Ministry of high Education and Scientific Research

Foundation of Technical Education

College Health &Medical Technology / Baghdad

Department of clinical laboratory techniques

Educational package

In

(Laboratories Equipments)

For

First year student

lecturer

by

Lacturer: Nazar Shiyaa Mohammed

Microbiotecnology

Over view

Target population :-

For students of first class

Department of: **Laboratories Equipments**

College of Health &Medical Technology / Baghdad

First week

General objective : To study the type Laboratories Equipments, and uses.

Laboratories Equipments:

The Microscope:

It a imaging instrument and it is primary toll for many material investigations, it in larges the minute details the objects under study.

Notes about microscope.

There's two types of microscope according to the number of eye piece (Ocular)

a- Monocular
b- Binocular

There's two types of microscope according to the source of the light.

a- Day light
b- Electric lamps

There's two types of microscope according to the technical uses:

a- Dark field microscope
b- Polarizing microscope
c- Fluorescent microscope
d- Phase contrast microscope
e- Interference microscope

Second & Third week

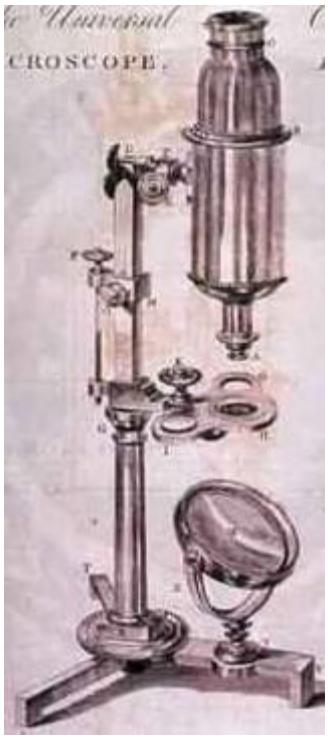
History of the Microscope

How the light microscope evolved.

By Mary Bellis, About.com Guide

See More About:

- microscope
- glass
- telescope
- anton van leeuwenhoek
- robert hooke



Light Microscope

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- See also: Timeline - History of the Light Microscope
  Timeline of simple and compound light microscopes.

During that historic period known as the Renaissance, after the "dark" Middle Ages, there occurred the inventions of printing, gunpowder and the mariner's compass, followed by the discovery of America. Equally remarkable was the invention of the light microscope: an instrument that enables the human eye, by means of a lens or combinations of lenses, to observe enlarged images of tiny objects. It made visible the fascinating details of worlds within worlds.

Invention of Glass Lenses

Long before, in the hazy unrecorded past, someone picked up a piece of transparent crystal thicker in the middle than at the edges, looked through it, and discovered that it made things look larger. Someone also found that such a crystal would focus the sun's rays and set fire to a piece of parchment or cloth. Magnifiers and "burning glasses" or "magnifying glasses" are mentioned in the writings of Seneca and Pliny the Elder, Roman philosophers during the first century A. D., but apparently they were not used much until the invention of spectacles, toward the end of the 13th century. They were named lenses because they are shaped like the seeds of a lentil.

The earliest simple microscope was merely a tube with a plate for the object at one end and, at the other, a lens which gave a magnification less than ten diameters -- ten times

the actual size. These excited general wonder when used to view fleas or tiny creeping things and so were dubbed "flea glasses."

Birth of the Light Microscope

About 1590, two Dutch spectacle makers, Zaccharias Janssen and his son Hans, while experimenting with several lenses in a tube, discovered that nearby objects appeared greatly enlarged. That was the forerunner of the compound microscope and of the telescope. In 1609, Galileo, father of modern physics and astronomy, heard of these early experiments, worked out the principles of lenses, and made a much better instrument with a focusing device.

Fourth & Fifth week

Centrifuge

Definition: Is a simple instrument use for separation or precipitation of particles by centrifugal action.

Types:

1- Manual centrifuge.

separation and precipitation large particle

2- Electric centrifuge.

a- Universal centrifuge.

separation and precipitation different particle

speed (300- 5000) RM

b- Heamatocrit centrifuge.

Used for PCV test (packed cell volume)

speed (15000) RM

c- Ultra centrifuge.

d- speed (20000- 100000) RM

Used for separated very small particles

Parts:

1- Motor

2- Head

3- Timer

4- Tachometer

5- Brake

6- Temperature controls keys

7- Green or red colors key

8- Cover

Maintenance:

1- Replace brushes from time to time.

2- Clean the motor.

3- Clean the test tube holders.

Laboratory centrifuge

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Laboratory centrifuge

A tabletop laboratory centrifuge

Uses	Separation
Related items	Gas centrifuge Ultracentrifuge

A **laboratory centrifuge** is a piece of laboratory equipment, driven by a motor, which spins liquid samples at high speed. There are various types of centrifuges, depending on the size and the sample capacity.

Like all other centrifuges, laboratory centrifuges work by the sedimentation principle, where the centripetal acceleration is used to separate substances of greater and lesser density.

Contents

[hide]

Operation

Increasing the effective gravitational force will more rapidly and completely cause the precipitate to gather on the bottom of the tube as a "pellet". The remaining solution is called the "supernate" or "supernatant".

The supernatant liquid is then either quickly decanted from the tube without disturbing the pellet, or withdrawn with a Pasteur pipette. The rate of centrifugation is specified by the acceleration applied to the sample, typically measured in revolutions per minute (RPM) or relative centrifugal force (RCF). The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity.

The use of a centrifuge is known as centrifugation.

Types

Laboratory centrifuge

There are various types of centrifugation:

- Differential centrifugation, often used to separate certain organelles from whole cells for further analysis of specific parts of cells
- Isopycnic centrifugation, often used to isolate nucleic acids such as DNA
- Sucrose gradient centrifugation, often used to purify enveloped viruses and ribosomes, and also to separate cell organelles from crude cellular extracts

There are different types of laboratory centrifuges:

- **Microcentrifuges**

(devices for small tubes from 0.2 ml to 2.0 ml (micro tubes), up to 96 well-plates, compact design, small footprint; up to 30.000 g)

- **Clinical centrifuges**

(devices used for clinical applications like blood collection tubes, low-speed devices)

- **Multipurpose benchtop centrifuges**

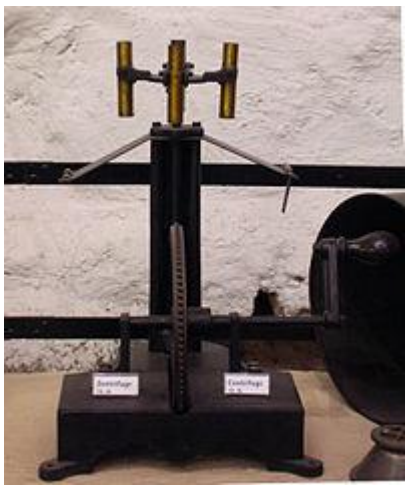
(devices for a broad range of tube sizes, high variability, big footprint)

- **Stand alone centrifuges**

(heavy devices like the ultracentrifuge)

Many centrifuges are available with (refrigerated device) or without cooling function. There are different providers of laboratory centrifuges like Eppendorf, Thermo-Heraeus, Thermo-Sorvall, Hettich, Beckmann-Coulter, MSE, Sigma and Awel.

