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Semester: 4<sup>th</sup>

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Program: BS (MLT)

Subject: Biomedical/

# Lab Instrumentation

# Date: 22 June 2020

#### **<u>Q1</u>**: Define the following.

- i. PH Meter
- ii. Vortex mixer
- iii. Balance iv. Water still
- v. deionizer

#### Answer:

## 1) PH Meter:

**Definition:** "It is a device used for the measurement of PH of a solution."  $\Box$  A simple and speedy device to measure the acidity and alkalinity of a fluid.

□ The PH meter was invented in 1934 by the by the American chemist Arnold O. Beckman to measure the sourness of lemons. 2) Vortex mixer:

**Definition:** A vortex mixer, or vortexer, is a simple device used commonly in laboratories to mix small vials of liquid.

3) Balance:

**Definition:** A weighing scale is a device for measuring weight.

□ Balance measure the mass of an object and are used in science. 4) Water still:

**Definition:** It is an instrument used in laboratory for Purification of water.

5) Deionizer:

**Definition:** It is an instrument used in laboratory for Purification of water.

#### **<u>Q2:</u>** Describe electrophoresis and its importants.

## Answer:

## **Electrophoresis:**

**Definition:** Electrophoresis involves the separation of components of a sample by differential rate of migration in the presence of electric field.

Theory was first proposed by Prof Ferdinand F reuss by doing experiment on migration of colloidal clay particles.

**Principle:** Molecules moves with the speed dependent on their charge, shape and size and get separated in the presence of an electric field.

## **Components:**

- Gel casting assembly.
- Buffer container or electrophoresis tank
- Power supply
- Glass plate to hold the gel
- Comb to load sample in the gel before solidification.

# **Operation:**

- Gel is prepared by adding powdered agarose to liquid boiling the mixture
- Comb is already placed which create rows of well for sample loading
- This agarose is then poured into casting tray and allow to solidify at room temp
- Gel is solidified and comb is removed and load standards and sample in wells
- Apply desired voltage to initiate electrophoresis
- Separated products can be seen by placing the gel on UV trans illuminator and calculated by comparing with standards <u>Quality Control:</u>
- Equipment calibration.
- Good quality and properly working of standards
- The standards should be run to check the validity
- Chemical must be purchased from suppliers who guarantee the purity
- Do not use expire reagent
- Buffers for QC of reagent must be tested according to SOPs <u>Applications:</u>
- Separation of proteins, DNA ,RNA and other macromolecules 

  Purifications and analysis of vaccine and antibiotics

## **<u>Q3:</u>** Write a note on flow cytometery?

## Answer:

## Flow cytometery:

**Definition:** Flow cytometery is a technology that is used to analyze physical and chemical characteristics of particles in a fluid as it passes through at least one laser.

- Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths.
- Up to thousands of particles per second can be analyzed as they pass through liquid stream.
- Examples of properties measured include particle's relative granularity, size and fluorescence intensity as well as its internal complexity.
- Optical-to-electronic coupling system is used to record the way in which particle emits fluorescence and scatters incident light from laser <u>Main Contents of Flow Cytometer:</u>

**Fluidics:** Purpose of the fluidics system is to transport the particles in a stream of fluid to laser beam where they are interrogated.

- If cells are from solid tissue, they require disaggregation before they can be analyzed.
- Although cells from animals, plants, bacteria, yeast or algae are usually measured, other particles such as chromosomes or nuclei can also be examined.
- Some particles such as marine algae are naturally fluorescent, but in general, fluorescent labels are required to tag components of the particle.
- Section of fluid stream that contains particles is referred as sample core.

#### **Optics System:**

- Lasers which illuminate particles present in stream as they pass through and scatter light from laser.
- Fluorescent molecules that are on particle emit fluorescence, which is detected by carefully positioned lenses.
- Light scattered from up to six or more fluorescence is determined for two different angles.
- Optical filters and beam splitters then direct light signals to relevant detectors, which emit electronic signals proportional to signals that hit them.
- Data collected on each particle or event and characteristics of those events or particles are determined based on their fluorescent and light scattering properties

**Electronics System:** Used to change light signals detected into electronic pulses that a computer can process.

- Data can then be studied to ascertain information about a large number of cells over a short period
- Information on the heterogeneity and different subsets within cell populations can be identified and measured.

#### **Parameters:**

• Cell pigments such as chlorophyll.

- Total DNA content (cell cycle analysis, cell kinetics, proliferation, ploidy, aneuploidy, end reduplication, etc.)
- total RNA content
- DNA copy number variation (by Flow-FISH technology)
- Protein expression and localization

## **<u>Q4:</u>** What do you know about beer lambert law (Uses, Principles)? <u>Answer:</u>

#### Beers Lambert's law.

The absorbance of light is directly proportional to the thickness of the media through which the light is being transmitted multiplied by the concentration of absorbing chromosphere; that is,  $A = \epsilon bc$  where A is the absorbance,  $\epsilon$  is the molar extinction coefficient, b is the thickness of the solution, and c is the concentration.

#### Uses:

The relation may be used to determine the concentration of a chemical species in a solution using a colorimeter or spectrophotometer. The relation is most often used in UV-visible absorption spectroscopy.

#### Working principle

The colorimeter is based on Beer-Lambert's law, according to which the absorption of light transmitted through the medium is directly proportional to the medium concentration.

n a colorimeter, a beam of light with a specific wavelength is passed through a solution via a series of lenses, which navigate the colored light to the measuring device. This analyzes the color compared to an existing standard. A microprocessor then calculates the absorbance or percent transmittance. If the concentration of the solution is greater, more light will be absorbed, which can be identified by measuring the difference between the amount of light at its origin and that after passing the solution.

## **<u>Q5:</u>** Explain autoclave, its uses and components?

## Answer:

# Autoclave:

An autoclave is a machine that is used to eradicate bio hazardous waste from the surface of tools or instruments. It was invented by Charles Chamber land in 1884. Autoclaves sterilize or disinfect through physical means by using pressure, temperature and steam. They are often referred to as steam sterilization machines.

Components of autoclave

Autoclave comprises of three parts: a pressure chamber, a lid and an electrical heater. Pressure chamber consists of -

Large cylinder (vertical or horizontal) in which the materials to be sterilized are placed. It is made up of gunmetal or stainless steel and placed in a supporting iron case ay through

A steam jacket (water compartment)

The lid is fastened by screw clamps and rendered airtight by an asbestos washer. The lid bears the following-

A discharge tap for air and steam discharge

A pressure gauge (sets the pressure at a particular level)

A safety valve (to remove the excess steam)

An electrical heater is attached to the jacket; that heats the water to produce steam.

# **Types of Autoclaves**

There are different types of autoclaves

Gravity displacement type autoclave: It is the most common type used in laboratories and is available in various sizes and dimensions. Vertical type (small volume capacity)

Horizontal autoclave (large volume capacity)

Positive pressure displacement type autoclave Negative

pressure (vacuum) displacement type.

# Uses of autoclave

An autoclave is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials. It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes. Autoclaves vary in size, shape and functionality. A very basic autoclave is similar to a pressure cooker; both use the power of steam to kill bacteria, spores and germs resistant to boiling water and powerful detergents.

# The end