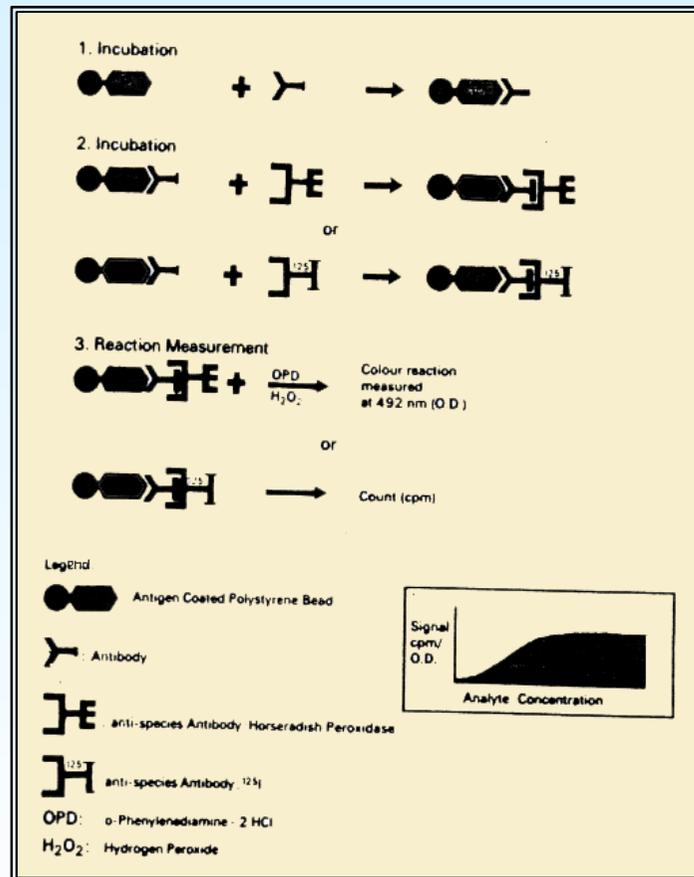


Rosalynne Yalow

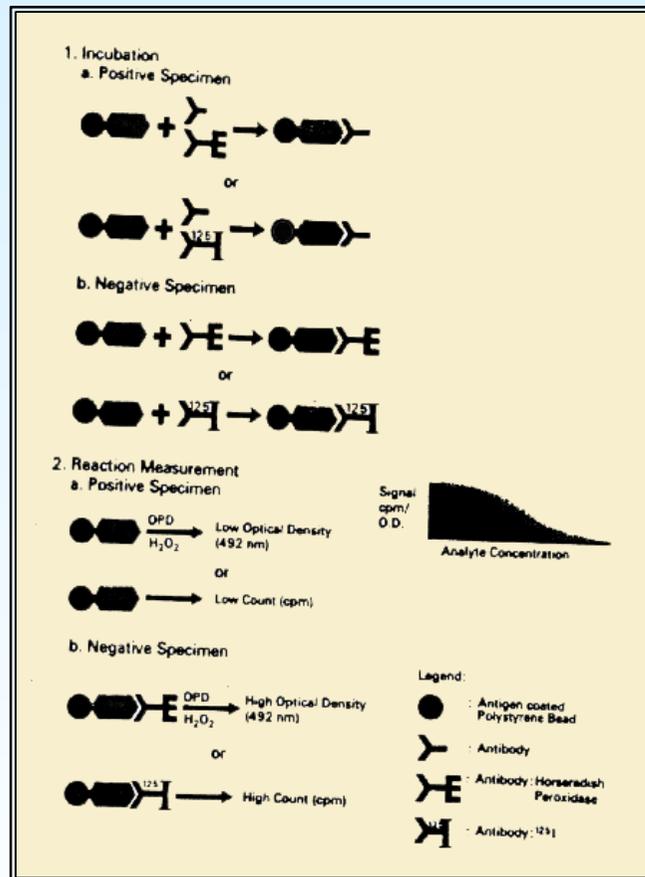
The main idea



The principle of sandwich type RIA/ELISA



The principle of competitive type RIA/ELISA

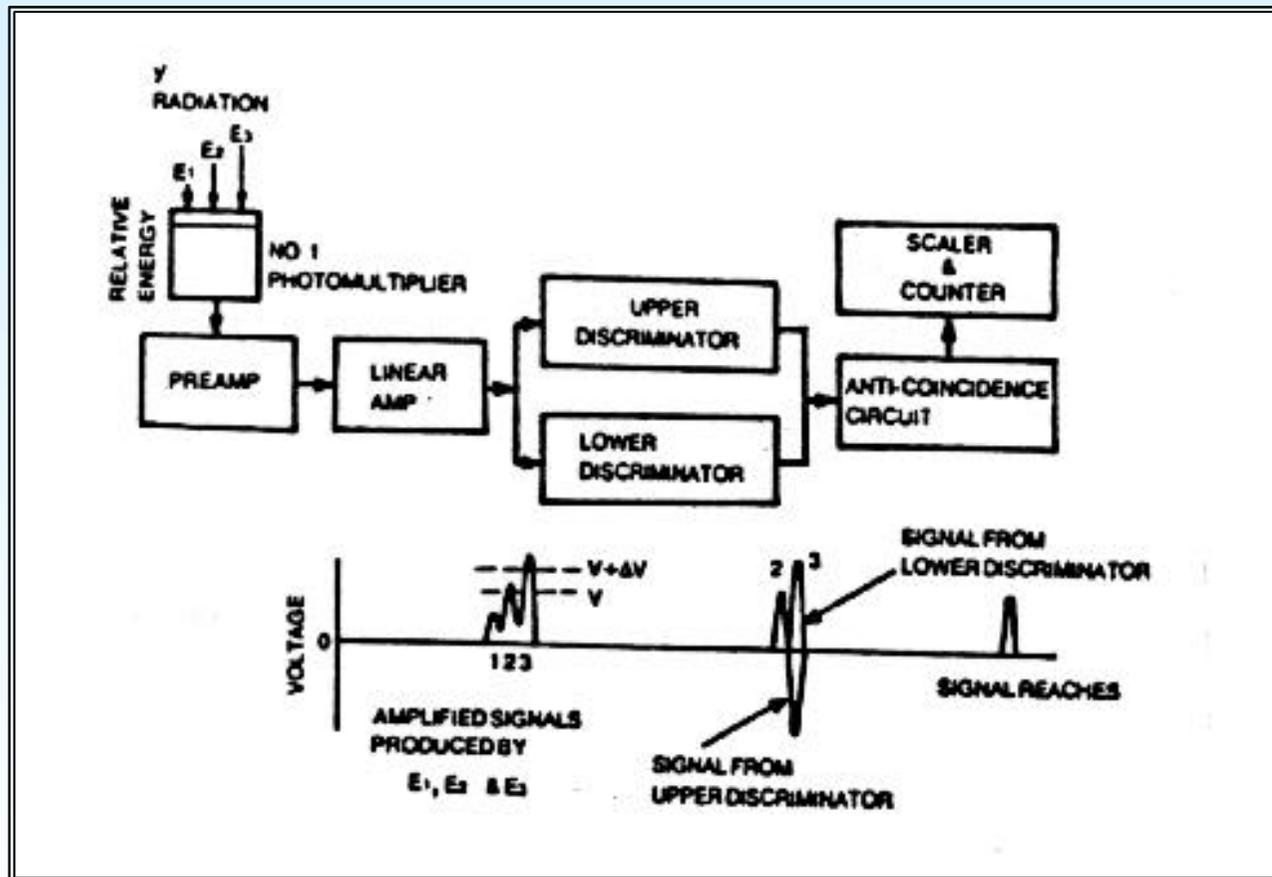




Radio Immuno Assay (RIA) or Immuno Radiometric Assay (IRMA) Equipment

- *A Scintillation Counter comprises a scintillator, i.e. a crystalline substance, which produces minute flashes of light, in the visible or near ultraviolet range, when it absorbs ionising radiation, along with a photomultiplier tube is used to detect α emitting radioactive labels such as I^{125} .*
- *Samples containing weak beta emitters, such as H^3 and C^{14} can be counted more efficiently by mixing the sample with a liquid scintillator, so that the scintillator is in intimate contact with the short-range beta rays.*
- *Counting is carried out again with a photomultiplier.*

NaI(Tl) Crystal γ - Counter

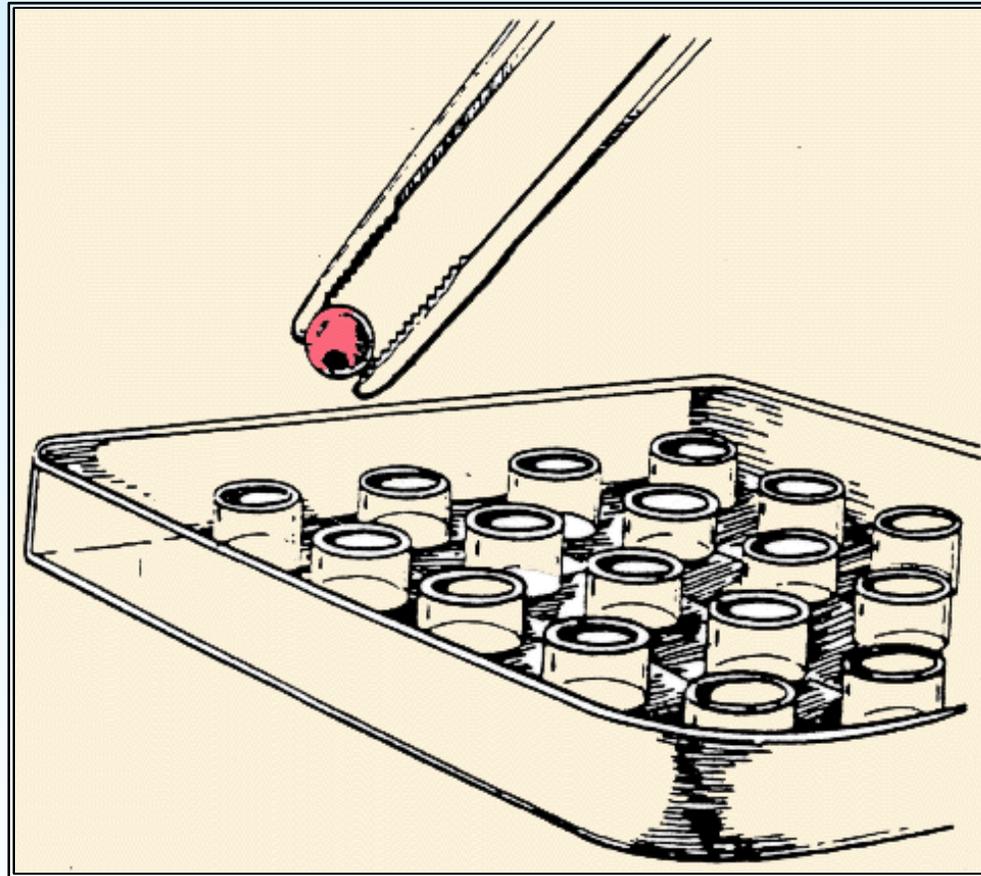




ELISA and LIA

- *Enzyme-Linked Immuno-Sorbent Assay (ELISA) or Immuno Enzymometric Assays (IEMA): Coloration due to the presence of the enzyme is measured using a spectrophotometer set at the appropriate wavelength.*
- *Luminescence Immuno Assay (LIA) or Immuno Luminometric Assay (ILMA): The chemiluminescence activity that determines the compound concentration is recorded using a photomultiplier system.*