History



A 19th century hand cranked laboratory centrifuge.

English military engineer Benjamin Robins (1707–1751) invented a whirling arm apparatus to determine drag. In 1864, Antonin Prandtl invented the first dairy centrifuge in order to separate cream from milk. In 1879, Gustaf de Laval demonstrated the first continuous centrifugal separator, making its commercial application feasible.

Different sizes of centrifuges were developed. The range of applications varied from Liter-scale to Milli-Liter-scale.

Regarding the laboratory microcentrifuge, in 1962 the Hamburg-based company "Netheler & Hinz Medizintechnik" (nowadays known as "eppendorf") developed the "Microliter System" for laboratory usage. Besides the first piston stroke pipette, based on the work of Dr. Schnittger (Marburg/ Germany), the plastic-made micro test tube and the first microcentrifuge (model 3200) were introduced for applications in routine

analysis labs in microliter scale. This first real microcentrifuge had one control knob for the time and space for up to 12 micro test tubes in a fixed-angle rotor. Common up-to-date features like cooling, programming, automatic imbalance detection, noise reduction, or changeable rotor systems were completely missing.

The "Microliter System" was the starting point for a broad range of tools for the molecular lab, developed by all different kinds of biotech and labware companies.

Design

A large laboratory centrifuge.

Laboratory centrifuges are used in chemistry, biology, and biochemistry for isolating and separating solids from liquids in a suspension. The solids can be insoluble compounds, biomolecules, cell organelles, or whole cells. They vary widely in speed and capacity. They usually comprise a rotor containing two, four, six, or many more numbered wells within which centrifuge tubes may be placed.

When a suspension in a centrifuge tube is centrifuged, the solids settle at the bottom of the centrifuge tube; having a tapered wall helps to concentrate the solids, making it easier to decant the supernatant solution, leaving the solids.

Generally spoken, there are two main types of rotors:

Fixed-angle rotor

The rotor (mainly made of aluminium) is very compact. There are boreholes with a specific angle (like 45°) within the rotor. These boreholes are used for the sample tubes.

Swing-out rotor (= horizontal rotor)

The rotor looks like a cross with gondolas, called buckets. Within these buckets, different tubes can be centrifuged. For a safe centrifugation, a specific adapter for every tube shape is mandatory.

The rotor is closed by a rotor lid. The rotor is located in a rotor chamber which is covered by a metall centrifuge lid. The open lid prevents the motor from turning the rotor when the rotor chamber is open. During the run, the lid is locked. The lid protects the user from being injured by touching a rapidly spinning rotor. The rotor chamber and the lid of high quality centrifuges are robust enough to survive a rotor failure at full speed. This robustness protects the user and the laboratory from crashing fragments in case the rotor fails catastrophically. After a rotor crash, a centrifuge should not be reused as the enormous forces during a crash may have damaged essential parts of the device.

The rotor must be balanced by placing samples or blanks of equal mass opposite each other. Since most of the mass is derived from the solvent, it is usually sufficient to place blanks or other samples of equal volume. As a safety feature, some centrifuges may stop turning when wobbling is detected (automatic imbalance detection, see Safety).

Centrifuge tubes:

Centrifuge tubes or **centrifuge tips** are tapered tubes of various sizes made of glass or plastic. They may vary in capacity from tens of millilitres, to much smaller capacities used in microcentrifuges used extensively in molecular biology laboratories. The most commonly encountered tubes are of about the size and shape of a normal test tube (~ 10 cm long). Microcentrifuges typically accommodate microcentrifuge tubes with capacities from 250 µl to 2.0 ml. These are exclusively made of plastic.

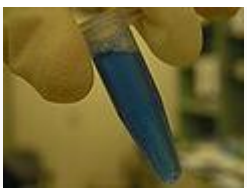
Glass centrifuge tubes can be used with most solvents, but tend to be more expensive. They can be cleaned like other laboratory glassware, and can be sterilized by autoclaving. Plastic centrifuge tubes, especially microcentrifuge tubes tend to be less expensive. Water is preferred when plastic centrifuge tubes are used. They are more difficult to clean thoroughly, and are usually inexpensive enough to be considered disposable.

Microcentrifuge tubes:

Microcentrifuge tubes or **microfuge tubes** are small, cylindrical plastic containers with conical bottoms, typically with an integral snap cap. They are used in molecular biology and biochemistry to store and centrifuge small amounts of liquid. As they are inexpensive and considered disposable, they are used by many chemists and biologists as convenient sample vials in lieu of glass vials; this is particularly useful when there is only a small amount of liquid in the tube or when small amounts of other liquids are being added, because microcentrifugation can be used to collect the drops together at the bottom of the tube after pipetting or mixing.

Made of polypropylene,[1] they can be used in very low temperature (-80 °C to liquid nitrogen temperatures) or with organic solvents such as chloroform. They come in many different sizes, generally ranging from 250 µL to 2.0 mL. The most common size is 1.5 mL. Disinfection is possible (1 atm, 120 °C, 20 minutes) and is commonly performed in work related to DNA or microbes, where purity of the sample is of utmost importance. Due to their low cost and the difficulty in cleaning the plastic surface, they are usually discarded after each use.

Eppendorf tube has become a genericized trademark for *microfuge tubes* or *microcentrifuge tubes*. Eppendorf is a major manufacturer of this item, but is not the only one.

- 

  Microcentrifuge tube with Coomassie Blue solution.

- 

  left|Three microcentrifuge tubes: 2 mL, 1.7 mL and 200 µL (for PCR).

- 

  Four screw-top microcentrifuge tubes.

Safety

The load in a laboratory centrifuge must be carefully balanced. This is achieved by using a combination of samples and balance tubes which all have the same weight or by using various balancing patterns without balance tubes.[2] Small differences in mass of the load can result in a large force imbalance when the rotor is at high speed. This force imbalance strains the spindle and may result in damage to the centrifuge or personal injury. Some centrifuges have an automatic rotor imbalance detection feature that immediately discontinues the run when an imbalance is detected.

Before starting a centrifuge, an accurate check of the rotor and lid locking mechanisms is mandatory. Centrifuge rotors should never be touched while moving, because a spinning rotor can cause serious injury. Modern centrifuges generally have features that prevent accidental contact with a moving rotor as the main lid is locked during the run.

