

Tuesday, November 29<sup>th</sup>  
[2022]

## INSTRUMENTATION IN MLS

### HAEMATOLOGY (MLS 331)

#### Synopsis

- Electrophoresis
- Staining Machine
- Coulter Counter Machine

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#### ⇒ ELECTROPHORESIS

This equipment or this process is used for running one's Genotype.

A = AO - Recessive

AA - Homozygous

B = BO -

BB -

Haemoglobin Genotype

Sickling Electrophoresis

This test is done with

- Sodium metabisulphide

Haemoglobin Electrophoresis  $\Rightarrow$  This is used to separate and identify the different haemoglobin by their migration with an electric field.

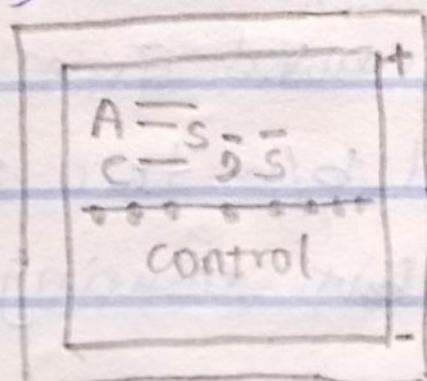
Haemoglobin variants separate at different rate due to differences in their surface electrical charge as determined by their amino acid structure.

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### Alkaline Cellulose - Acetate Electrophoresis

Several techniques are available to separate haemoglobin variant by electrophoresis. For routine work, electrophoresis in an alkaline buffer at pH of 8.4 - 8.6, using a cellulose acetate membrane. This gives a good separation of Hb A (Adult Hb), Hb F (Fetal), C, HbS, Hb H, HbD, etc.

On alkaline electrophoresis, these haemoglobin variants migrate at various electric field and speed (On alkaline electrophoresis HbD & S migrate at the same mobility WHILE HbC, E & O migrates at the same speed)



\* Genotype test is run from negative to positive

\* Use a control, which is A & S

In Specialist's laboratories, Agarose gel electrophoresis with an acid pH of 6 can be used to separate these haemoglobins.

Reagents for Alkaline Cellulose Acetate Electrophoresis

(1) Tris Buffer: (10.2g)

Tris Hydroxyl (Methyl Amino) Methane

(2) EDTA (Ethylene Diamine Tetra-acetic acid) (0.2g)

(3) Boric acid (3.2g)

(4) Distilled water

These reagents can be stored in a refrigerator at 4-6°C.

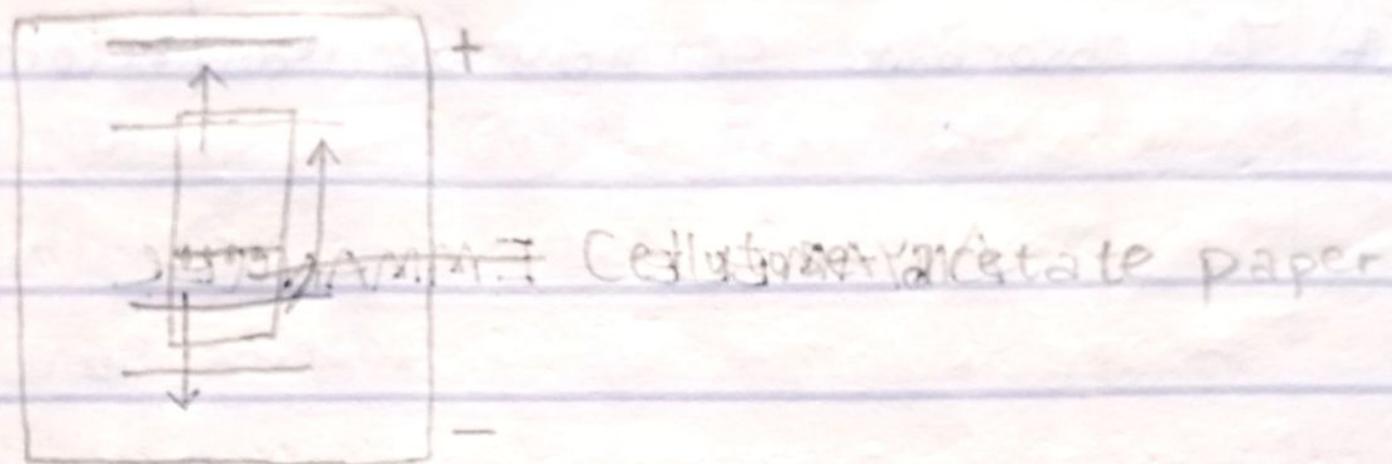
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#### METHOD OF RUNNING AN ELECTROPHORETIC TEST