Solid Phase: Beds



Solid Phase: Multi - well plate

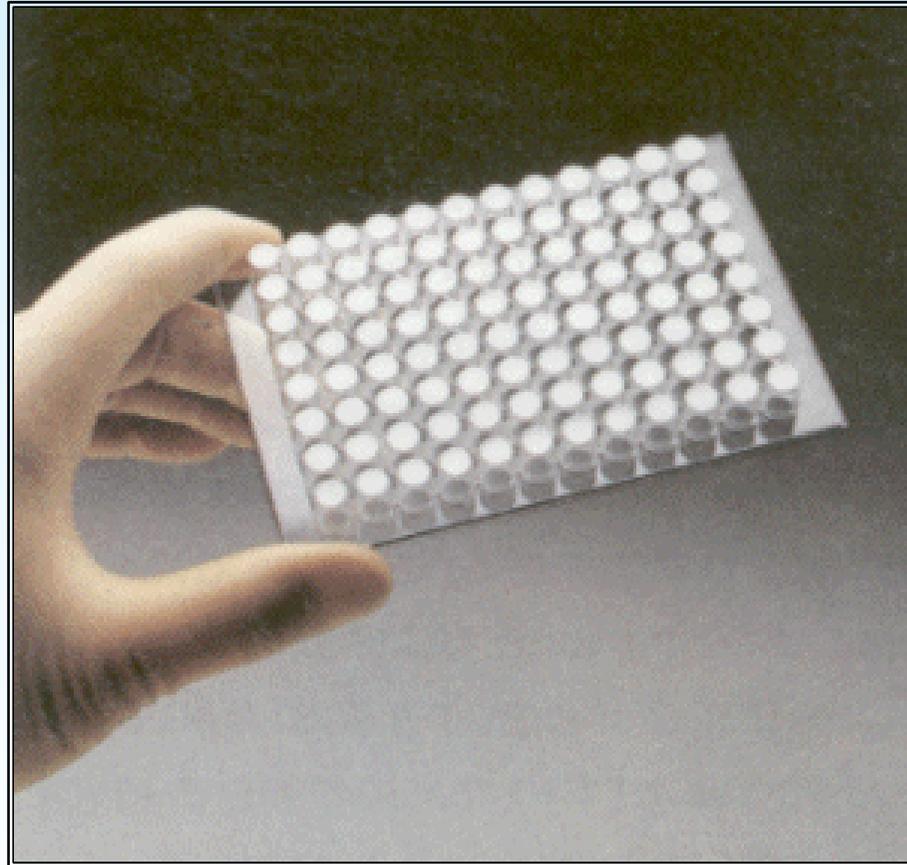
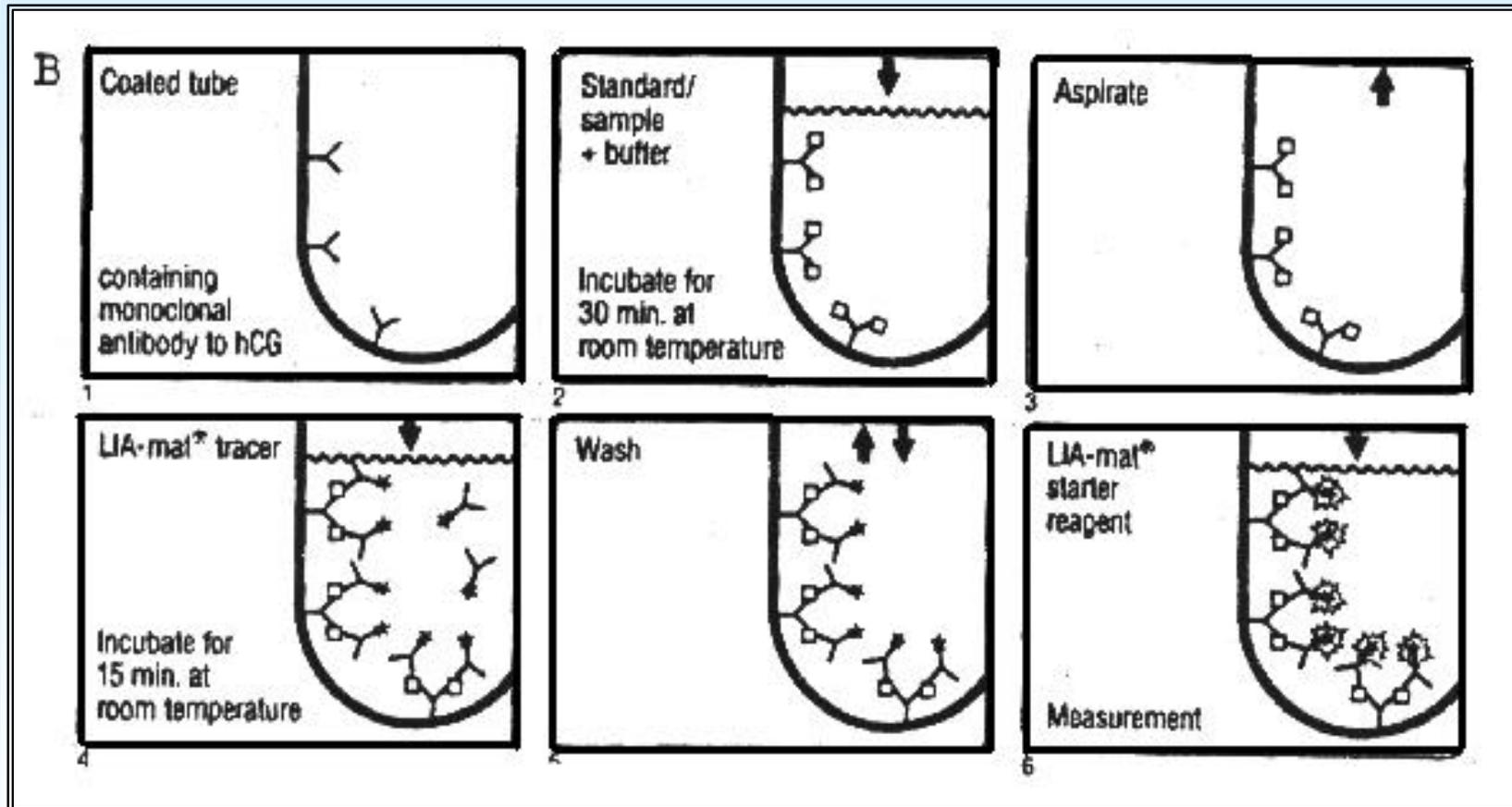


Plate Washing / Reading Device



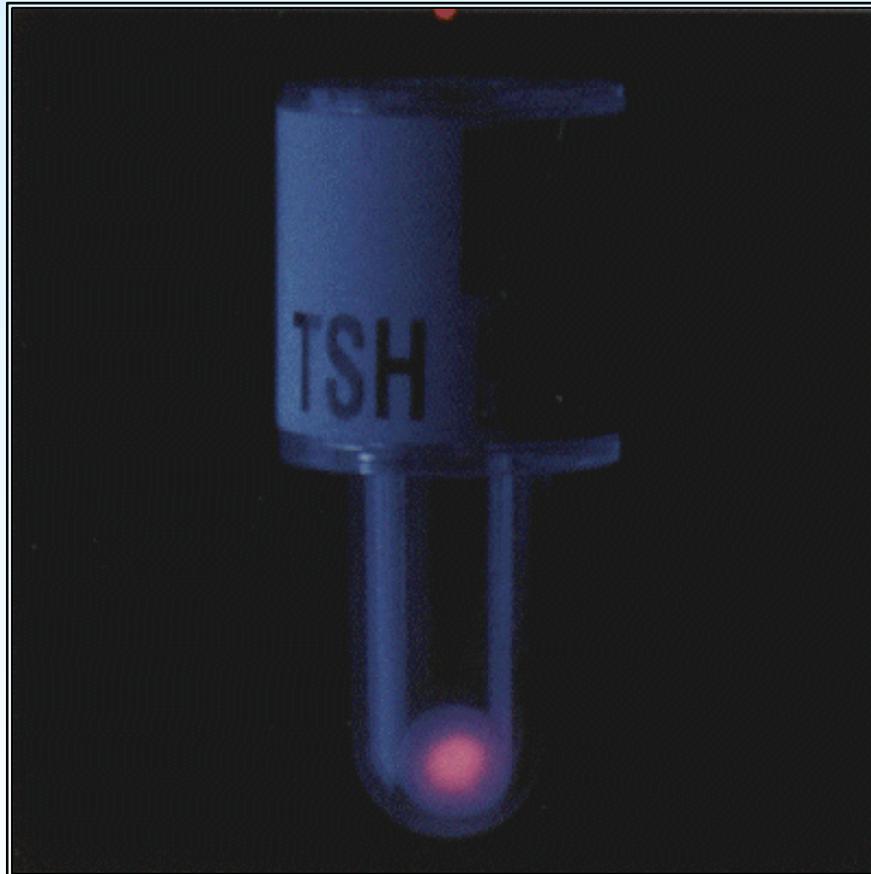
Luminescence Immuno Assay (LIA)



LIA Autoanalyzer



LIA Autoanalyzer: Detail

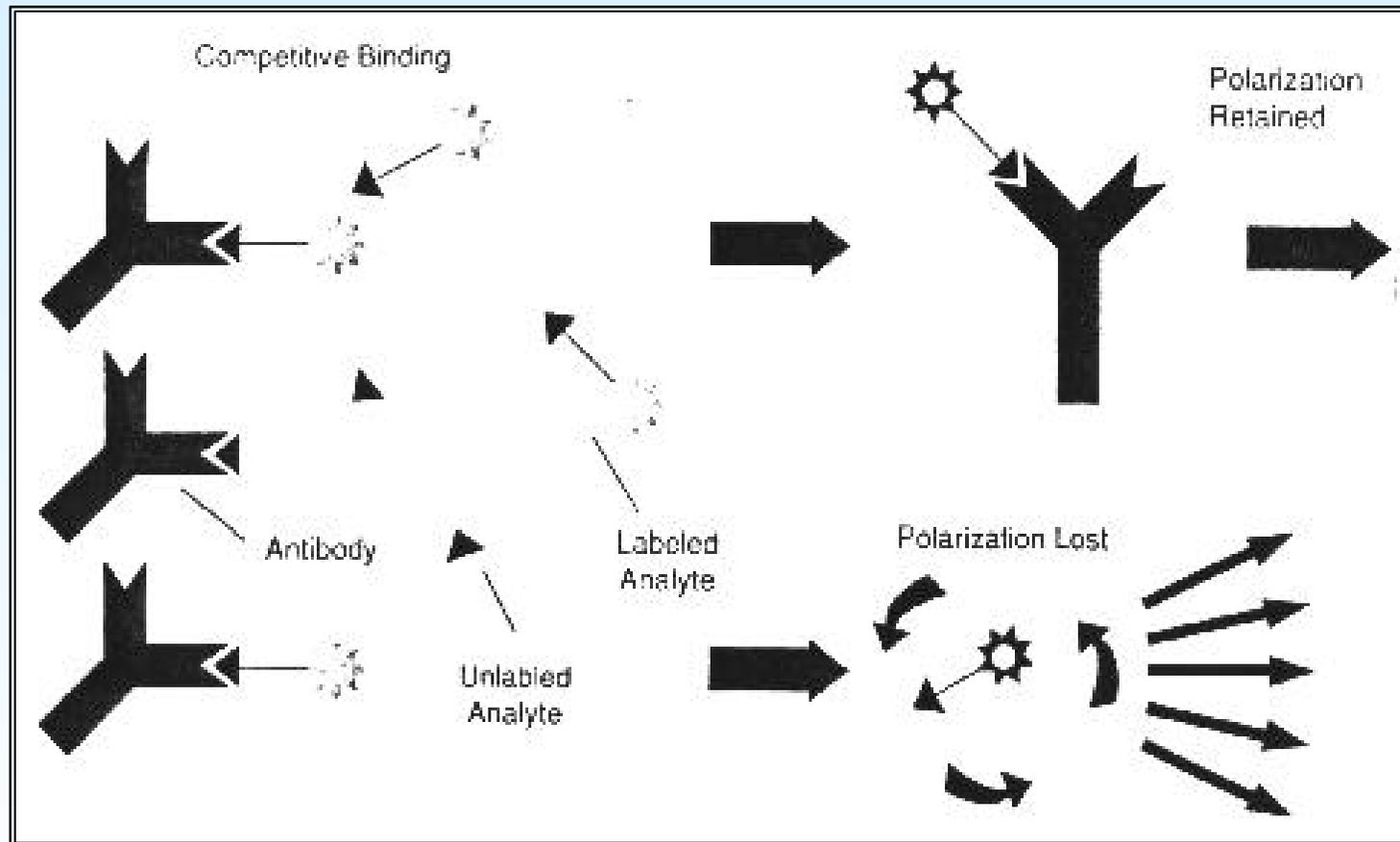




Fluorescence Polarization Immuno Assay (FPIA)

- *A Fluorometer equipped with a polarized light source is needed.*
- *The excitation of the fluorescent conjugates by the polarized light results in the transition of fluorescent conjugate electrons to a higher energy level and the orientation of these electrons in the same direction as the polarized light.*
- *The bound fluorescent conjugate emits an increased amount of polarized fluorescence, compared to the fast rotating free fluorescent conjugate, that is detected and is proportional to the immuno-complex concentration.*

Polarized Immuno Fluorescence (PIF)