Centrifuge rotors have tremendous kinetic energy during high speed rotation. Rotor failure, caused by mechanical stress from the high forces imparted by the motor, can occur due to manufacturing defects, routine wear and tear, or improper use and maintenance. Such a failure can be catastrophic failure, especially with larger centrifuges, and generally results in total destruction of the centrifuge. While centrifuges generally have safety shielding to contain these failures, such shielding may be inadequate, especially in older models, or the entire centrifuge unit may be propelled from its position, resulting in damage to nearby personnel and equipment. Uncontained rotor failures have shattered laboratory windows and destroyed refrigerators and cabinetry. To reduce the risk of rotor failures, centrifuge manufactures specify operating and maintenance procedures to ensure that rotors are regularly inspected and removed from service or derated (only operated at lower speeds) when they are past their expected lifetime.[3]

Another potential hazard is the aerosolization of hazardous samples during centrifugation. To prevent contamination of the laboratory, rotor lids with special aerosol-tight gaskets are available. The rotor can be loaded with the samples

within a hood and the rotor lid fixed on the rotor. Afterwards, the aerosol-tight system of rotor and lid is transferred to the centrifuge. The rotor can then be fixed within the centrifuge without opening the lid. After the run, the entire rotor assembly, including the lid, is removed from the centrifuge to the hood for further steps, maintaining the samples within a closed system.

Theory

Protocols for centrifugation typically specify the amount of acceleration to be applied to the sample, rather than specifying a rotational speed such as revolutions per minute. The acceleration is often quoted in multiples of g, the acceleration due to gravity at the Earth's surface. This distinction is important because two rotors with different diameters running at the same rotational speed will subject samples to different accelerations.

The acceleration can be calculated as the product of the radius and the square of the angular velocity.

Relative centrifugal force (RCF) is the measurement of the force applied to a sample within a centrifuge. This can be calculated from the speed (RPM) and the rotational radius (cm) using the following calculation.

$$g = RCF = 0.00001118 \times r \times N^2$$

Seven & Eight Week

Temperature Controlled Instruments

It included the following instruments:

1- Water bath

2- Incubator

3- Dry oven

4- Autoclave

Sterilization: Is the freeing of an article from all living organisms including bacteria and their spores.

Disinfection: Is a process having a narrow spectrum of activity, effective principally on nor acid fast vegetative and with an uncertain action on bacterial endospores.

Types of sterilization:

Physical methods:

1- Heat

a- Dry Heat

- Flame

- Hot air oven

- Red Heat

2- Radiation

a- Ionizing

b- non- Ionizing

Chemical Methods:

1- Bactericidal

2- Bacteriostatic

Sterilization by Heat:

1- Dry Heat

a- Red Heat

b- Flaming

c- Hot air oven

d- infra- red radiation

2- Wet Heat

a- at temp below 100C

b- at temp of 100C

c- at temp below 100C

nine week

Water Bath:

Principle of work depend on heater which heating water pleased in the tank informally the temperature degree controlled by thermostat within a range (25-100C) and there's thermometer is also providing to check up the accuracy of thermostat control.

Parts:

1- on- off switch

2- Thermostat

3- Electrical heater

5- Thermometer

Maintenance:

1- Keep it clean always and prevent all the secreting materials like salts and acids to reach it.

2- Keep on the thermostat away from water.

Uses:

It used in the researching and analytical laboratories to elevated liquid temperature degree to create a chemical – enzymatic…….etc reaction.

Week ten

Incubator:

It is same principle work to the water bath except there's no water tank and temperature degree ranged (25-60C).

Parts:

It the same parts the water bath but no contain water tank.

Types:

1- **Ordinary incubator:**

   It work by electrical power and thermostat connected with electrical heater to elevated temperature degree and there's thermostat.

2- **Co2 incubator:**

   It work within Co2 gas conditions with gas concentration(5-20%) this type used in bacteriological growth that need to this conditions and their parts included adjusters for Co2 liberation, heater, thermometer, On-Off switch.

Maintenance:

1- Keep it clean always and prevent all the secreting materials like salts and acids to reach it.

2- Keep on the thermostat away from water.

Week Eleven & Twelve

Gel electrophoresis

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Gel electrophoresis

Gel electrophoresis refers to using a gel as an anticonvective medium and or sieving medium during electrophoresis. Gel electrophoresis is most commonly used for separation of biological macromolecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein; however, gel electrophoresis can be used for separation of nanoparticles. Electrophoresis refers to the movement of a charged particle in an electrical field. Gels suppress the thermal convection caused by application of the electric field, and can also act as a sieving medium, retarding the passage of molecules; gels can also simply serve to maintain the finished separation, so that a post electrophoresis stain can be applied.[1] DNA Gel electrophoresis is usually performed for analytical purposes, often after amplification of DNA via PCR, but may be used as a preparative technique prior to use of other methods such as mass spectrometry, RFLP, PCR, cloning, DNA sequencing, or Southern blotting for further characterization.

Contents:

Separation:

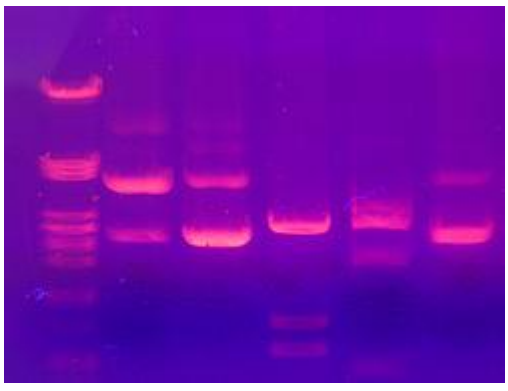
In simple terms: Electrophoresis is a procedure which enables the sorting of molecules based on size and charge. Using an electric field, molecules (such as DNA) can be made to move through a gel made

of agar. The molecules being sorted are dispensed into a well in the gel material. The gel is placed in an electrophoresis chamber, which is then connected to a power source. When the electric current is applied, the larger molecules move more slowly through the gel while the smaller molecules move faster. The different sized molecules form distinct bands on the gel.[*citation needed*]

The term "gel" in this instance refers to the matrix used to contain, then separate the target molecules. In most cases, the gel is a crosslinked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed. When separating proteins or small nucleic acids (DNA, RNA, or oligonucleotides) the gel is usually composed of different concentrations of acrylamide and a cross-linker, producing different sized mesh networks of polyacrylamide. When separating larger nucleic acids (greater than a few hundred bases), the preferred matrix is purified agarose. In both cases, the gel forms a solid, yet porous matrix. Acrylamide, in contrast to polyacrylamide, is a neurotoxin and must be handled using appropriate safety precautions to avoid poisoning. Agarose is composed of long unbranched chains of uncharged carbohydrate without cross links resulting in a gel with large pores allowing for the separation of macromolecules and macromolecular complexes.[*citation needed*]