- (1) The cellulose acetate strips are marked with pencil at the point of application of the sample approximately 4cm at one end which will be the cathode end.
- (2) They are often well labeled for later identification and then placed lightly on the surface of the buffer allowing the buffer to impregnate.
- (3) The impregnated strip is removed from the buffer using forceps and lightly blotted b/w two sheets of paper to remove excess moisture but ensuring that no excess opaque.

If this happens, the strip should be returned to the buffer and start afresh.

- (4) The strips are then positioned in the tank with the point of application with the cathode side and wick of filter paper is placed over either ends of the strip dipping into the buffer of the tank.



- (5) Apply the different samples, on your strip and at the margin, leaving a slight margin between each sample, also apply the control.
- (6) The lead is replaced on top of the tank and the power supply connected. Usually, the voltage is about 185-350 volt.
- (7) Leave for 25 minutes or shorter.
- (8) The strips are removed from the tank and dried.
- (9) Stain with Ponceau solution for 10 minutes and remove & dry. ANYAMELE EMMANUEL
- (10) After staining is complete, the strips are placed in a small tank containing 5% acetic acid.
- (11) Replace the acid several times to ensure the dye has

been removed from the background completely.

- (ii) Dry and cellotape each strip to the laboratory or patients form.
- (iii) Fractions can be eluted from the strip by the cutting out the individual fraction and placing a cut in a test tube containing 10% Tropole.
- (iv) The absorbance can now be read/recorded at 220 nm

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## STAINING MACHINE

When staining large numbers of blood film or slide, it is easier to use a staining machine, the advantage is that they will not only stain quickly or quicker but it will also stain more slide, the staining is uniform and it will take less time.

### ADVANTAGES OF STAINING MACHINE

- ① It stains quickly / more quicker.
- ② It stains more slides
- ③ The staining is uniform
- ④ It takes less time
- ⑤ Machines has been designed to stain both slide and cover thin film

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### PRINCIPLE

The principle of staining machine is the same as staining manually (Romanovsky's stain). The machine incorporates a central shaft which has a plastic disk filled at the top end. This disc is filled with hooks at regular intervals. The slides to be stained are placed in stainless steel containers which are hung on the hooks. The beakers containing the reagents are placed at stations underneath

the plastic disk in such a way that when the machine is at rest, the hook is directly opposite the reagent beakers.

A timing mechanism similar to that on the tissue processing machine allows the central shaft to be raised at intervals.

$$\text{Total staining} = 7-12 \text{ mins}$$

This lifts the slide, clears of the beaker and transfers them to the next one. For the purpose of staining blood films, the last two beaker are filled with buffered water. The surface of the buffer tends to become covered with a stain precipitate which deposits on the film when it is lifted clear of the fluid. For this reason, air is bubbled through this two beaker in order to keep the fluid in motion. At the end, the slide holder is lowered into a ramp so that it leans over slightly and it release from the hook. The ramp then revolves and removes the holder so that the next one is not lowered on top of it. Staining machines may have 8, 12 or 23 stations;

- ① The first two beakers contain absolute methyl alcohol.
- ② The next three beakers contains the Romanovsky stain diluted with buffered water of pH 6.8
- ③ The next three beakers also contain stain and it is diluted with buffered water.

(A) The last two beakers are filled with buffered distilled water at pH 6.8 and the bubbling device fitted. The timing device is so programmed that the slides spend 2 minutes in each beaker, the film is therefore fixed for 4 minutes, stained for 6 minutes, the second is stained also for 6 minutes ( $6+6+4 = 16$  mins). Wash in two changes of buffer for 2 mins (i.e. Each beaker is for 1 mins).