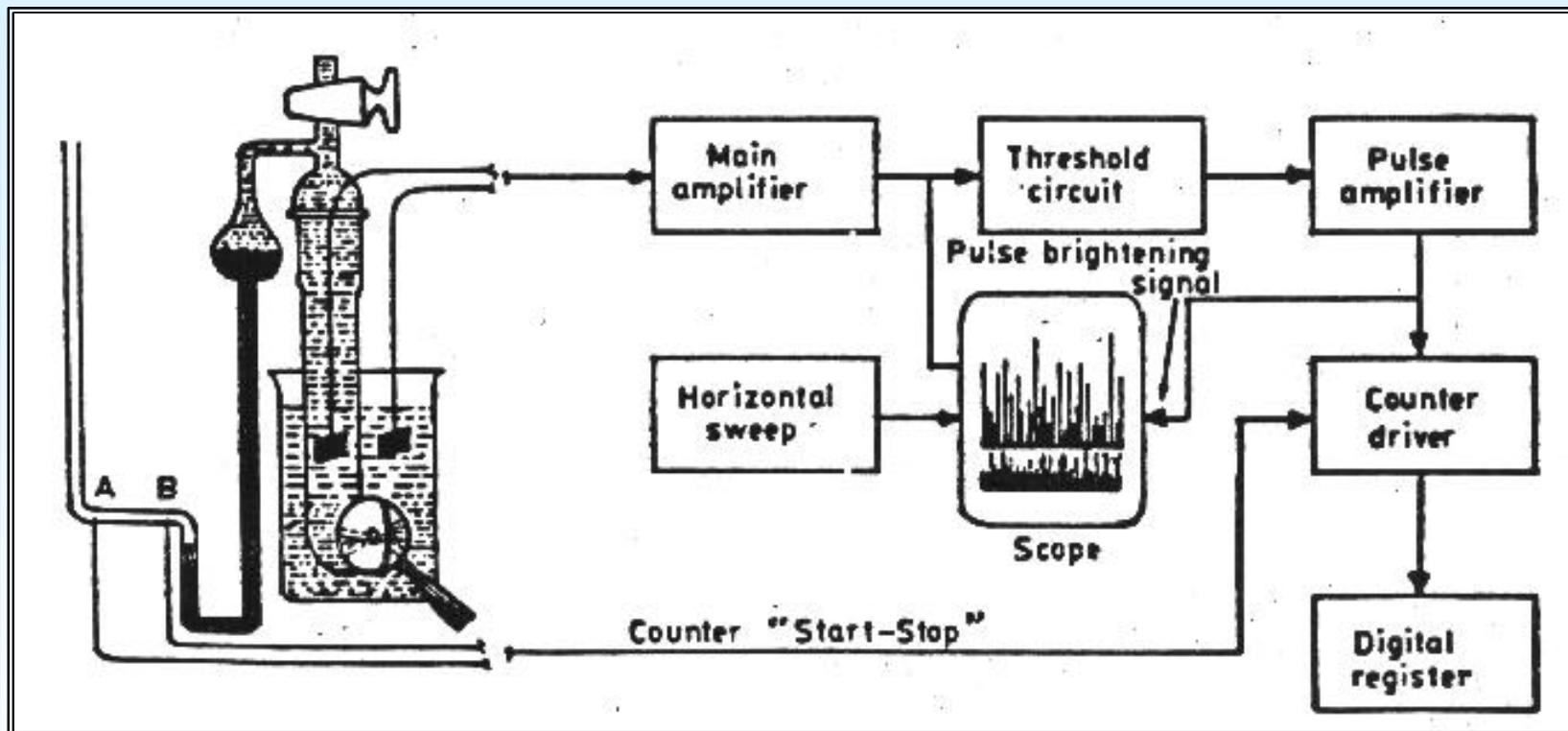




Hematology Analyzers

- Hematology includes, beyond blood biochemistry, that is covered by the above described methods and equipment, cell counting and classification and coagulation chemistry.
- Hematology Analyzers are based either:
 - ◆ *On the Coulter Principle i.e. the haematocytes are suspended in isotonic NaCl solution and are counted by means of a measurement of electrical resistance of the suspension.*
 - ◆ *On the scattering of a LASER-beam on the flowing cell sample.*

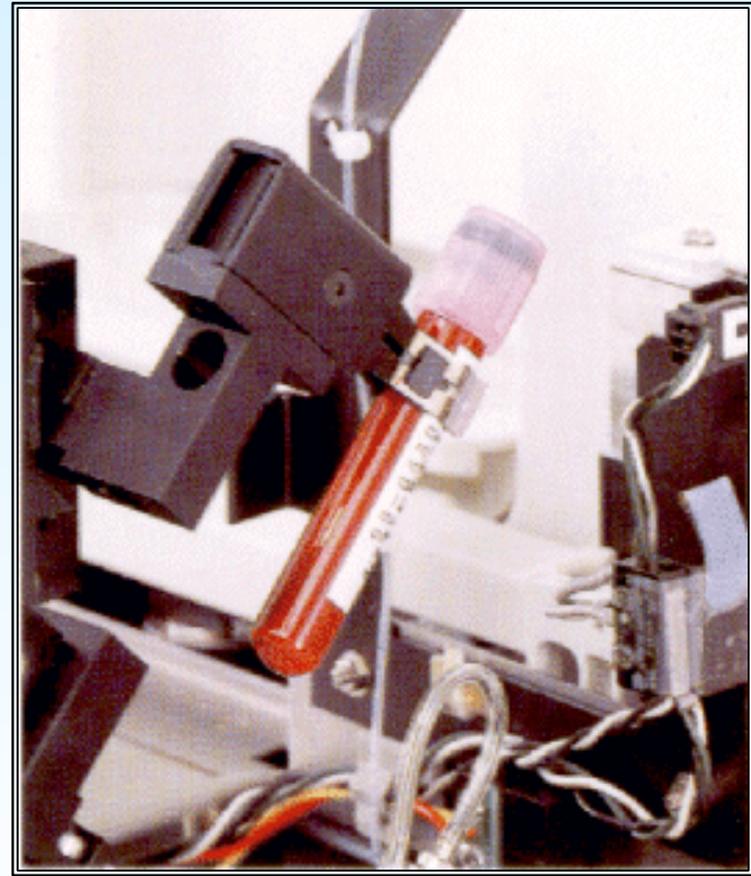
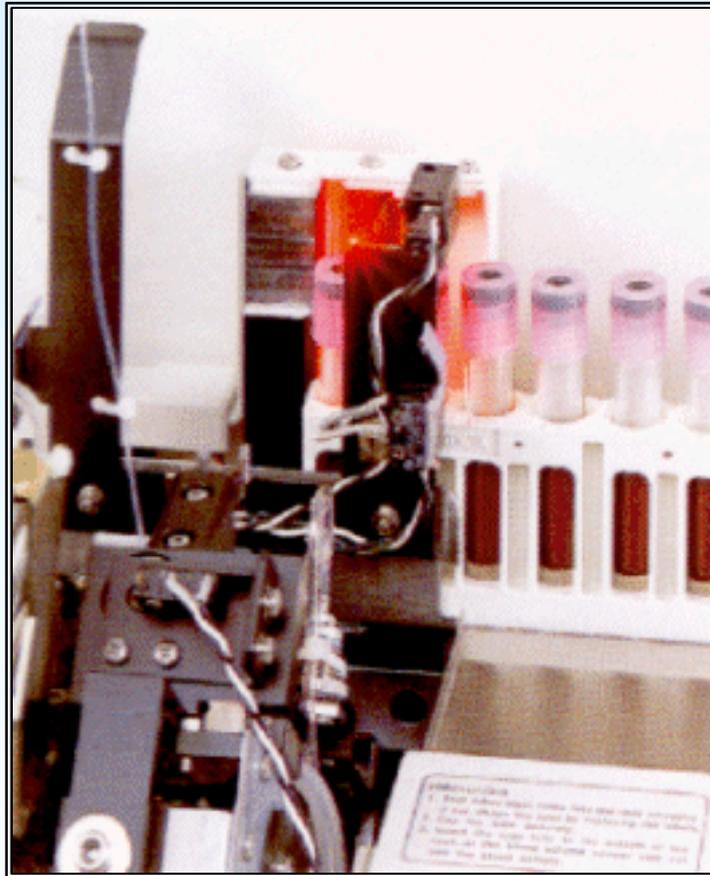
Block diagram of an Hematology Analyzer based on the *Coulter principle*



Typical Coulter Counter



Sample introduction into an Hematology Analyzer

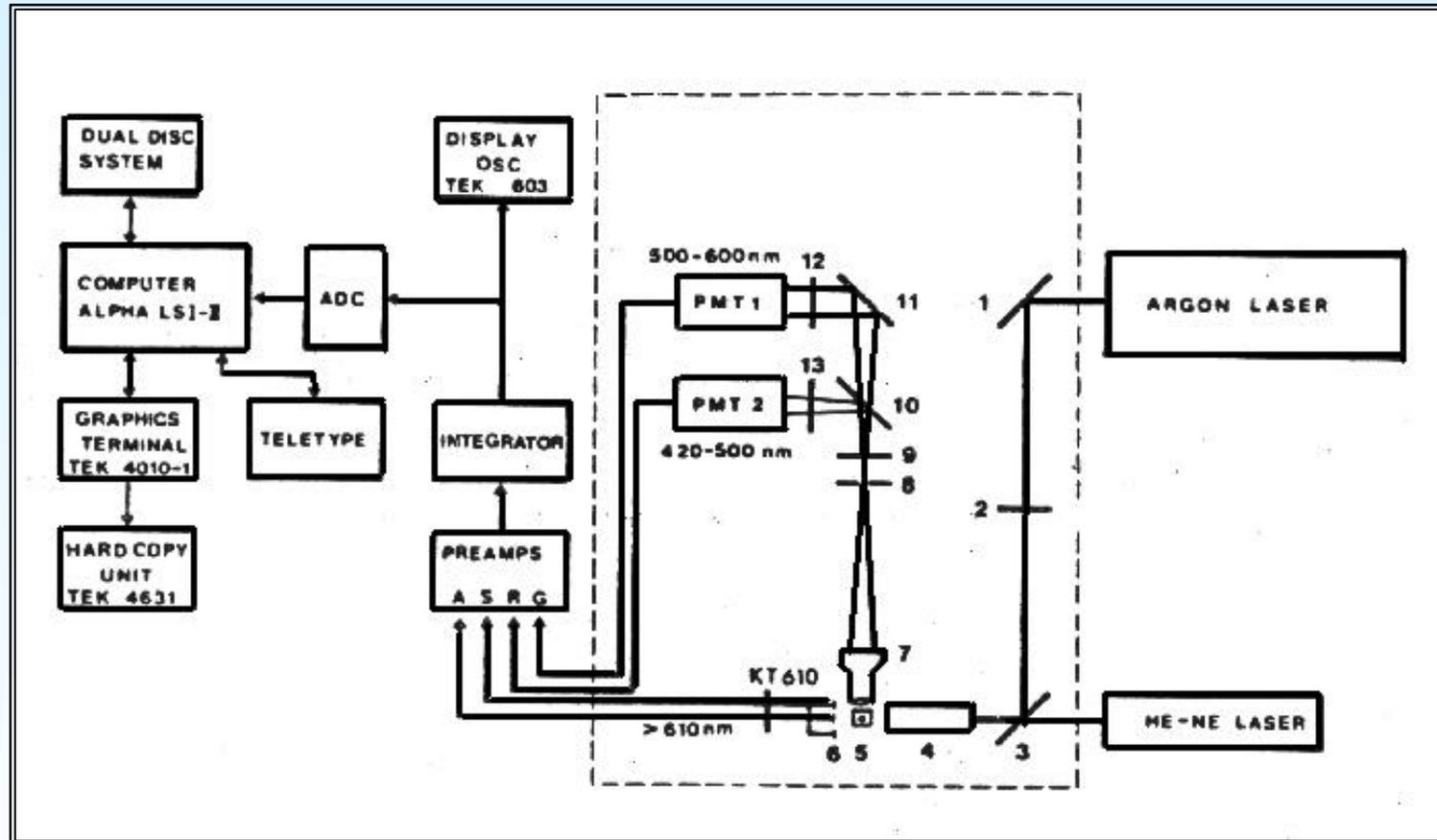




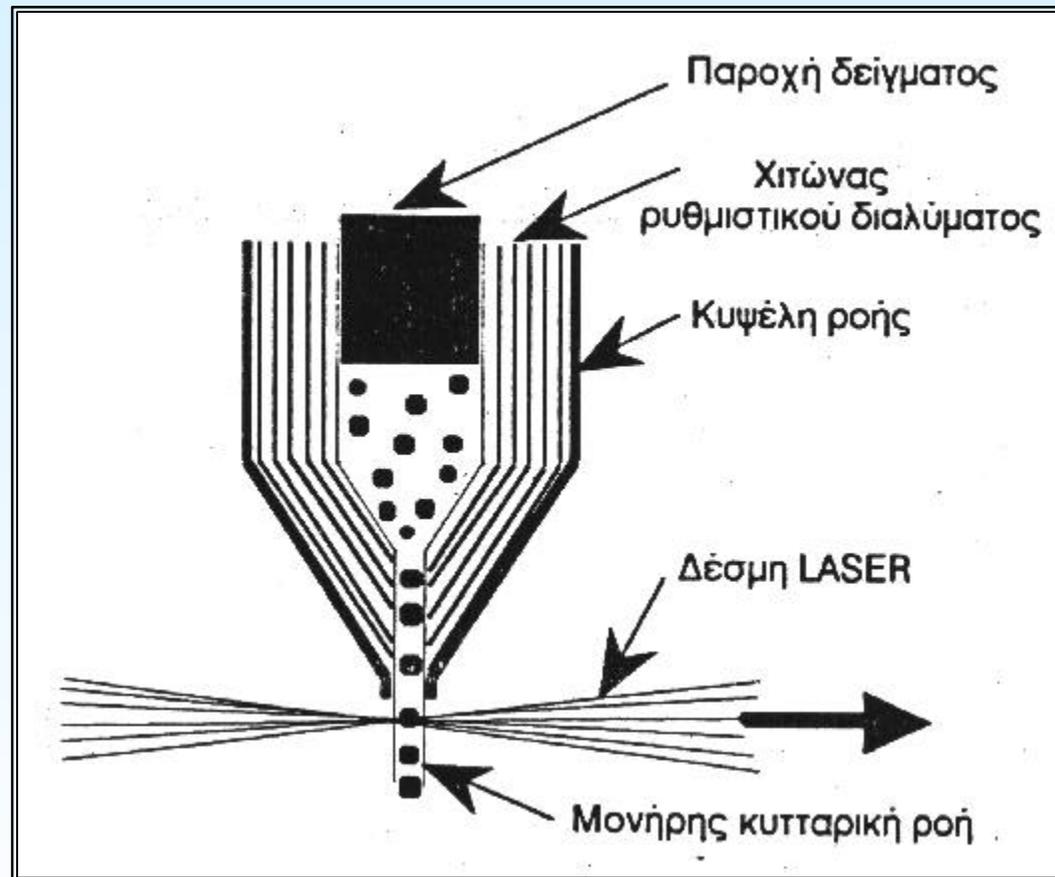
Other Hematology Equipment

- *Flow Cytometers* are based on LASER excitation using crossed cylindrical to focus the beam as a sheet of light through which fluorochrome tagged cells pass single file for analysis.
- *Microscopy* is still an important complementary method in Hematology, applied on any case of doubt, concerning the results of automated methods.
- *Coagulometers* determine parameters such as Partial Thromboplastin Time (PTT), Prothrombin Time (PT) etc. which reflect the coagulation status of a patient by estimating manually, photometrically, mechanically or magnetically the time needed for clot formation.