"Electrophoresis" refers to the electromotive force (EMF) that is used to move the molecules through the gel matrix. By placing the molecules in wells in the gel and applying an electric field, the molecules will move through the matrix at different rates, determined largely by their mass when the charge to mass ratio (Z) of all species is uniform, toward the anode if negatively charged or toward the cathode if positively charged.[2]

Visualization:



Gel electrophoresis

After the electrophoresis is complete, the molecules in the gel can be stained to make them visible. Ethidium bromide, silver, or Coomassie Brilliant Blue dye may be used for this process. Other methods may also be used to visualize the separation of the mixture's components on the gel. If the

analyte molecules fluoresce under ultraviolet light, a photograph can be taken of the gel under ultraviolet lighting conditions, often using a Gel Doc. If the molecules to be separated contain radioactivity added for visibility, an autoradiogram can be recorded of the gel.[*citation needed*]

If several samples have been loaded into adjacent wells in the gel, they will run parallel in individual lanes. Depending on the number of different molecules, each lane shows separation of the components from the original mixture as one or more distinct bands, one band per component. Incomplete separation of the components can lead to overlapping bands, or to indistinguishable smears representing multiple unresolved components.[*citation needed*]

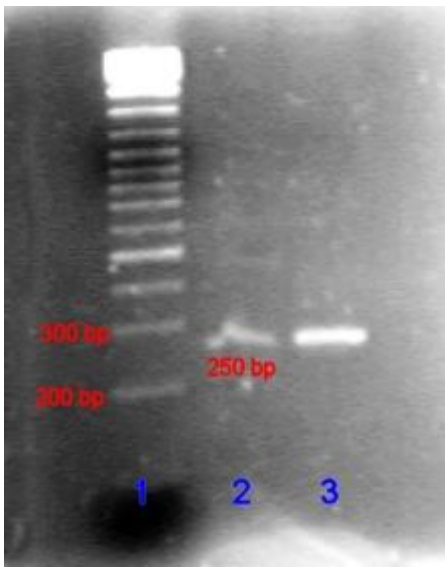
Bands in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size. There are molecular weight size markers available that contain a mixture of molecules of known sizes. If such a marker was run on one lane in the gel parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size. The distance a band travels is approximately inversely proportional to the logarithm of the size of the molecule.[*citation needed*]

Applications:

Gel electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. The image is recorded with a computer operated camera, and the intensity of the band or spot of interest is measured and compared against standard or markers loaded on the same gel. The measurement and analysis are mostly done with specialized software.

Depending on the type of analysis being performed, other techniques are often implemented in conjunction with the results of gel electrophoresis, providing a wide range of field-specific applications.

Nucleic acids:



An agarose gel of a PCR product compared to a DNA ladder.

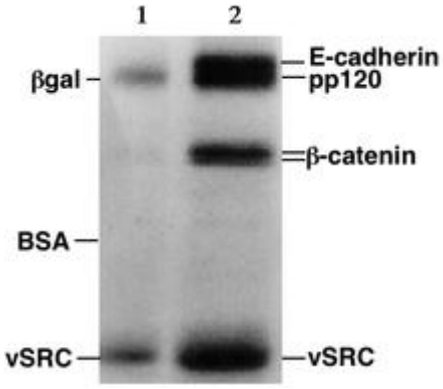
In the case of nucleic acids, the direction of migration, from negative to positive electrodes, is due to the naturally-occurring negative charge carried by their sugar-phosphate backbone.[3]

Double-stranded DNA fragments naturally behave as long rods, so their migration through the gel is relative to their size or, for cyclic fragments, their radius of gyration. Single-stranded DNA or RNA tend to fold up into molecules with complex shapes and migrate through the gel in a complicated manner based on their tertiary structure. Therefore, agents that disrupt the hydrogen bonds, such as sodium hydroxide or formamide, are used to denature the nucleic acids and cause them to behave as long rods again.[4]

Gel electrophoresis of large DNA or RNA is usually done by agarose gel electrophoresis. See the "Chain termination method" page for an example of a polyacrylamide DNA sequencing gel. Characterization through ligand interaction of nucleic acids or fragments may be performed by mobility shift affinity electrophoresis.

Electrophoresis of RNA samples can be used to check for genomic DNA contamination and also for RNA degradation. RNA from eukaryotic organisms shows distinct bands of 28s and 18s rRNA, the 28s band being approximately twice as intense as the 18s band. Degraded RNA has less sharpely defined bands, has a smeared appearance, and intensity ratio is less than 2:1.

Proteins:



SDS-PAGE autoradiography – The indicated proteins are present in different concentrations in the two samples.

Proteins, unlike nucleic acids, can have varying charges and complex shapes, therefore they may not migrate into the polyacrylamide gel at similar rates, or at all, when placing a negative to positive EMF on the sample. Proteins therefore, are usually denatured in the presence of a detergent such as sodium dodecyl sulfate/sodium dodecyl phosphate (SDS/SDP) that coats the proteins with a negative charge.[1] Generally, the amount of SDS bound is relative to the size of the protein (usually 1.4g SDS per gram of protein), so that the resulting denatured proteins have an overall negative charge, and all the proteins have a similar charge to mass ratio. Since denatured proteins act like long rods instead of having a complex tertiary shape, the rate at which the resulting SDS coated proteins migrate in the gel is relative only to its size and not its charge or shape.[1]

Proteins are usually analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), by native gel electrophoresis, by quantitative preparative native continuous polyacrylamide gel electrophoresis (QPNC-PAGE), or by 2-D electrophoresis.

Characterization through ligand interaction may be performed by electroblotting or by affinity electrophoresis in agarose or by capillary electrophoresis as for estimation of binding constants and determination of structural features like glycan content through lectin binding.

Week Thirteen & Fourteen

Spectrophotometer:

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Spectrophotometer

In chemistry, **spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.[1] It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range of absorption or reflectance measurement.

A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases. However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200nm -

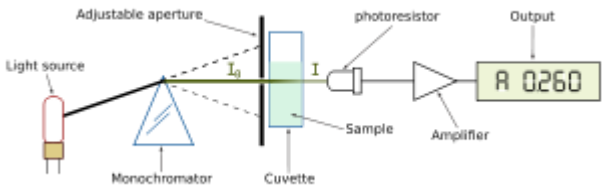
2500nm using different controls and calibrations.[2] Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the *photometric determination*.[3]

An example of an experiment in which spectrophotometry is used is the determination of the equilibrium constant of a solution. A certain chemical reaction within a solution may occur in a forward and reverse direction where reactants form products and products break down into reactants. At some point, this chemical reaction will reach a point of balance called an equilibrium point. In order to determine the respective concentrations of reactants and products at this point, the light transmittance of the solution can be tested using spectrophotometry. The amount of light that passes through the solution is indicative of the concentration of certain chemicals that do not allow light to pass through.