(NB) → The stain should be replaced daily and the buffered water changed at least twice a day.

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→ COULTER COUNTER MACHINE

This is an equipment used in the laboratory to count cells and their sizes. A Coulter Counter is a machine, apparatus or equipment used for counting & sizing of a cell suspended in solution (electrolyte).

→ PRINCIPLE

The Coulter counter principle is based on the fact that particles moving in an electric field cause measurable disturbances in that field. The magnitude of these disturbances are proportional to the size of the particles.

Coulter identifies several requirements necessary for practical application, of this phenomenon;

① The particle should be suspended in a conducting liquid like

## Normal Saline

- (2) The electrical field should be physically restricted so that the movement of particles in the field can cause change in the current.
- (3) The particles should be diluted enough so that only one at a time passes the orifice preventing an artifact known as coincidence.

There are two types of Coulter Counter

(1) Aperture Format

(2) Flow-cell Format

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(1) APERTURE FORMAT - This is used by most commercial Coulter counters. In the set-up, an orifice of defined size is created in a jewel disc using special manufacturing process. The resulting aperture is then embedded in the wall of a glass tube. While in use, the aperture tube is placed in a liquid so that the jewel disc is completely submerged and the tube can filled with liquid.

Electrodes are positioned both inside and outside the aperture tube which allows current to flow through the aperture. A pump is used to create a vacuum at the top of the tube which draws the liquid to the aperture.

Samples to be analyzed are then slowly added to the

conducting liquid surrounding the aperture tube. At the start of the experiment, the electric field is turned on and the pump begins to draw the dilute suspension through the aperture. The resulting data are collected by recording the electrical pulses generated as the particles transverse the aperture. While the basic physical setup formats consistent in every Coulter Counter System as a function of the signal processing circuit implemented.

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(2) The Flow-cell Format- This is the most commonly used in haematology and sometimes in flowometers. In this format, electrodes are embedded at either ends of a flow channels  $\frac{1}{3}$  the electric field is applied to the channel. This format has several advantages when compared with the aperture format.

#### ADVANTAGES

- (1) It allows for continuous sample analysis where as the aperture format is single batch format.
- (2) Cells length itself to addition of a sheet flow which keep particles centered in the middle of the channel. This allows additional measurement to be performed simultaneously such as probing the object with a laser.

## DISADVANTAGES

- (1) It is expensive
- (2) It is fixed to one channel width whereas the aperture format offer a variety of aperture sizes.

The counter can range from a single machine which will count the whole blood, RBC & WBC to complex by automatic dilution system which accounts whole blood and in 20secs produces a seven parameter printed results of WBC, RBC, MCV, Hb, MCH & MCHC.

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## PIPETTES

In the medical laboratory, we measure liquid/fluid by putting them in the special bottles which will hold the liquids e.g. PIPETTE:

Pipettes are used to measure volumes up to 50 ml. Pipetting must not be done by mouth. There are various devices obtainable to fill empty conventional pipetting.

The pipette fillers includes:-

- (1) Rubber Bulbs
- (2) Glass sleeves - Over the ends of specially designed pipette that act in a similar way to a Syringe and

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various value operated System. Only the most commonly used pipettes are discussed below;

(1) ONE-MARK PIPETTE - Also referred to a Volumetric or transfer pipette. These pipettes are covered by BS (British Standard) 1583. The capacity is defined as the volume of water at  $20^{\circ}$  delivered by the pipette when used in prescribed manner which is first to rinse it with the fluid to be used, then fill to the mark of graduation.

The Meniscus is carefully adjusted to the mark (at eye level) and the outside of the pipette is wiped with clean tissue holding the pipette vertically, the contents are allowed to run out, then the tip of the pipette is touched at the sides of the receiver. No attempt should be made to increase the speed of delivery or to expel small amount of fluid remaining after draining. MAYWA

A class "A" pipette used for most accurate work are marked with both delivery time and the rate. Since the tip plays a major part in the accurate delivery of Calibrated volume, any pipette with chipped or broken end should be discarded.