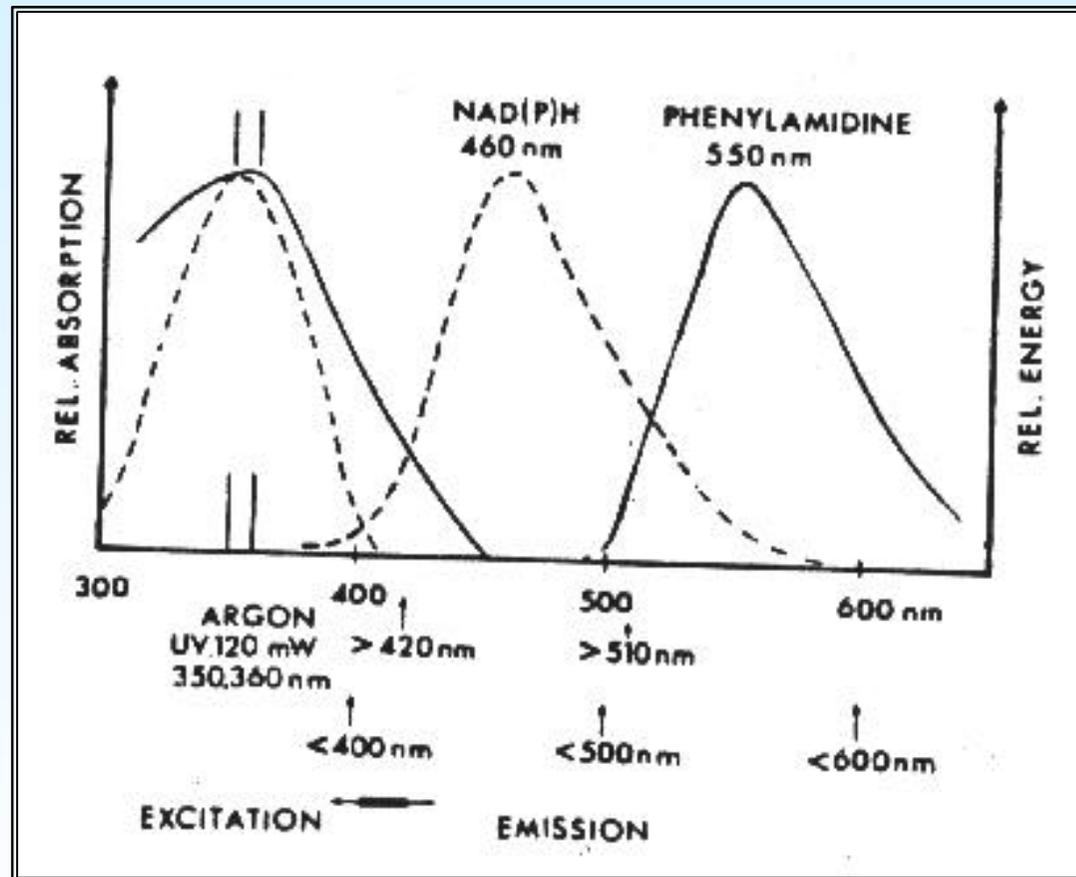
Block diagram of a Flow Cytometer



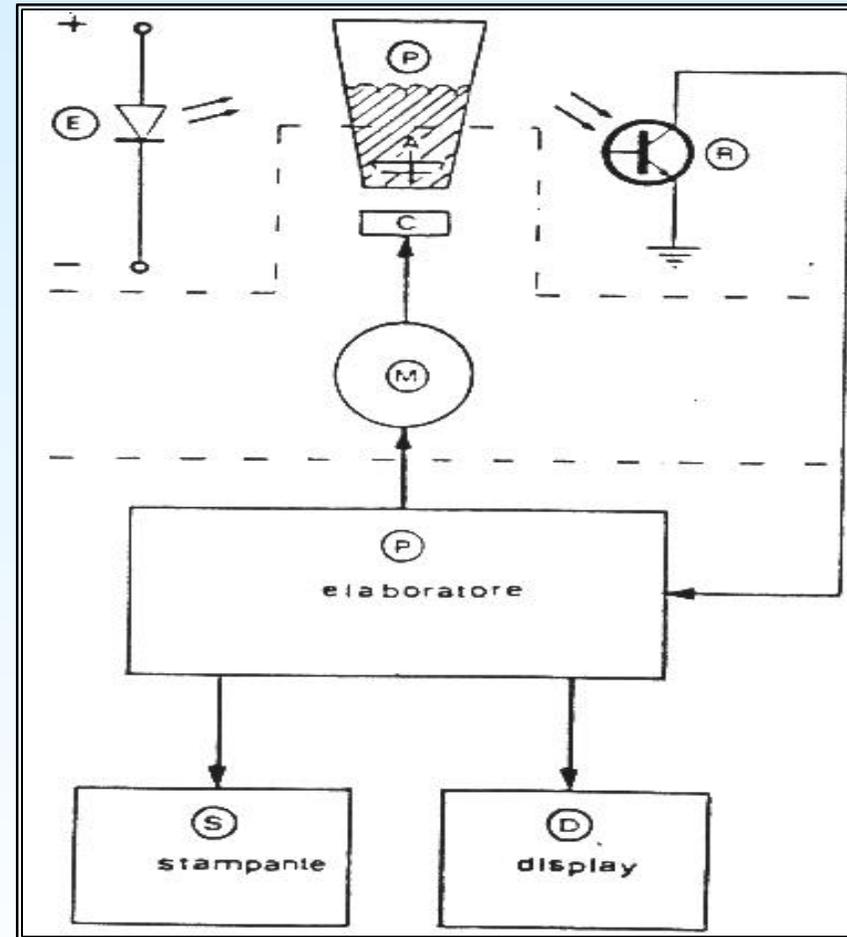
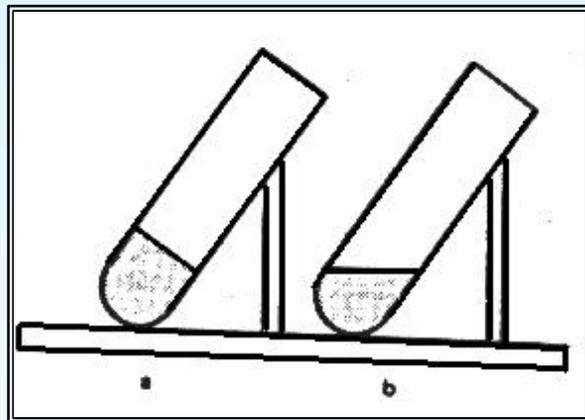
Flow Cytometry: LASER Scattering



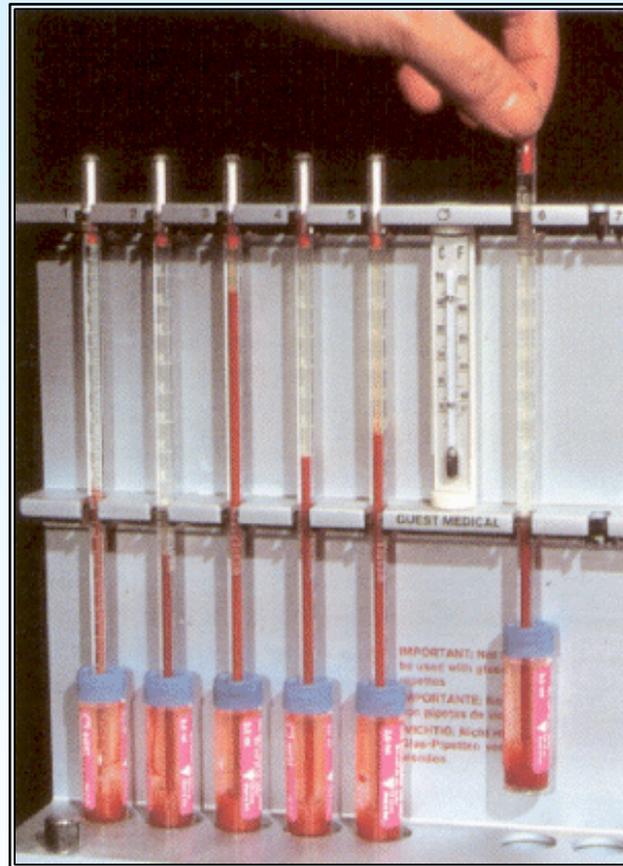
Flow Cytometry: Emission Spectra of Fluorescent Dyes (Fluorochrome)



Coagulometers



Sedimentation Rate Measurement

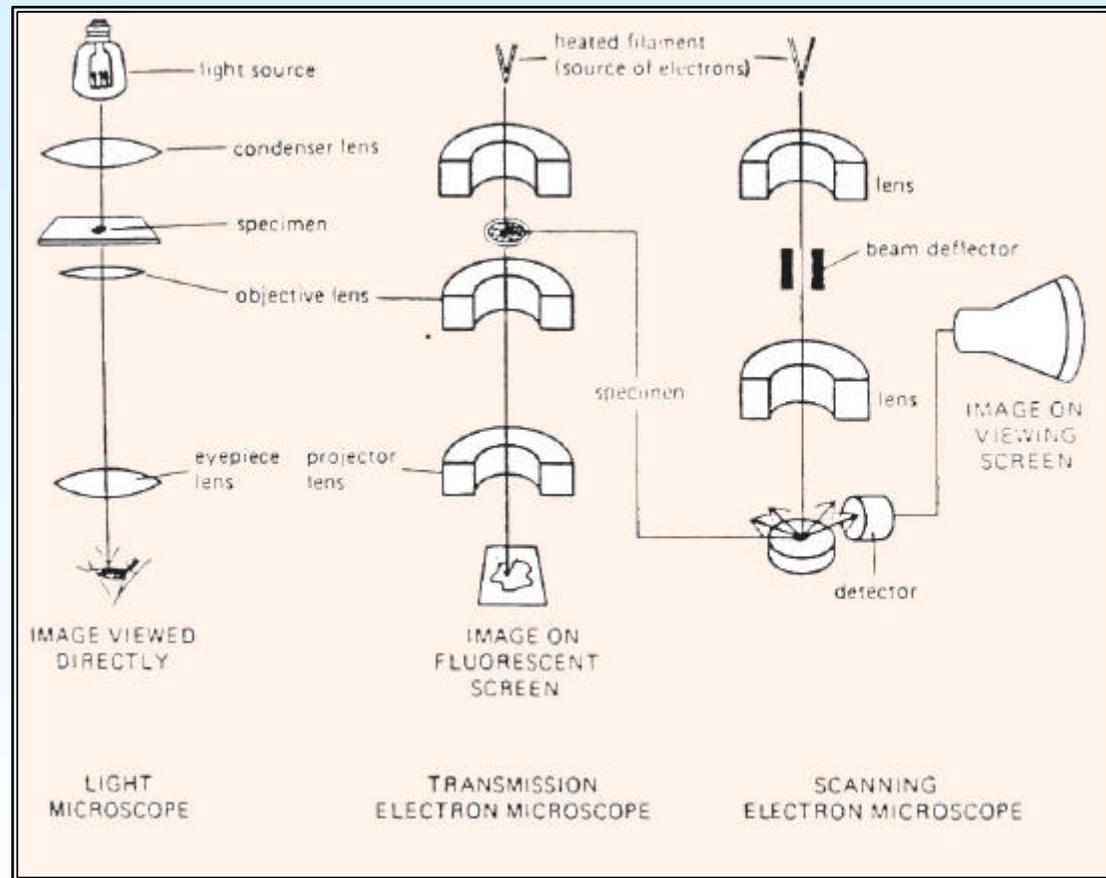




Microbiology

- Microbiology includes bacteriology, virology, parasitology, and possesses much less equipment requirements compared to Clinical Chemistry, such as centrifuges, microscopes of different types, incubators and minor auxiliary equipment.
- However, it possesses specific environmental requirements with regard to air pressure, segregation, air movement, ventilation, lighting, hoods, storage areas, refrigeration etc.
- In this department, automation of test performance has been slower than other areas. Therefore, Microbiology is considered a people-intensive department.