The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, and molecular biology.[4] They are widely used in many industries including semiconductors, laser and optical manufacturing, printing and forensic examination, as well in laboratories for the study of chemical substances. Ultimately, a spectrophotometer is able to determine, depending on the control or calibration, what substances are present in a target and exactly how much through calculations of observed wavelengths.

Contents:

Design:



Single beam spectrophotometer

There are two major classes of devices: single beam and double beam. A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample. A single beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted. Although comparison measurements from double beam instruments are easier and more stable, single beam instruments can have a larger dynamic range and are optically simpler and more compact. Additionally, some specialized instruments, such as spectrophotometer built onto microscopes or telescopes, are single beam instruments due to practicality.

Historically, spectrophotometers use a monochromator containing a diffraction grating to produce the analytical spectrum. The grating can either be movable or fixed. If a single detector, such as a photomultiplier tube or photodiode is used, the grating can be scanned stepwise so that the detector can measure the light intensity at each wavelength (which will correspond to each "step"). Arrays of detectors, such as charge coupled devices (CCD) or photodiode arrays (PDA) can also be used. In such systems, the grating is fixed and the intensity of each wavelength of light is measured by a different detector in the array. Additionally, most modern mid-infrared spectrophotometers use a Fourier transform technique to acquire the spectral information. The technique is called Fourier Transform Infrared.

When making transmission measurements, the spectrophotometer quantitatively compares the fraction of light that passes through a reference solution and a test solution. For reflectance measurements, the spectrophotometer quantitatively compares the fraction of light that reflects from the reference and test samples. Light from the source lamp is passed through a monochromator, which diffracts the light into a "rainbow" of wavelengths and outputs narrow bandwidths of this diffracted spectrum. Discrete frequencies are transmitted through the test sample. Then the photon flux density (watts per metre squared usually) of the transmitted or reflected light is measured with a photodiode, charge coupled device or other light sensor. The transmittance or reflectance value for each wavelength of the test sample is then compared with the transmission (or reflectance) values from the reference sample.

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample
2. A fraction of the light is transmitted or reflected from the sample
3. The light from the sample is imaged upon the entrance slit of the monochromator

4. The monochromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially

Many older spectrophotometers must be calibrated by a procedure known as "zeroing." The absorbancy of a reference substance is set as a baseline value, so the absorbancies of all other substances are recorded relative to the initial "zeroed" substance. The spectrophotometer then displays % absorbancy (the amount of light absorbed relative to the initial substance).

Week Fifteen & sixteen

Week seventeen

UV-visible spectrophotometer:

Main article: Ultraviolet-visible spectroscopy

The most common spectrophotometers are used in the UV and visible regions of the spectrum, and some of these instruments also operate into the near-infrared region as well.

Visible region 400–700 nm spectrophotometry is used extensively in colorimetry science. Ink manufacturers, printing companies, textiles vendors, and many more, need the data provided through colorimetry. They take readings in the region of every 5–20 nanometers along the visible region, and produce a spectral reflectance curve or a data stream for alternative presentations. These curves can be used to test a new batch of colorant to check if it makes a match to specifications e.g., ISO printing standards.

Traditional visible region spectrophotometers cannot detect if a colorant or the base material has fluorescence. This can make it difficult to manage color issues if for example one or more of the printing inks is fluorescent. Where a colorant contains fluorescence, a bi-spectral fluorescent spectrophotometer is used. There are two major setups for visual spectrum spectrophotometers, d/8 (spherical) and 0/45. The names are due to the geometry of the light source, observer and interior of the measurement chamber. Scientists use this instrument to measure the amount of compounds in a sample. If the compound is more concentrated more light will be absorbed by the sample; within small ranges, the Beer-Lambert law holds and the absorbance between samples vary with concentration linearly. In the case of printing measurements two alternative settings are commonly used-without/with uv filter to control better the effect of uv brighteners within the paper stock.

Samples are usually prepared in cuvettes; depending on the region of interest, they may be constructed of glass, plastic, or quartz.

IR spectrophotometer:

Main article: Infrared spectroscopy

Spectrophotometers designed for the main infrared region are quite different because of the technical requirements of measurement in that region. One major factor is the type of photosensors that are available for different spectral regions, but infrared measurement is also challenging because virtually everything emits IR light as thermal radiation, especially at wavelengths beyond about 5 μm.

Another complication is that quite a few materials such as glass and plastic absorb infrared light, making it incompatible as an optical medium. Ideal optical materials are salts, which do not absorb strongly. Samples for IR spectrophotometry may be smeared between two discs of potassium bromide or ground with potassium bromide and pressed into a pellet. Where aqueous solutions are to be measured, insoluble silver chloride is used to construct the cell.

Week seventeen

Sahli's apparatus

Alternative eponyms

- Sahli’s tube

Related people

- Hermann Sahli

Instrument used in Sahli's method for determination of haemoglobin.

Description

Used in Sahli's method for determination of haemoglobin. Sahli's instrument was based on a design by the British neurologist Sir William Richard Gowers (1845-1915). **Bibliography**

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Week: Eight teen

Glucose meter:

From Wikipedia, the free encyclopedia

Jump to: navigation, search

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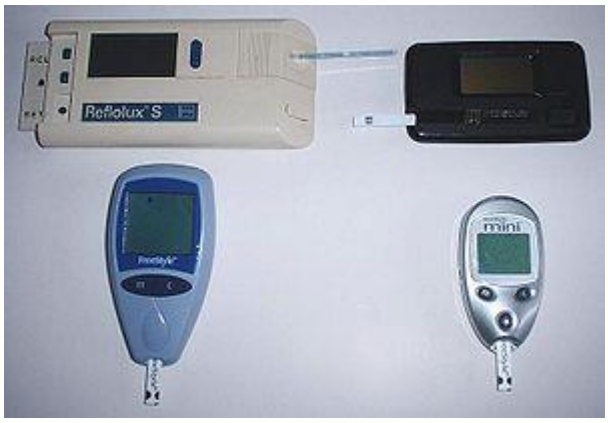

Four generations of blood glucose meter, c. 1993-2005. Sample sizes vary from 30 to 0.3 µl. Test times vary from 5 seconds to 2 minutes (modern meters typically provide results in 5 seconds).

A **glucose meter** (or **glucometer**) is a medical device for determining the approximate concentration of glucose in the blood. It is a key element of home blood glucose monitoring (HBGM) by people with diabetes mellitus or hypoglycemia. A small drop of blood, obtained by pricking the skin with a lancet, is placed on a disposable test strip that the meter reads and uses to calculate the blood glucose level. The meter then displays the level in mg/dl or mmol/l.