(2) CAPILLARY PIPETTE - British Standard (BS) 1428 recognized this pipettes. They are used for small volume. Type I is graduated for delivery and therefore used the same way as

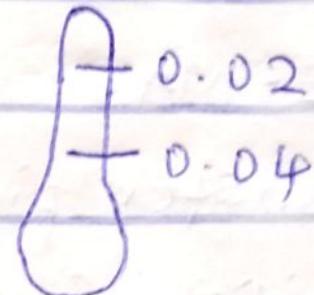
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marked pipette. However, Type II, III & IV are graduated for contents.

Type II is fully graduated

Type III has only one mark

Type IV has only two marks



These types requires washing out. The content are blown out in to the diluent and the diluent is drawn up into the graduated mark to wash out the remainder.

This washing procedure should be repeated at least three times. Another type of graduated Capillary pipette is the Blow-out Pipette. As the name implies, it does not require rinsing. This type has a number of useful application particularly in serology.

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#### (3) GRADUATED PIPETTE

There are three types of graduated pipette recognized by BS 300. Type I belongs to pipette Calibrated for delivery from 0- down to any graduation.

Type II pipettes are graduated for delivery from any graduation line to any graduation.

Type III is graduated to contain a given Capacity from the Jet up to the Calibration line Corresponding capacity to the

Capacity:

It is used in the same way as the capillary pipette.

Type II graduated pipette is one-Mark pipette.

For greatest accuracy, the graduation should be made up to the Jet. Type I is filled the same way, the content are allowed to run down.

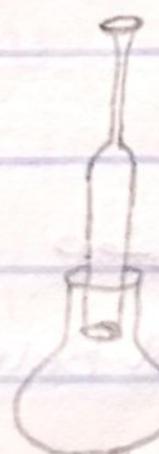
The flow is then stopped and the tip is slightly touched at the edge to remove any drop that may have been collected on delivery.

For the greatest accuracy, each delivery should start at zero(0)

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#### (A) PASTEUR PIPETTE

These are uncalibrated pipettes that can easily be made in the laboratory or obtained commercially.



They can also deliver a set number of drops per milliliter. Plastic Pasteur pipette manufactured with an integral part feature can be obtained and these are ideal for transfer of biological fluids.

To prevent the formation of frosts & to ensure complete delivery of the liquid been transferred by the pasteur pipette. The plunger should be pressed slowly & release in the same way.

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(5) DISPOSABLE TIP PIPETTE - A number of pipetting systems has been designed using disposable plastic tips. They are controlled by plunger, & the liquid been pipetted is separated from the plunger by an air gap. They are quicker to use than the conventional pipette. Fixed or variable patterns can be obtainable. There is some variation in the mode of action of disposable pipette.

- (A) FORWARD MODE  $\Rightarrow$  The method is disposable for use with aqueous solution.
- (i) Put a clean tip in the pipette.
  - (ii) Depress the plunger to first step
  - (iii) Immerse deep to 2-3mm (at below surface of solution) and allow the plunger to return.
  - (iv) Remove tip from solution.
  - (v) Place end of the tip against side of the receiving vessels and depress plunger readily.
  - (vi) Remove pipette from vessel & allow plunger to return to a

Starting position under control.

(vii) Discard tip.

(B) REVERSE MODE  $\Rightarrow$  This method should be used for Viscous sample e.g. Plasma.

- (i) Put a clean tip into the pipette.
- (ii) Depress plunger through both steps
- (iii) Immerse tip 2-3 mm and allow plunger to return under control to its starting position.
- (iv) Remove tip from solution
- (v) Place end of tip against the receiving vessel and Depress plunger at the initial position to disperse the solution.
- (vi) Remove tip and allow plunger to return to starting point.
- (vii) Discard tip.

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Mention the 5 types of Pipette & Discuss.

- (1) One-mark / Volumetric / Transfer Pipette
- (2) Capillary Pipette
- (3) Graduated Pipette
- (4) Pasteur Pipette
- (5) Disposable Pipette.