Optical and Electron Microscopy: Physical Principles



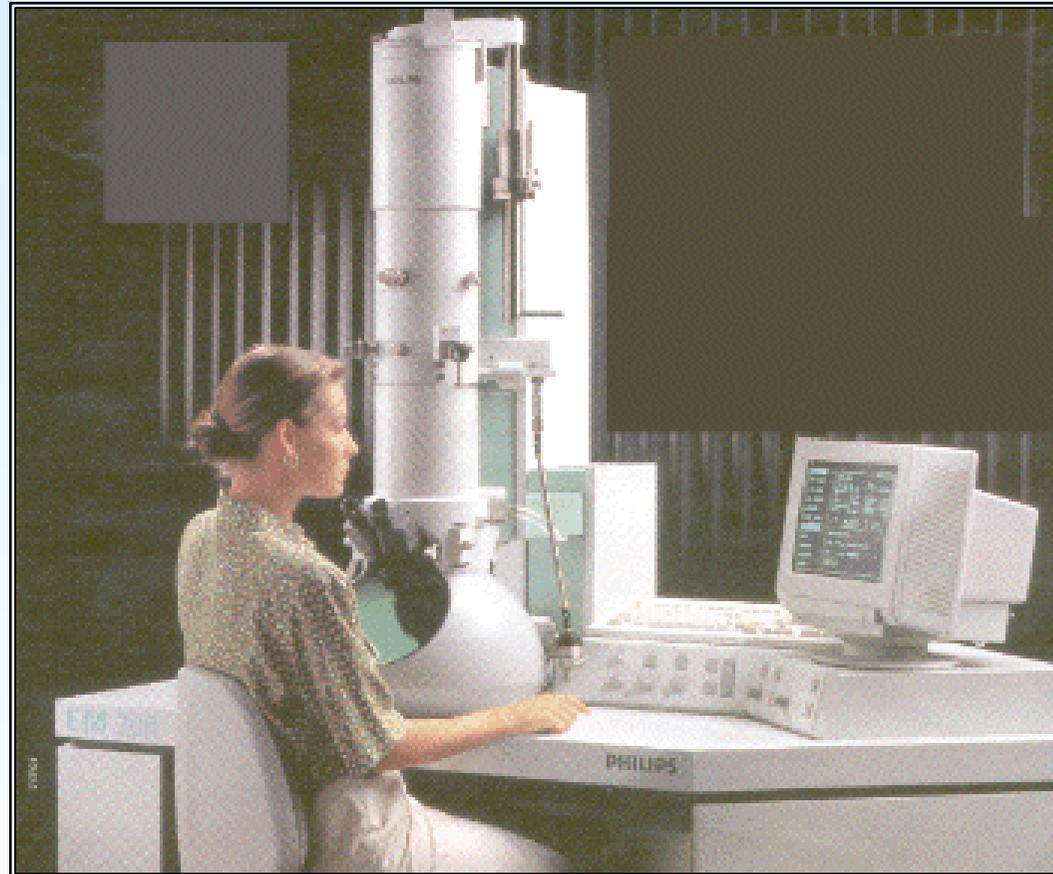
Classical Microscopy



Digital Microscopy Image Processing



Electron Microscopy



Biohazard Chamber





Histopathology and Cytology

- Surgical pathology, a main activity of the department, possesses two main elements, routine gross and microscopic tissue analysis and frozen tissue examinations performed for the surgeon at the time of the operation. It is convenient to have an area within the surgical suite for frozen tissue examinations.
- Major hospitals must possess an autopsy room, for performance of post-mortem examinations.
- Cytology laboratories mainly are examining Papanicolau (pap) smears and other smear tests and a good slides archive is necessary.



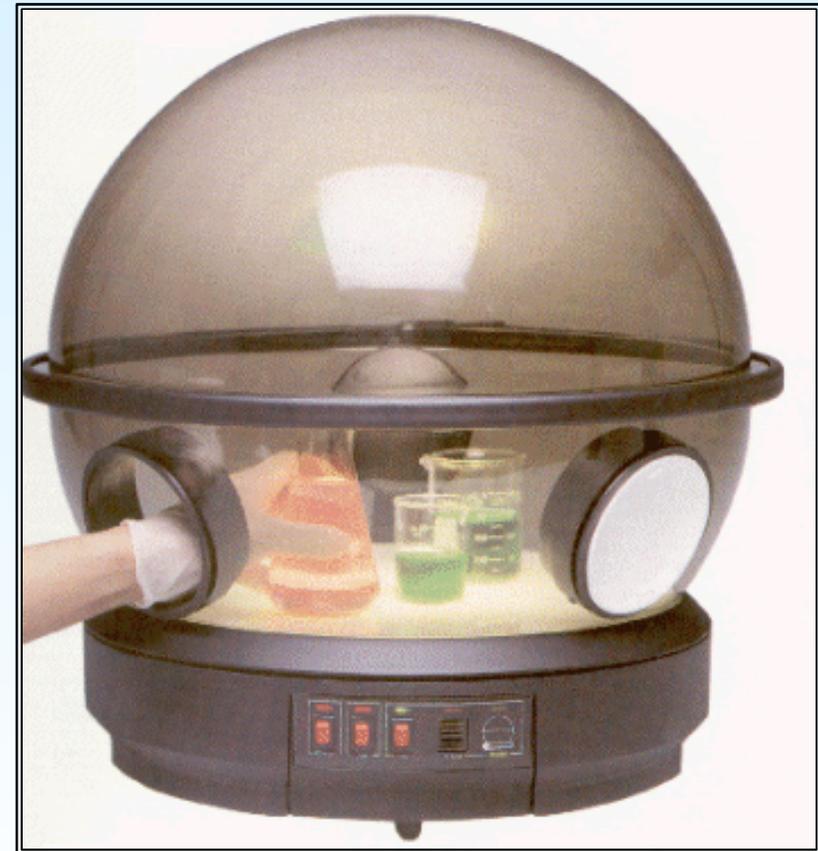
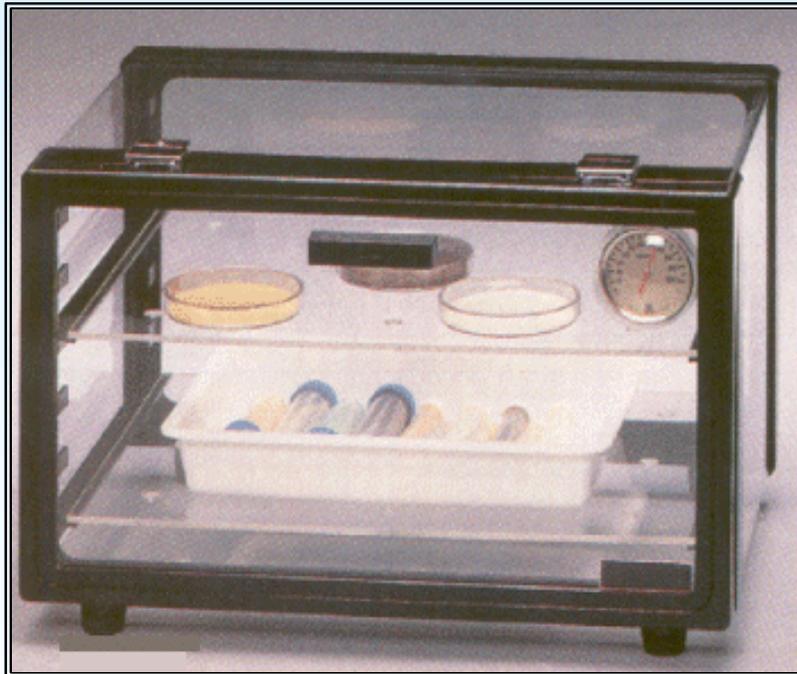
Histopathology and Cytology Equipment

- The necessary apparatus include microtomes, staining systems, microscopes, incubators, hoods and the morgue's equipment.
- Although pattern recognition systems connected to microscopes and video-(image) processing equipment have been developed, Histopathology still remains a specialized staff intensive department.

Refrigerated Microtome



Exsiccators





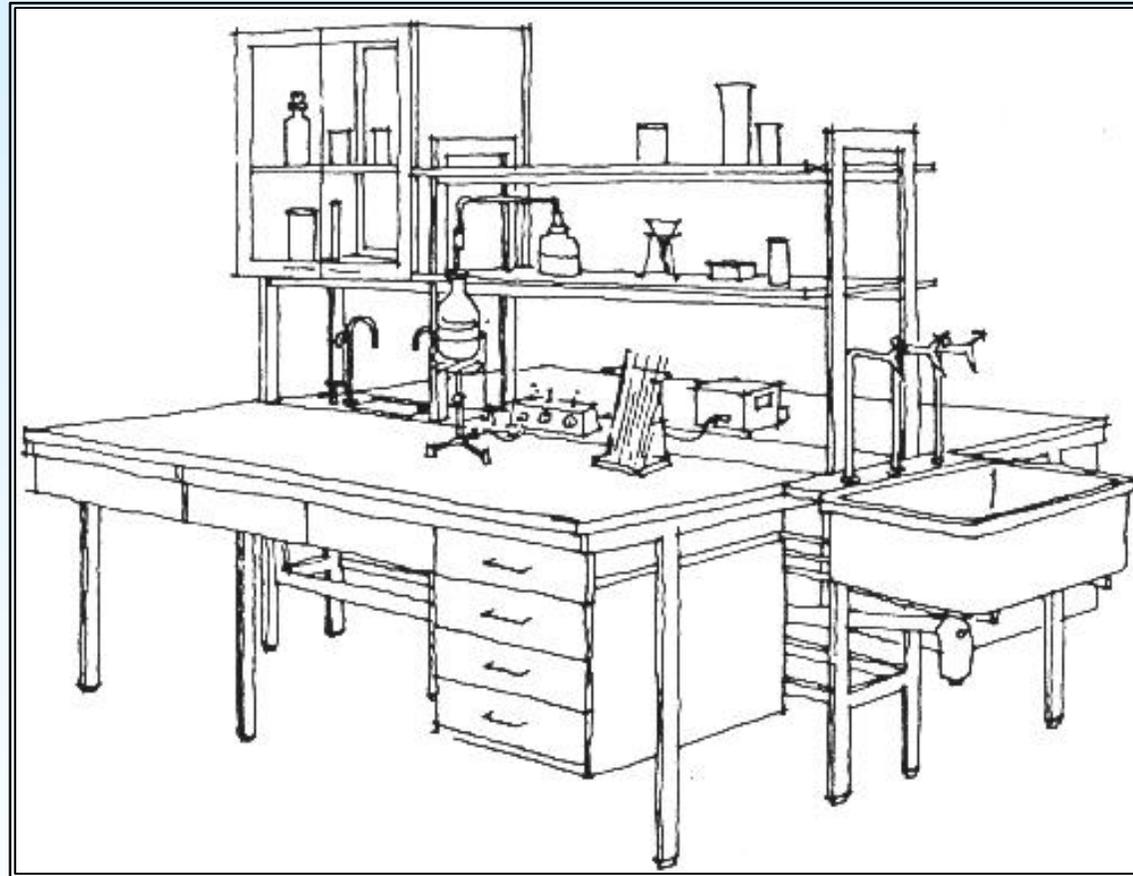
Blood Bank (Transfusion Medicine Department)

- *The blood bank is another specialized area which use some of the in vitro Diagnostics techniques, into which many ambulant people enter from the outside world daily, making traffic control and cleanliness extremely vital.*
- *The deparment includes an immuno-hematology laboratory, as described above, blood-drawing room, equipped with reclining couches, blood separation and processing devices and storage refrigerators.*