Since approximately 1980, a primary goal of the management of type 1 diabetes and type 2 diabetes mellitus has been achieving closer-to-normal levels of glucose in the blood for as much of the time as possible, guided by HBGM several times a day. The benefits include a reduction in the occurrence rate and severity of long-term complications from hyperglycemia as well as a reduction in the short-term, potentially life-threatening complications of hypoglycemia.

Contents:

Characteristics:

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There are several key characteristics of glucose meters which may differ from model to model:

- **Size**: The average size is now approximately the size of the palm of the hand, though some are smaller or larger. They are battery-powered.
- **Test strips**: A consumable element containing chemicals that react with glucose in the drop of blood is used for each measurement. For some models this element is a plastic test strip with a small spot impregnated with glucose oxidase and other components. Each strip is used once and then discarded. Instead of strips, some models use discs that may be used for several readings.
    - **Coding**: Since test strips may vary from batch to batch, some models require the user to manually enter in a code found on the vial of test strips or on a chip that comes with the test strip. By entering the coding or chip into the glucose meter, the meter will be calibrated to that batch of test strips. However, if this process is carried out incorrectly, the meter reading can be up to 4 mmol/L (72 mg/dL) inaccurate. The implications of an incorrectly coded meter can be serious for patients actively managing their diabetes. This may place patients at increased risk of hypoglycemia. Alternatively, some test strips contain the code information in the strip; others have a microchip in the vial of strips that can be inserted into the meter. These last two methods reduce the possibility of user error.
- **Volume of blood sample**: The size of the drop of blood needed by different models varies from 0.3 to 1 µl. (Older models required larger blood samples, usually defined as a "hanging drop" from the fingertip.) Smaller volume requirements reduce the frequency of unproductive pricks.

- **Alternative site testing**: Smaller drop volumes have enabled "alternate site testing" — pricking the forearms or other less sensitive areas instead of the fingertips. Although less uncomfortable, readings obtained from forearm blood lag behind fingertip blood in reflecting rapidly changing glucose levels in the rest of the body.

- **Testing times**: The times it takes to read a test strip may range from 3 to 60 seconds for different models.

- **Display**: The glucose value in mg/dl or mmol/l is displayed in a small window. The preferred measurement unit varies by country: mg/dl are preferred in the U.S., France, Japan, Israel, and India. mmol/l are used in Canada, Australia, China and the UK. Germany is the only country where medical professionals routinely operate in both units of measure. (To convert mmol/l to mg/dl, multiply by 18. To convert mg/dl to mmol/l, divide by 18.) Many machines can toggle between both types of measurements; there have been a couple of published instances in which someone with diabetes has been misled into the wrong action by assuming that a reading in mmol/l was really a very low reading in mg/dl, or the converse.

Week :nineteen

Colorimeter (chemistry):

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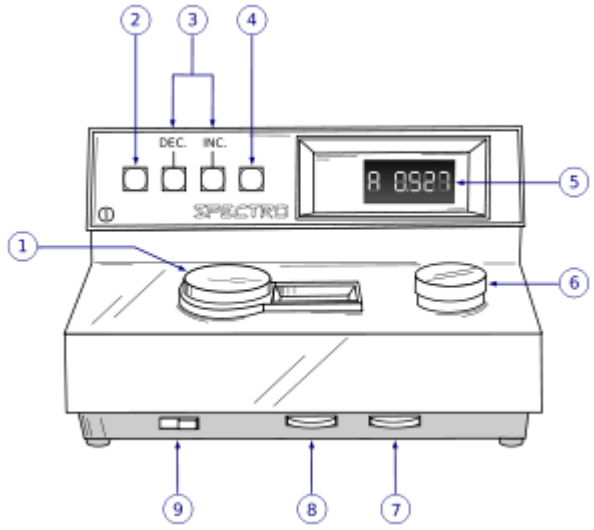
A **colorimeter** is a device used in colorimetry. In scientific fields the word generally refers to the device that measures the absorbance of particular wavelengths of light by a specific solution. This device is most commonly used to determine the concentration of a known solute in a given solution by the application of the Beer-Lambert law, which states that the concentration of a solute is proportional to the absorbance.

Contents:

Construction:



(1) Wavelength selection, (2) Printer button, (3) Concentration factor adjustment, (4) UV mode selector (Deuterium lamp), (5) Readout, (6) Sample compartment, (7) Zero control (100% T), (8) Sensitivity switch.

The essential parts of a colorimeter are:

- a **light source** (often an ordinary low-voltage filament lamp)
- an adjustable aperture
- a set of colored filters
- a cuvette to hold the working solution
- a detector (usually a photoresistor) to measure the transmitted light
- a meter to display the output from the detector

In addition, there may be:

- a voltage regulator, to protect the instrument from fluctuations in mains voltage.
- a second light path, cuvette and detector. This enables comparison between the working solution and a "blank", consisting of pure solvent, to improve accuracy.

Filters:

Changeable optics filters are used in the colorimeter to select the wavelength of light which the solute absorbs the most, in order to maximize accuracy. The usual wavelength range is from 400 to 700 nanometers (nm). If it is necessary to operate in the ultraviolet range (below 400 nm) then some

modifications to the colorimeter are needed. In modern colorimeters the filament lamp and filters may be replaced by several light-emitting diodes of different colors.

Cuvettes:

Main article: Cuvette

In a manual colorimeter the cuvettes are inserted and removed by hand. An automated colorimeter (as used in an AutoAnalyzer) is fitted with a **flowcell** through which solution flows continuously.

Week :Twenty , Twenty one& Twenty two

AutoAnalyzer:

AutoAnalyzer is an automated analyzer using a special flow technique named "continuous flow analysis (CFA)" first made by the *Technicon Corporation*. The instrument was invented 1957 by Leonard Skeggs, PhD and commercialized by Jack Whitehead's Technicon Corporation. The first applications were for clinical analysis, but methods for industrial analysis soon followed.

The AutoAnalyzer profoundly changed the character of the chemical testing laboratory by allowing significant increases in the numbers of samples that could be processed. The novel design based on separating a continuously flowing stream with air bubbles all but eliminated slow, clumsy, and error prone manual methods of analysis. This instrument single handedly changed the concept of days per sample to a mindset that hundreds, or even thousands, of tests are possible per day.

Contents:

Clinical analysis:

AutoAnalyzers were used mainly for routine repetitive medical laboratory analyses, but they had been replaced during the last years more and more by discrete working systems which allow lower reagent consumption. These instruments typically determine levels of albumin, alkaline phosphatase, aspartate transaminase (AST), blood urea nitrogen, bilirubin, calcium, cholesterol, creatinine, glucose, inorganic phosphorus, proteins, and uric acid in blood serum or other bodily samples. AutoAnalyzers automate repetitive sample analysis steps which would otherwise be done manually by a technician, for such medical tests as the ones mentioned previously. This way, an AutoAnalyzer can analyze hundreds of samples every day with one operating technician. Early AutoAnalyzer instruments each tested multiple samples sequentially for individual analytes. Later model AutoAnalyzers such as the SMAC tested for multiple analytes simultaneously in the samples.

In 1959 a competitive system of analysis was introduced by Hans Baruch of Research Specialties Company. That system became known as Discrete Sample Analysis and was represented by an instrument known as the "Robot Chemist." Over the years the

Discrete Sample Analysis method slowly replaced the Continuous Flow system in the clinical laboratory.

Industrial analysis:

The first industrial applications - mainly for water, soil extracts and fertilizer - used the same hardware and techniques as clinical methods, but from the mid 1970s special techniques and modules were developed so that by 1990 it was possible to perform solvent extraction, distillation, on-line filtration and UV digestion in the continuously flowing stream. In 2005 about two thirds of systems sold worldwide were for water analysis of all kinds, ranging from sub-ppb levels of nutrients in seawater to much higher levels in waste water; other common applications are for soil, plant, tobacco, food, fertilizer and wine analysis.

Operating Principle:

Continuous Flow Analyzer:

In Continuous Flow Analysis (CFA) a continuous stream of material is divided by air bubbles into discrete segments in which chemical reactions occur. The continuous stream of liquid samples and reagents are combined and transported in tubing and mixing coils. The tubing passes the samples from one apparatus to the other with each apparatus performing different functions, such as distillation, dialysis, extraction, ion exchange, heating, incubation, and subsequent recording of a signal. An essential principle of the system is the introduction of air bubbles. The air bubbles segment each sample into discrete packets and act as a barrier between packets to prevent cross contamination as they travel down the length of the tubing. The air bubbles also assist mixing by creating turbulent flow (bolus flow), and provide operators with a quick and easy check of the flow characteristics of the liquid. Samples and standards are treated in an exactly identical manner as they travel the length of the tubing, eliminating the necessity of a steady state signal, however, since the presence of bubbles create an almost square wave profile, bringing the system to steady state does not significantly decrease throughput (third generation CFA analyzers average 90 or more samples per hour) and is desirable in that steady state signals (chemical equilibrium) are more accurate and reproducible.[7]

A continuous flow analyzer (CFA) consists of different modules including a sampler, pump, mixing coils, optional sample treatments (dialysis, distillation, heating, etc), a detector, and data generator. Most continuous flow analyzers depend on color reactions using a flow through colorimeter, however, many methods have been developed that use ISE, flame photometry, ICAP, fluorometry, and so forth.

Week: Twenty Tree& Twenty Four

Flow Injection Analyzer:

Main article: Flow Injection Analyzer

Flow Injection Analysis (FIA), as introduced in 1975 by Ruzicka and Hansen,[8] has been described in over 18,000 scientific papers[9] and almost 20 monographs. The Japanese Society for Flow Injection Analysis (JAFIA) has been in existence for 20 years and publishes a scientific journal devoted to research in this rapidly growing field of automated chemical analysis, now closely related to microfluidics.

The first generation of FIA technology, termed flow injection (FI), was inspired by the AutoAnalyzer technique invented by Skeggs in early 1950s.While Skeggs' AutoAnalyzer uses air segmentation to separate a flowing stream into numerous discrete segments to establish a long train of individual samples moving through a flow channel, FIA systems separate each sample from subsequent sample with a carrier reagent. While the AutoAnalyzer mixes sample homogeneously with reagents, in all FIA techniques sample and reagents are merged to form a concentration gradient that yields analysis results. Removal of air segmentation opened the door to instrument miniaturization and inspired further progress towards analytical microfluidics, sometimes termed as "lab-on-chip".

FIA methods can be used for both fast reactions as well as slow reactions. For slow reactions, a heater is often utilized. The reaction does not need to reach completion since all samples and standards are given the same period to react. For typical assays commonly measured with FIA (e.g., nitrite, nitrate, ammonia, phosphate) it is not uncommon to have a throughput of 60-120 samples per hour.[*citation needed*]

FIA methods are limited by the amount of time necessary to obtain a measurable signal since travel time through the tubing tends to broaden peaks to the point where samples can merge with each other. As a general rule, FIA methods should not be used if an adequate signal cannot be obtained within two minutes, and preferably less than one. Reactions that need longer reaction times should be segmented. However,considering the number of FIA publications and wide variety of uses of FIA for serial assays, the "one minute" time limitation does not seem to be a serious limitation for most real life assays. Yet, assays based on slow chemical reactions have to be carried either in stopped flow mode (SIA) or by segmenting the flow.

OI Analytical, in its gas diffusion amperometric total cyanide method, uses a segmented flow injection analysis technique that allows reaction times of up to 10 minutes by flow injection analysis.

Technicon experimented with FIA long before it was championed by Ruzicka and Hansen. Andres Ferrari reported that analysis was possible without bubbles if flow rates were increased and tubing diameters decreased.[11] In fact, Skegg's first attempts at the auto analyzer did not segment. Technicon chose to not pursue FIA because, at the time, it increased reagent consumption and the cost of analysis.

The second generation of the FIA technique, called Sequential Injection Analysis (SIA), was conceived in 1990 and has been further developed and miniaturized over the course of the following decade. It uses flow programming instead of the continuous flow regime (as used by CFA and FIA), that allows the flow rate and flow direction to be tailored to the need of individual steps of analytical protocol. Reactants are mixed by flow reversals and a measurement is carried out while the reaction mixture is arrested within the detector by stepping the flow. Microminiaturized chromatography is carried out on microcolumns that are automatically renewed by microfluidic manipulations. The discrete pumping and metering of microliter sample and reagent volumes used in SI only generates waste per each sample injection. The enormous volume of FI and SI literature documents the versatility of FI and SI and their usefulness for routine assays (in soil, water, environmental, biochemical and biotechnological assays) has demonstrated their potential to be used as a versatile research tool.

Dialyzer module:

In medical testing applications and industrial samples with high concentrations or interfering material, there is often a dialyzer module in the instrument in which the analyte permeates through a dialysis membrane into a separate flow path going on to further analysis. The purpose of a dialyzer is to separate the analyte from interfering substances such as protein, whose large molecules do not go through the dialysis membrane but go to a separate waste stream. The reagents, sample and reagent volumes, flow rates, and other aspects of the instrument analysis depend on which analyte is being measured.