Blood Group Determination



Laboratory Furniture

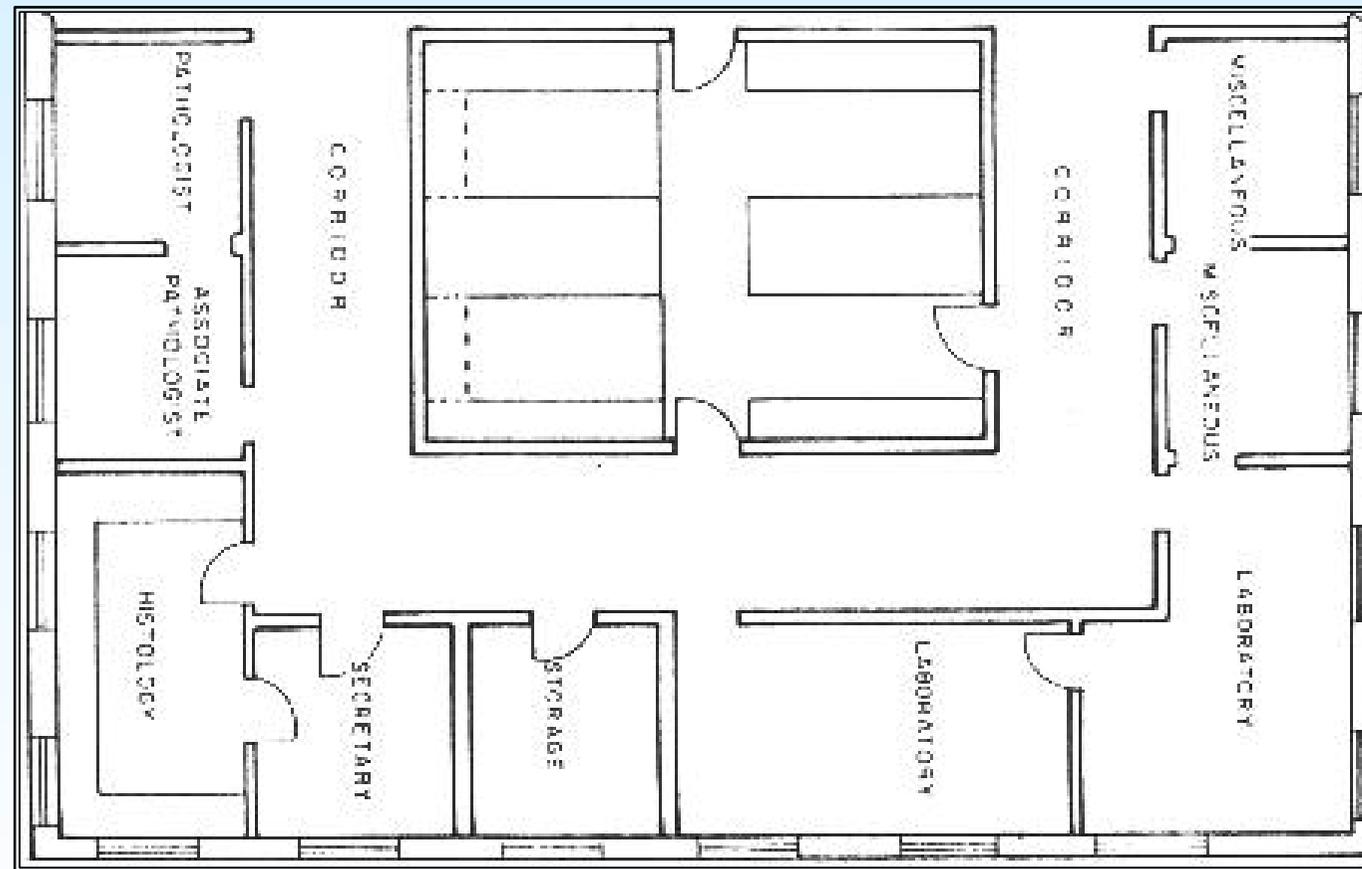




Laboratory Modular Design

- *In designing laboratories it is important to determine the anticipated volume of testing to be carried out in each department.*
- *For any given group of tests about of 1.8 m of counter to work on are required.*
- *The size of an optimal working module in a hospital laboratory is about 18 m² and this area permits approximately 4.5 m of counter on each side of the module, accommodating also the necessary casework, free-standing equipment, sinks, etc.*

Typical example of Laboratory Modular Design





Open and closed areas

- *The counters are usually lined up back to back and the modules are arranged in an orderly repetitive fashion.*
- *The system works out quite well in a large raboratory room that permitts sharing f personnel and specimens, interchange of information about tests, specimen, patients and technology, superior supervision and much more effective air conditioning, heating, ventilation, lighting, and sound proofing.*
- *However, there are areas in the laboratory in which a large, jointly shared area cannot be permitted, as in the case of pathology, RIA laboratories, PCR laboratories, Fluoromicroscopy etc.*

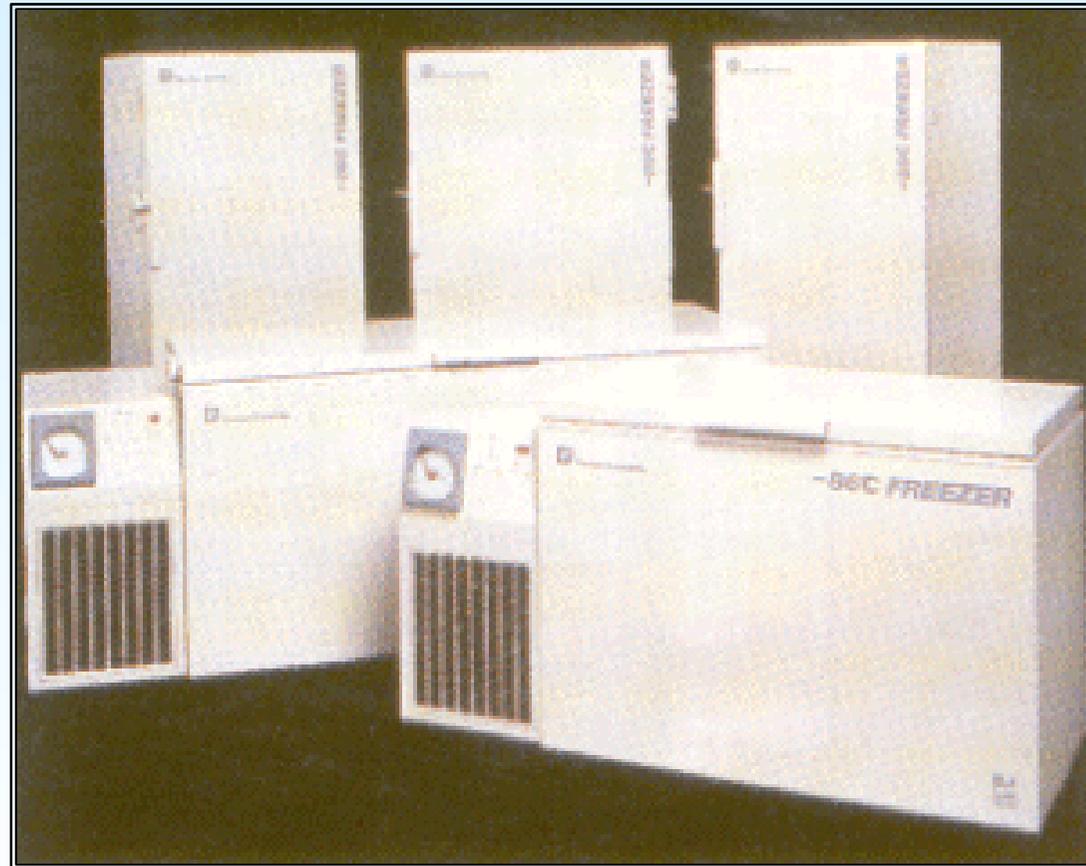
Auxilliary Equipment



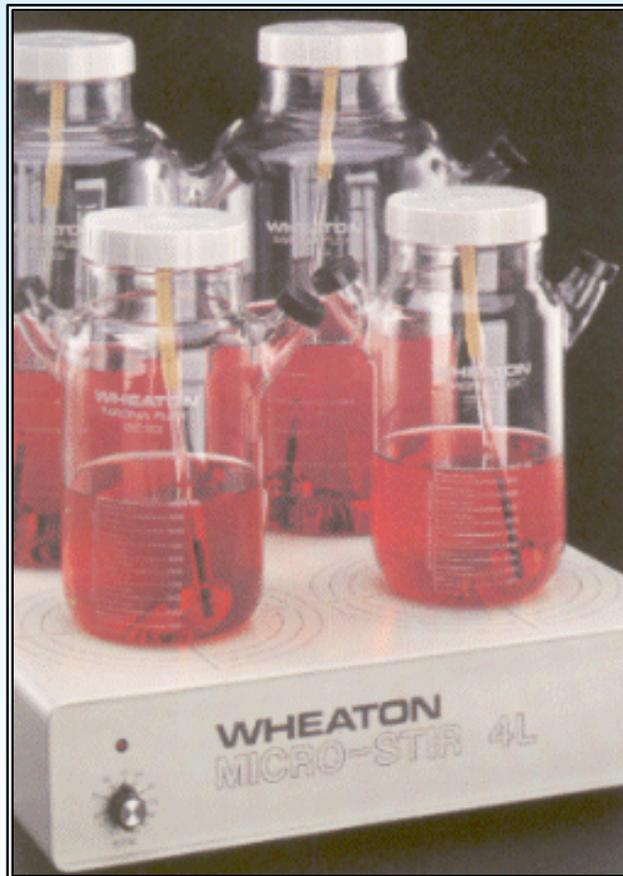
Centrifuges



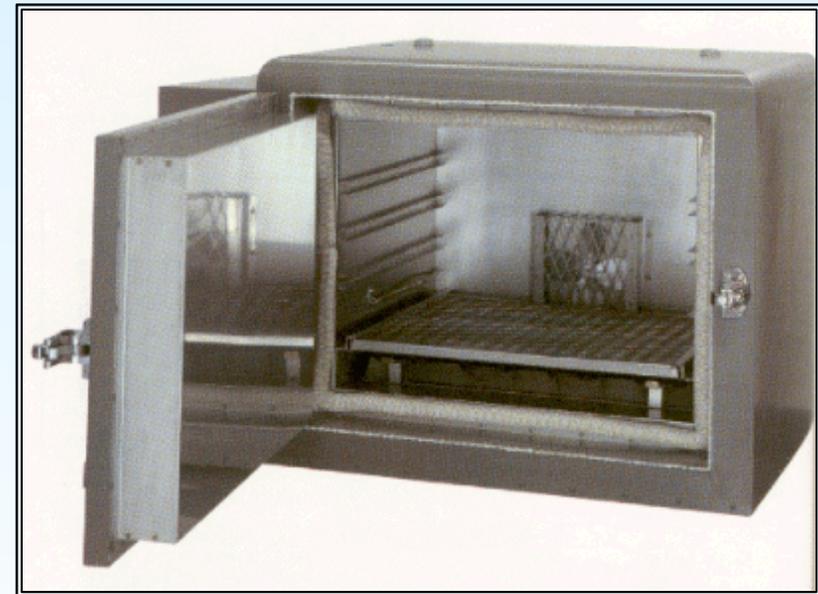
Refrigerators and Deep Freezers



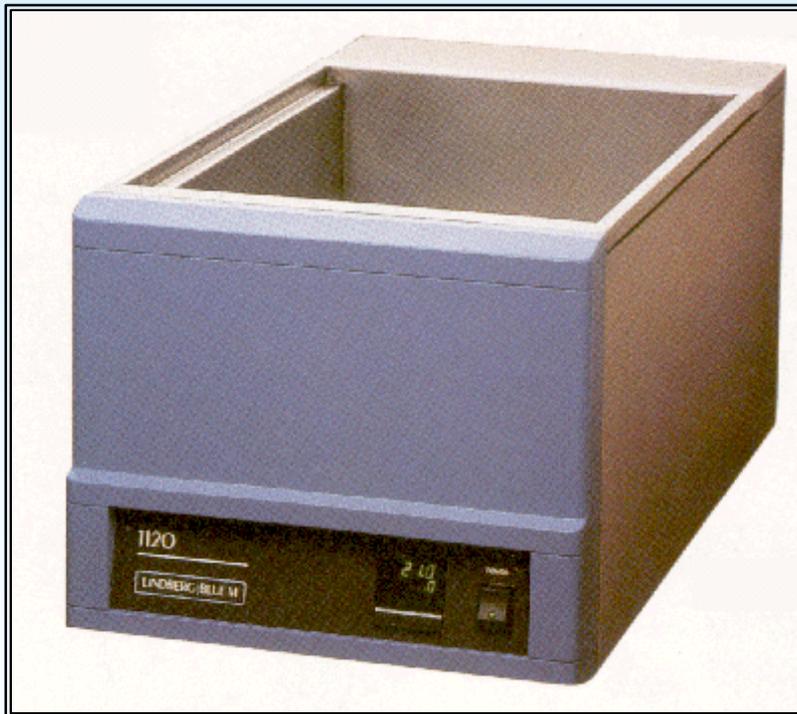
Laboratory Glassware



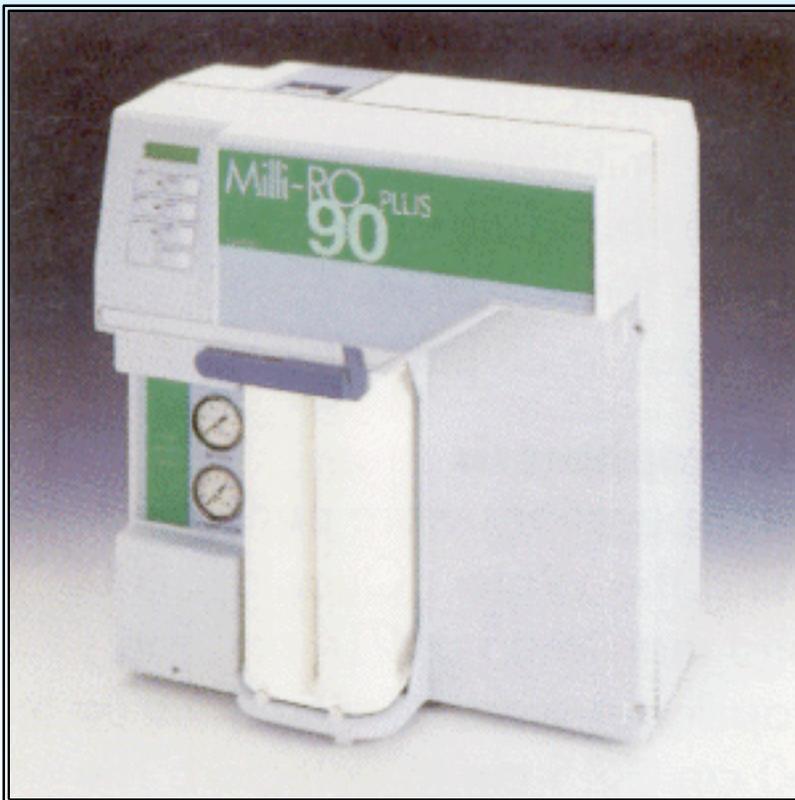
Incubators and Laboratory Sterilizers



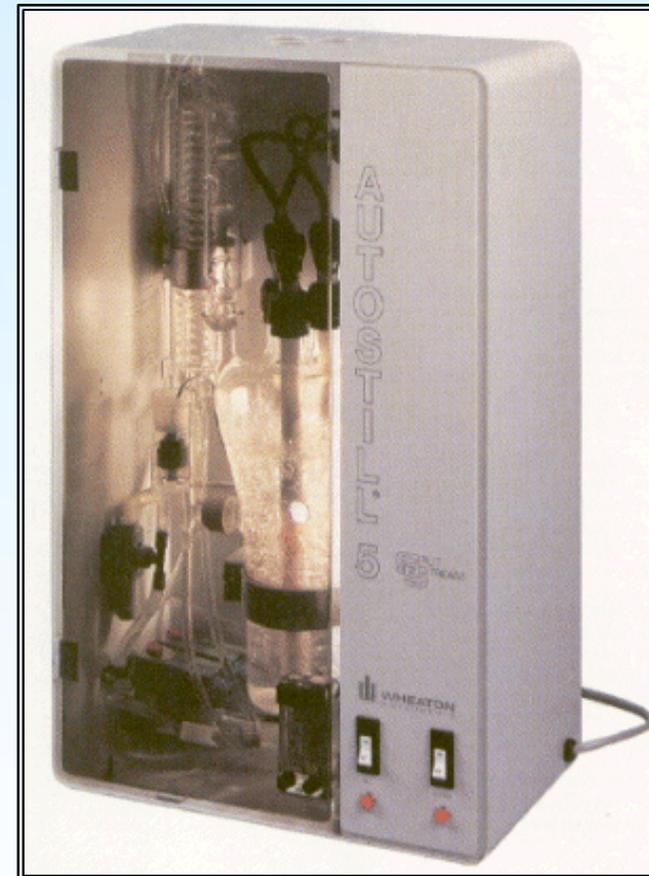
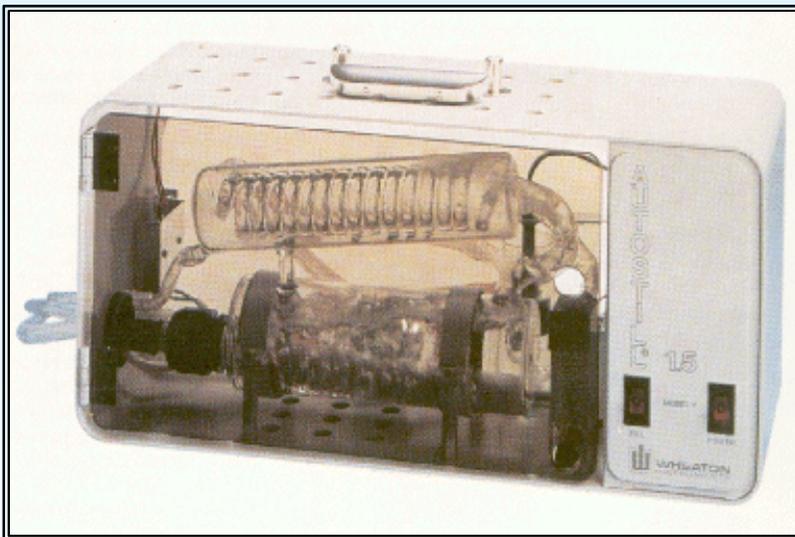
Waterbaths



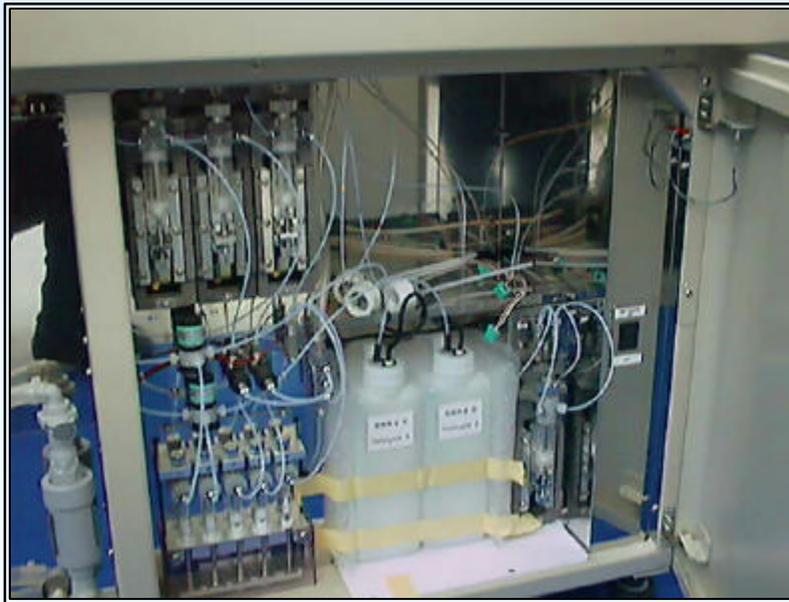
Deionization Devices (Ion Exchange Resins)



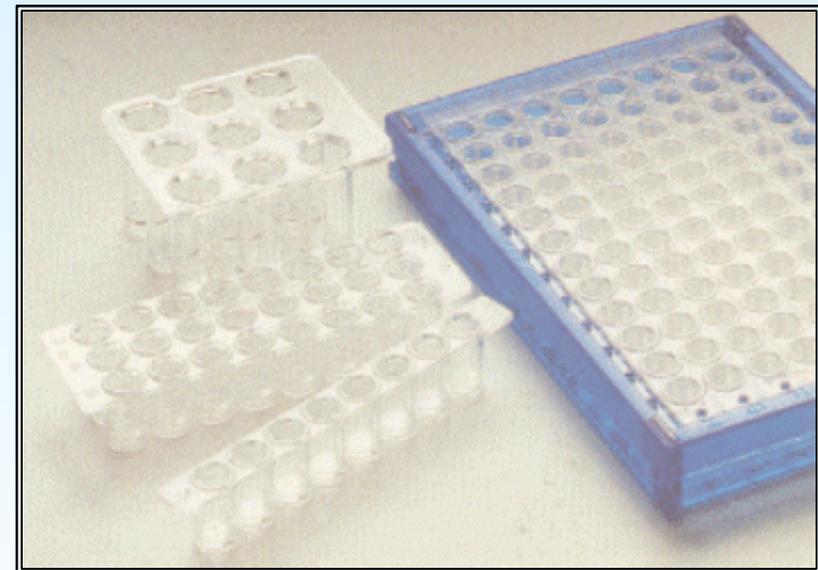
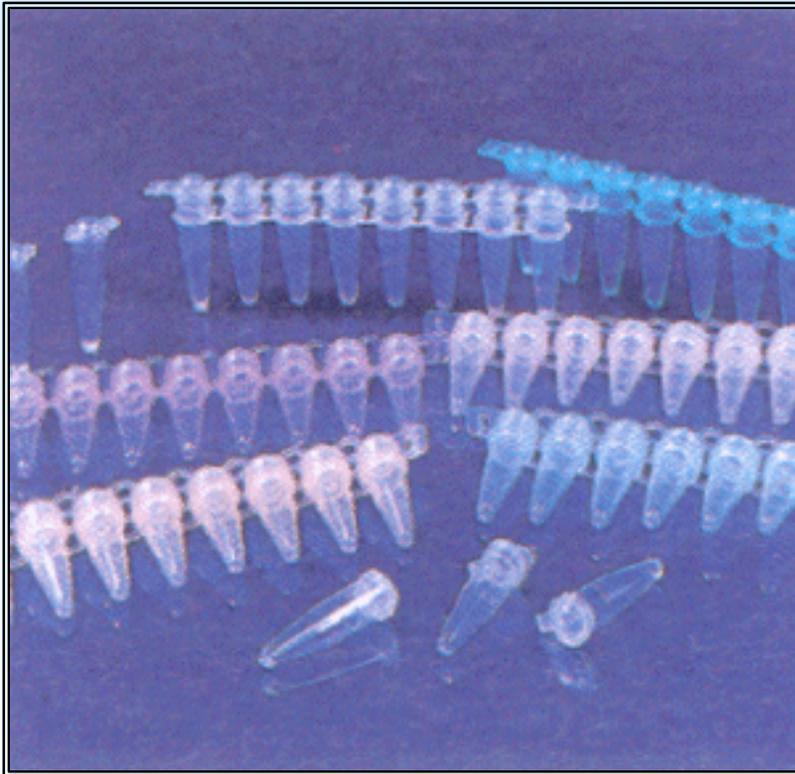
Distillation Devices



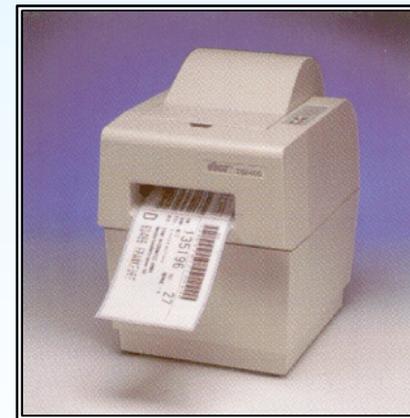
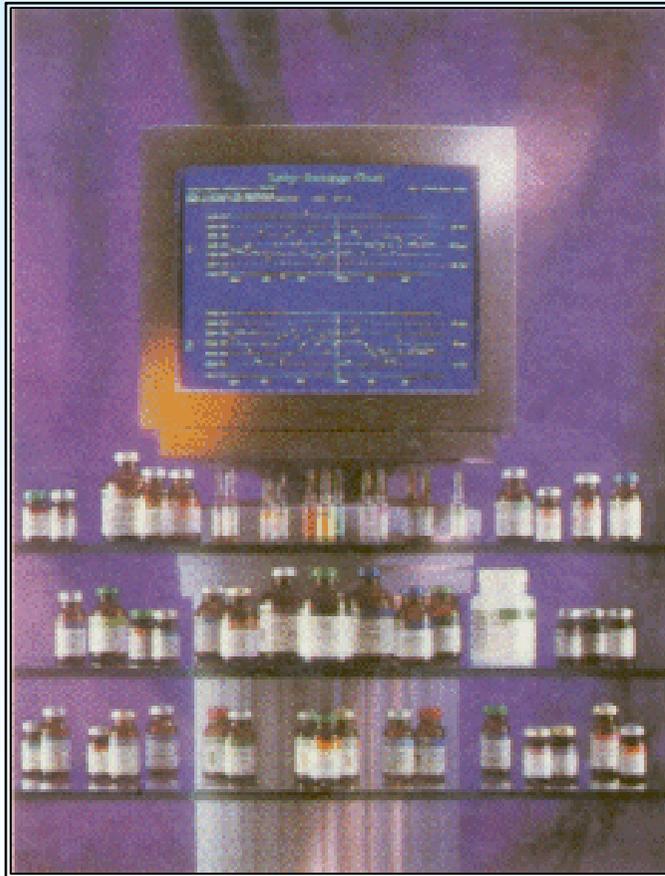
Equipment and Reagent Management



Laboratory Disposable Items



Laboratory Load Management Techniques



Laboratory Safety



- *Safety practices in the clinical laboratory must be followed by all personnel who work in or enter the laboratory.*
- *National & International Regulations & Standards address most of the following safe work practices.*



General practice

- Smoking, eating and application of cosmetics are prohibited in laboratory and a protective gown should be worn over clothing.
- Contact lenses should be discouraged. Dangling jewelry, long hair and beards may pose a safety hazard.
- Drawing specimens, reagents or other substances through a pipet by mouth should be prohibited. Hand washing is one of the major ways of preventing spread of infectious agents.
- Trash, infectious waste and dirty glassware should not be allowed to accumulate and containers of discarded specimens should be covered, working surfaces should be frequently cleaned and disinfected.
- Needles and other sharps should be discarded into a puncture-resistant and leak-proof container. All containers should be clearly labeled and all hazardous areas should be clearly marked.



Chemical Safety

- A Chemical Hygiene Plan is required in clinical and research laboratories including (Anderson, 1993):
- Criteria for and methods of monitoring chemical exposure.
- Standard operating procedures for handling hazardous chemicals.
- Criteria for implementing engineering controls (e.g. fume hoods).
- Use of personal protective equipment and other hygiene practices.
- Special precautions for extremely hazardous chemicals.
- Specific measures to ensure that protective equipment are properly working.
- Provision for employee information, training, and medical consultation.
- Designation of a chemical hygiene officer.