Recording of results:

Previously a chart recorder and more recently a data logger or personal computer records the detector output as a function of time so that each sample output appears as a peak whose height depends on the analyte level in the sample.

Current Uses:

AutoAnalyzers are still used for a few clinical applications such as neonatal screening or Anti-D, but the majority of instruments are now used for industrial and environmental work. Standardized methods published by the ASTM (ASTM International), the US Environmental Protection Agency (EPA) as well as the International Organization for Standardization (ISO) for environmental analytes such as nitrite, nitrate, ammonia, cyanide, and phenol. Autoanalyzers are also commonly used in soil testing laboratories, fertilizer analysis, process control, seawater analysis, air contaminants, and tobacco leaf analysis.

Autoanalyzers are used because they decrease costs, save time, conserve reagents and materials, minimize errors, and improve productivity. A laboratory should consider using an autoanalyzer if there is a significant backlog of samples, a lot of overtime just to get things done on time, or continuous repeating of mistakes due to human error. Not all laboratories should consider continuous flow. If the sample load is less than 20 samples per week, other options should be considered. Before adding an autoanalyzer, management needs to seriously consider that the operators need to understand the basic concepts of flow analysis. Instrument manufacturers, eager to make a sale, will tout simplicity, rapid start up and shut down, and flat learning curves. While these things may be possible when running standards, the laboratory runs real samples that have an effect on the reagents used. In the real world, methods may need to be modified and slight modifications can have significant impacts on the basic operation of the chemical system. Once an operator understands flow analysis the incredible capabilities of the instrument can be realized, allowing methods to be added, improved, enhanced, and developed.

Week :Twenty Five &Twenty Six

Photoelectric flame photometer:

A **photoelectric flame photometer** is a device used in inorganic chemical analysis to determine the concentration of certain metal ions, among them sodium, potassium, lithium, and calcium.

In principle, it is a controlled flame test with the intensity of the flame colour quantified by photoelectric circuitry. The sample is introduced to the flame at a constant rate. Filters select which colours the photometer detects and exclude the influence of other ions. Before use, the device requires calibration with a series of standard solutions of the ion to be tested.

Flame photometry is crude but cheap compared to flame emission spectroscopy, where the emitted light is analysed with a monochromator. Its status is similar to that of the colorimeter (which uses filters) compared to the spectrophotometer (which uses a monochromator). It also has the range of metals that could be analysed and the limit of detection are also considered

Week :Twenty seven &twenty Eight

1. Balance:

2.

3. What is Balance ?

4. **Balance** is our (still) suprisingly successful load balancing solution being a simple but powerful generic tcp proxy with round robin load balancing and failover mechanisms. Its behaviour can be controlled at runtime using a simple command line syntax.

5. **Balance** supports IPv6 on the listening side which makes it a very useful tool for IPv6 migration of IPv4 only services and servers.
6. **Balance** successfully runs at least on Linux(386), Linux(Itanium), FreeBSD, BSD/OS, Solaris, Cygwin, Mac-OS X, HP-UX and many more.
7. **Balance** is Open Source Software and released under GPL licensing terms.
8. **BalanceNG**® is our commercial Software Load Balancer for Linux and Solaris which is available under a "Free Basic Trial License". Click **here** for more information.

Week :Twenty nine & thirty

Erythrocyte sedimentation rate:

From Wikipedia, the free encyclopedia

Jump to: navigation, search The **erythrocyte sedimentation rate** (ESR), also called a **sedimentation rate** or **Biernacki Reaction**, is the rate at which red blood cells sediment in a period of 1 hour. It is a common hematology test that is a non-specific measure of inflammation. To perform the test, anticoagulated blood is placed in an upright tube, known as a Westergren tube, and the rate at which the red blood cells fall is measured and reported in mm/h.

Since the introduction of automated analyzers into the clinical laboratory, the ESR test has been automatically performed.

The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential). When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called 'rouleaux,' which settle faster. Rouleaux formation can also occur in association with some lymphoproliferative disorders in which one or more immunoglobulin are secreted in high amounts. Rouleaux formation can, however, be a normal physiological finding in horses, cats, and pigs.

The ESR is increased by any cause or focus of inflammation. The ESR is increased in pregnancy or rheumatoid arthritis, and decreased in polycythemia, sickle cell anemia, hereditary spherocytosis, and congestive heart failure. The basal ESR is slightly higher in female.

Contents:

History:

This test was invented in 1897 by the Polish doctor Edmund Biernacki.[2] In some parts of the world the test continues to be referred to as the Biernacki Test (Polish abbreviation: OB = *Odczyn Biernackiego*.) In 1918 the Swedish pathologist Robert Sanno Fåhræus declared the same and along with Alf Vilhelm Albertsson Westergren are eponymously remembered for the Fåhræus-Westergren test (abbreviated as FW test; in the UK, usually termed Westergren test),[3] which uses sodium citrate-coagulated specimens.[4]

Uses:

Although it is frequently ordered, ESR is of limited use as a screening test in asymptomatic patients. It is useful for diagnosing diseases, such as multiple myeloma, temporal arteritis, polymyalgia rheumatica, various auto-immune diseases, systemic lupus erythematosus, rheumatoid arthritis, and chronic kidney diseases. In many of these cases, the ESR may exceed 100 mm/hour.[5]

It is commonly used for a differential diagnosis for Kawasaki's disease and it may be increased in some chronic infective conditions like tuberculosis and infective endocarditis. It is a component of the PDCAI, an index for assessment of severity of inflammatory bowel disease in children.

The clinical usefulness of ESR is limited to monitoring the response to therapy in certain inflammatory diseases such as temporal arteritis, polymyalgia rheumatica and rheumatoid arthritis. It can also be used as a crude measure of response in Hodgkin's lymphoma. Additionally, ESR levels are used to define one of the several possible

adverse prognostic factors in the staging of Hodgkin's lymphoma. There is also a wintrobe method.

The use of the ESR as a screening test in asymptomatic persons is limited by its low sensitivity and specificity. When there is a moderate suspicion of disease, the ESR may have some value as a "sickness index.

An elevated ESR in the absence of other findings should not trigger an extensive laboratory or radiographic evaluation.

Normal values:

Westergren's original normal values (men 3mm and women 7mm)[6] made no allowance for a person's age and in 1967 it was confirmed that ESR values tend to rise with age and to be generally higher in women.[7] Values are increased in states of anemia,[8] and in black populations.[9]

Adults:

The widely used[10] rule for calculating normal maximum ESR values in adults (98% confidence limit) is given by a formula devised in 1983:[11]

$$\text{ESR } (mm/hr) \leq \frac{\text{Age } (in\ years) + 10\ (if\ female)}{2}$$

ESR reference ranges from a large 1996 study with weaker confidence limits:

Age	20	55	90
Men	12	14	19
Women	18	21	23