The hazard categories of chemicals

- ◆ *Corrosives with pH 12.5 or pH 2.0.*
- ◆ *Toxic substances, i.e. poisons, irritants, and asphyxiants.*
- ◆ *Carcinogens.*
- ◆ *Mutagens and teratogens.*
- ◆ *Ignitable, i.e. flammables and combustibles.*
- ◆ *Reactive, i.e. explosives and oxidizers.*

All chemicals in primary or secondary containers must be labeled with the name of the chemical and hazard, if any, and should be stored in an uncluttered area that is properly ventilated and away from a heat source. A written plan for containing and cleaning up chemical spills and spill pillows or other absorbent materials should be available.



Storage and Disposal Policies

- In general only small amounts, less than 100ml, of water-soluble, non-flammable, and no heavy-metal containing chemicals can be put into the sewer drains.
- The drain should be flushed with at least 100-fold excess of water and should flow into a wastewater treatment plant.
- Most laboratory chemicals will need to be disposed by a licenced chemical hauler or disposal company.
- Compressed gas cylinders should never be stored in flammable safety cabinets with flammable and combustible liquids.
- They should be grouped by types and stored in a ventilated room reserved exclusively for cylinder storage.



Biologic Safety

- Clinical laboratory workers are recognized as high-risk group for job-related infection with *Hepatitis B Virus (HBV)* and *Human Immunodeficiency Virus (HIV)*.
- The laboratory must supply personal protective equipment that does not permit potentially infectious materials, to reach clothes, skin, eyes and mouth under normal conditions of use.
- During biologic spill cleanup, gloves and a gown or lab-coat must always be worn, disposable towels should be placed over the spill and disinfectant poured on the towel and after standing a few minutes, the biologic material and disinfectant can be absorbed with absorbent material.



The categories of infectious waste

- The contaminated material is placed in abiohazard container and the spill-site is cleaned with high-level disinfectant.
- Infectious waste should be segregated from the regular trash and placed in a clearly marked infectious waste container.
- These containers should be disposed of by incineration, autoclaving, or discharge into a sanitary sewer system.
- The categories of infectious waste are:
 - ◆ *Isolation room waste.*
 - ◆ *Cultures and stocks of infectious agents and biological material.*
 - ◆ *Human blood and blood products.*
 - ◆ *Pathologic waste (e.g., tissue specimens).*
 - ◆ *Contaminated sharps.*
 - ◆ *Contaminated animal carcasses, body parts, and bedding.*



Radiation safety

- In clinical laboratory practice ^3H , ^{14}C , ^{32}P and ^{125}I are commonly used.
- Receipt of radioisotopes should be documented and broken packages monitored for significant leakage.
- Procedures using radioisotopes should be performed in a separate room, over an absorber pad.
- Waste containers should be clearly labeled.
- All personnel should wear an exposure-monitoring device such as a film or a TLD badge.
- Spills should be cleaned up with soap and water, beginning cleaning at the outer edge of the spill and work inward, until monitoring shows acceptable radiation levels.



RIA in vitro tests

- Effluents from RIA in vitro tests may be flushed into sanitary sewer and diluted with large amounts of water, designating one sink for this purpose.
- Other material such as disposable tubes and pipets that have been in contact with the radioisotopes may be safely discarded in the routine trash after all radioactive labels are removed.
- If the waste also contains biohazardous material, it may be autoclaved before disposal into the routine trash.



Quality Assurance

- *Quality assurance* is a process by which a laboratory ensures quality in laboratory results by closely monitoring the preanalytical, analytical, and postanalytical stages of laboratory testing.
- *Quality control* is a section of the analytical stage of quality assurance and is the process of monitoring results from control samples to verify quality in results from patients samples run alongside the controls.
- Quality control processes have both *internal* and *external* components.



Internal Quality Control

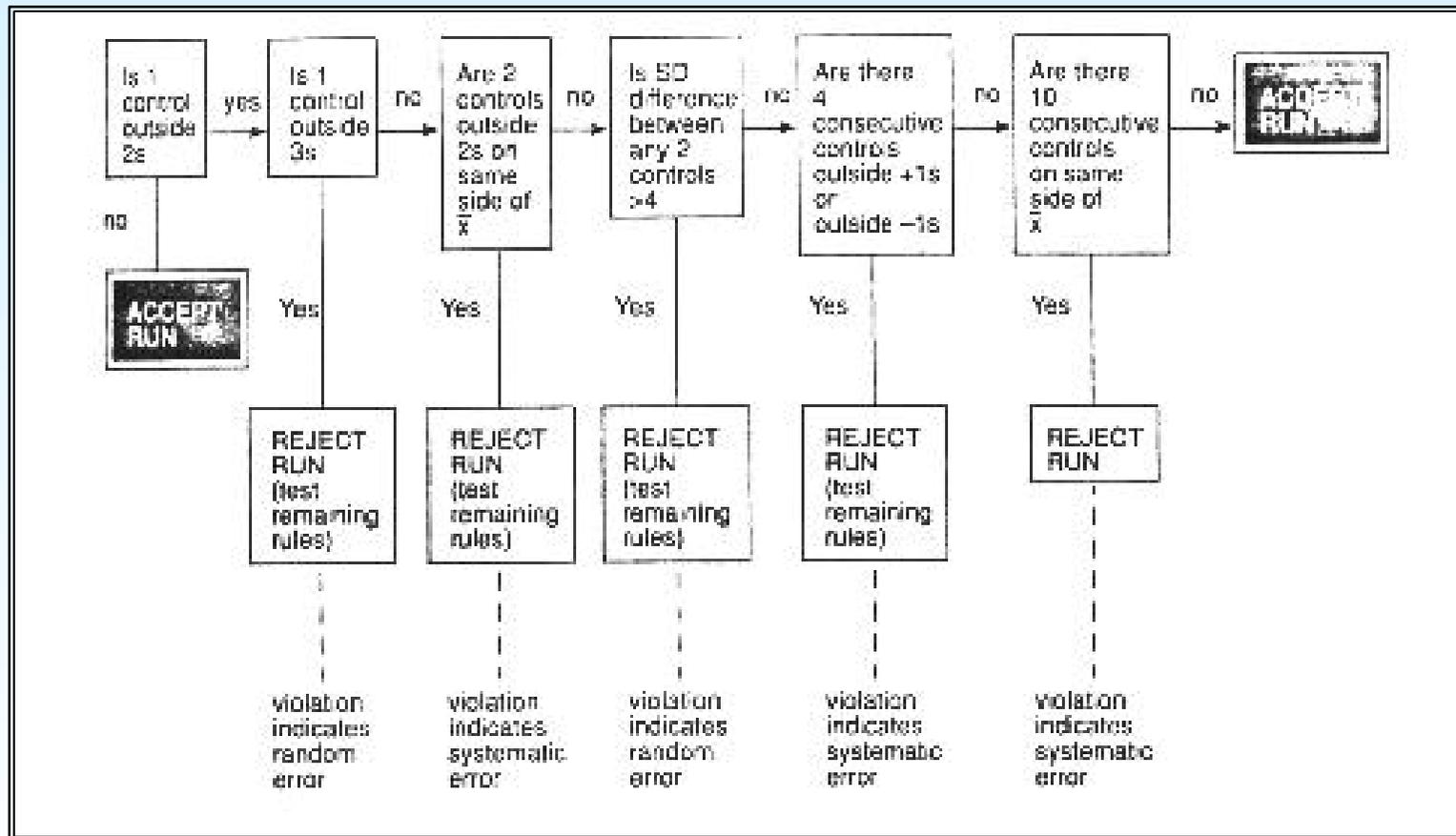
- The first step in an *internal Quality Control* process is to establish the target range for the control sample. The target range is set by assaying the control repeatedly and verifying the existence of a point of central tendency and a tight distribution in the resulting data set.
- If the point of central tendency is near the actual value of the control, the control can be used to monitor accuracy in lab results. If the repeat measurements produce a data set with a tight distribution about the point of central tendency, the control can also be used to monitor precision in lab results.



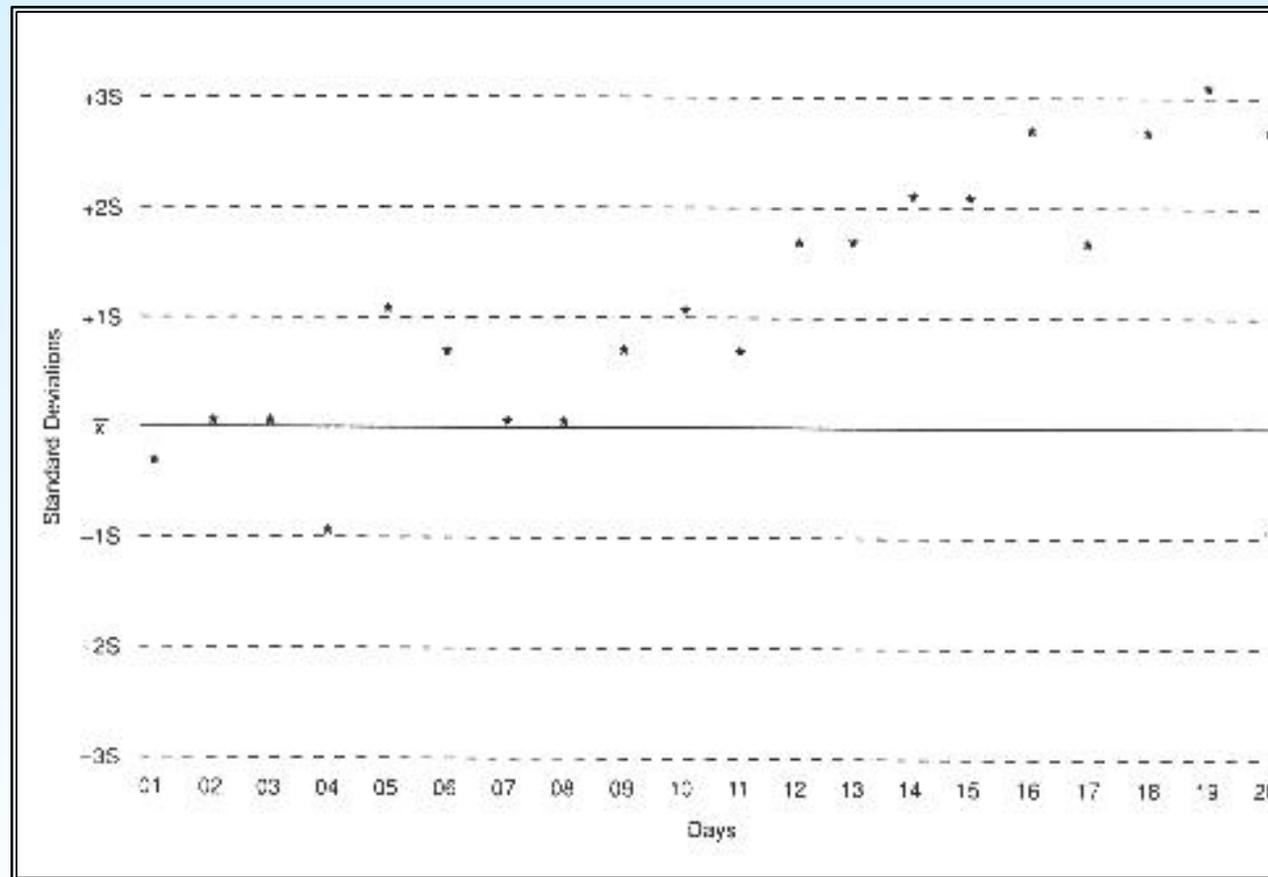
Monitoring of control results

- Once the range has been established, control results are monitored over time to detect any significant change in values that might indicate variability in method performance and a possible imprecision or inaccuracy in patient results.
- Imprecision in results is known as random error and inaccuracy is termed systematic error.
- The monitoring of control results to detect random and systematic error is done by visual methods such as the Levey-Jennings charts or by multirule (e.g. the Westgard multirule system) methods that rely on rule violation to indicate that an error has occurred.

The Westgard multirule system



Levey - Jennings charts





External Quality Control

- *External Quality Control* is the process of assaying unknown samples from an outside agency and thus verifying accuracy of testing by comparison to an established value for the outside sample or comparison to the average result obtained on the sample by laboratories within a peer group. The most common external Quality Control systems are proficiency testing programs.
- *Method evaluation* is a part of preanalytic Quality Assurance.
 - ◆ *Evaluation studies verify that a method is accurate and precise before it is incorporated into a laboratory's testing menu.*
 - ◆ *Linearity and precision studies are done to verify manufacturers claims. Accuracy is verified by patient comparison studies.*
 - ◆ *Diagnostic evaluation studies are sometimes performed to test the ability of a new method to correctly diagnose disease.*



Quality and Reference Range

- Quality in the final lab result depends heavily on using the correct reference range. Verification of this range is considered part of postanalytical Quality Assurance.
- Subjects for reference interval studies must represent the population of interest, and since data often do not follow a normal distribution, nonparametric statistics are often used to calculate the interval.
- A complete and effective Quality Assurance program verifies quality at every step in testing process (preanalytical, analytical and postanalytical).