

KARNATAKA STATE

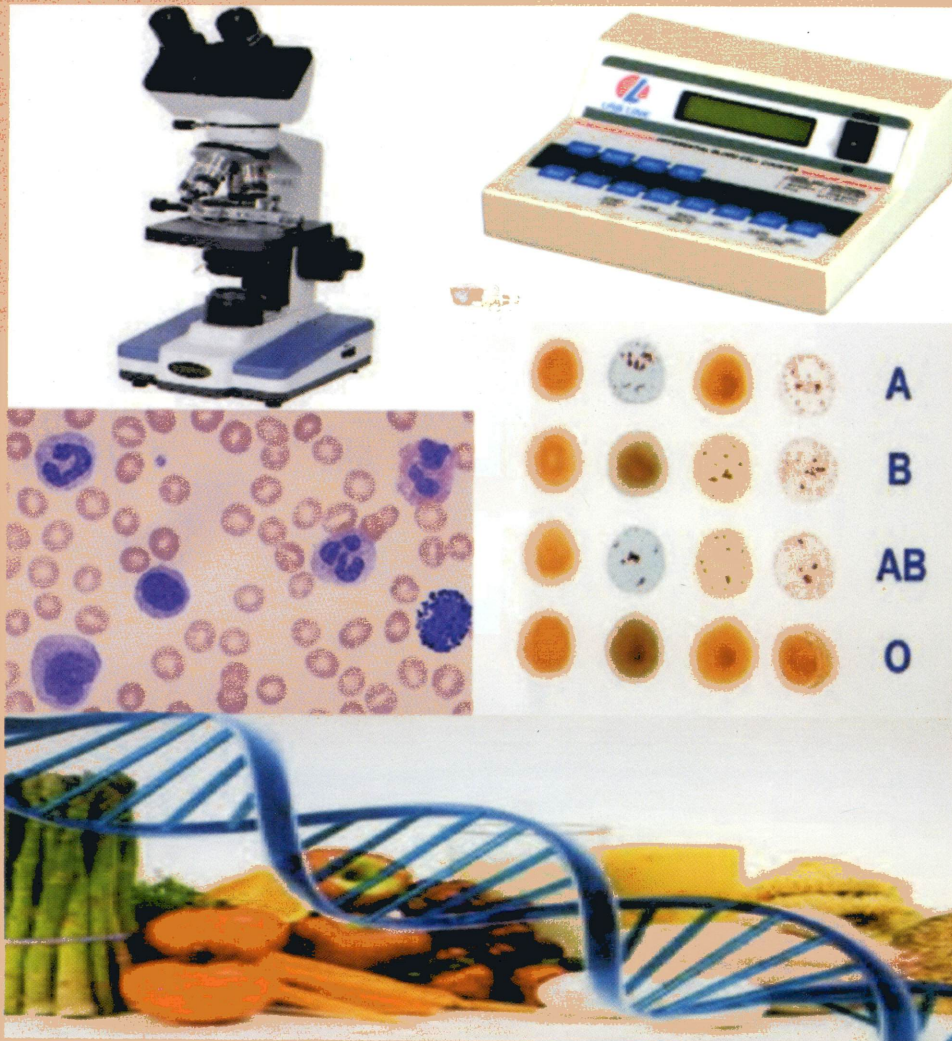


OPEN UNIVERSITY

MUKTHAGANGOTRI, MYSORE - 570 006

M.Sc in Clinical Nutrition and Dietetics

I Semester



PRACTICAL: PHYSIOLOGICAL CHEMISTRY

ಕರಾಮವಿ

ರಾಷ್ಟ್ರೀಯ
ಅಂತಾರಾಷ್ಟ್ರೀಯ
ಮಾನ್ಯತೆ



- * ಕರ್ನಾಟಕ ರಾಜ್ಯ ಮುಕ್ತ ವಿಶ್ವವಿದ್ಯಾನಿಲಯವು ಜೂನ್ ೧, ೧೯೯೬ ರಂದು ಸರ್ಕಾರಿ ಆದೇಶ ಸಂಖ್ಯೆ : ED1/UOV/dated 12th February 1996 (Karnataka State Open University Act-1992)ರ ಪ್ರಕಾರ ಕರ್ನಾಟಕ ರಾಜ್ಯಪಾಲರ ಅನುಮೋದನೆಯೊಂದಿಗೆ ಪೂರ್ಣಪ್ರಮಾಣದ ವಿಶ್ವವಿದ್ಯಾನಿಲಯವಾಗಿ ಸ್ಥಾಪನೆಗೊಂಡಿತು. ರಾಜ್ಯದ ಶೈಕ್ಷಣಿಕ ಪದ್ಧತಿಯಲ್ಲಿ 'ದೂರ ಶಿಕ್ಷಣ ಪದ್ಧತಿ'ಯನ್ನು ಆರಂಭಿಸುವ ಮತ್ತು ಉತ್ತೇಜಿಸುವ ದೃಷ್ಟಿಯಿಂದ ಈ ಮುಕ್ತ ವಿಶ್ವವಿದ್ಯಾನಿಲಯವನ್ನು ಅಧಿನಿಯಮದ ಮೂಲಕ ಸ್ಥಾಪಿಸಲಾಯಿತು.
- * ಕರ್ನಾಟಕ ರಾಜ್ಯ ಮುಕ್ತ ವಿಶ್ವವಿದ್ಯಾನಿಲಯದ ಅಧಿನಿಯಮ ೧೯೯೨ ರಂತೆ ಈ ವಿಶ್ವವಿದ್ಯಾನಿಲಯವು ಕರ್ನಾಟಕ ರಾಜ್ಯದ ಒಳಗೆ ಸಂಸ್ಥೆಗಳನ್ನು, ಕಾಲೇಜುಗಳನ್ನು, ಪ್ರಾದೇಶಿಕ ಕೇಂದ್ರಗಳನ್ನು ಮತ್ತು ಅಧ್ಯಯನ ಕೇಂದ್ರಗಳನ್ನು ಸ್ಥಾಪಿಸುವ, ನಿರ್ವಹಿಸುವ ಮತ್ತು ಮಾನ್ಯತೆ ಕೊಡುವ ಅಧಿಕಾರವನ್ನು ಹೊಂದಿದೆ. ಅಗತ್ಯವಿದ್ದ ಸಂದರ್ಭಗಳಲ್ಲಿ ಕರ್ನಾಟಕ ರಾಜ್ಯದ ಹೊರಗಿನ ಸ್ಥಳಗಳಲ್ಲೂ ಪ್ರಾದೇಶಿಕ ಕೇಂದ್ರ ಮತ್ತು ಅಧ್ಯಯನ ಕೇಂದ್ರಗಳನ್ನು ತೆರೆಯಲು ಅಧಿಕಾರವನ್ನು ಪಡೆದಿದೆ.
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ಉನ್ನತ ಶಿಕ್ಷಣ ಎಲ್ಲರಿಗೂ ಎಲ್ಲೆಡೆ

**Karnataka State Open University**

Mukthagangothri, Mysore - 570 006

Department of Studies in Food Science and Nutrition

M.Sc. Clinical Nutrition and Dietetics

FIRST SEMESTER

LAB MANUAL: PHYSIOLOGICAL BIOCHEMISTRY

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Publisher

Registrar
Karnataka State Open University
Mukthagangothri, Mysore - 570 006

Developed by Academic Section, KSOU, Mysore

Karnataka State Open University, 2013

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Printed and Published on behalf of Karnataka State Open University, Mysore-6.

INTRODUCTION

Nutrition science is multi disciplining and requires knowledge about related field in order to fully understand its physiological, biochemical and medical mechanisms. Primarily, the nutritional influence on physiological aspects of blood cells is very important. Most nutrients influence blood parameters, morphological changes of RBC is affected by many micro nutrients. Bleeding time and clotting time is under the influence of nutrients. Other factors such as infection and allergy alter WBC count; this has an indirect association to nutrition. In view of this few basic experiments have been included in the present practical course. The experiments are useful for you in assessing nutritional status of healthy individuals as well as sick.

Dr. Anitha C

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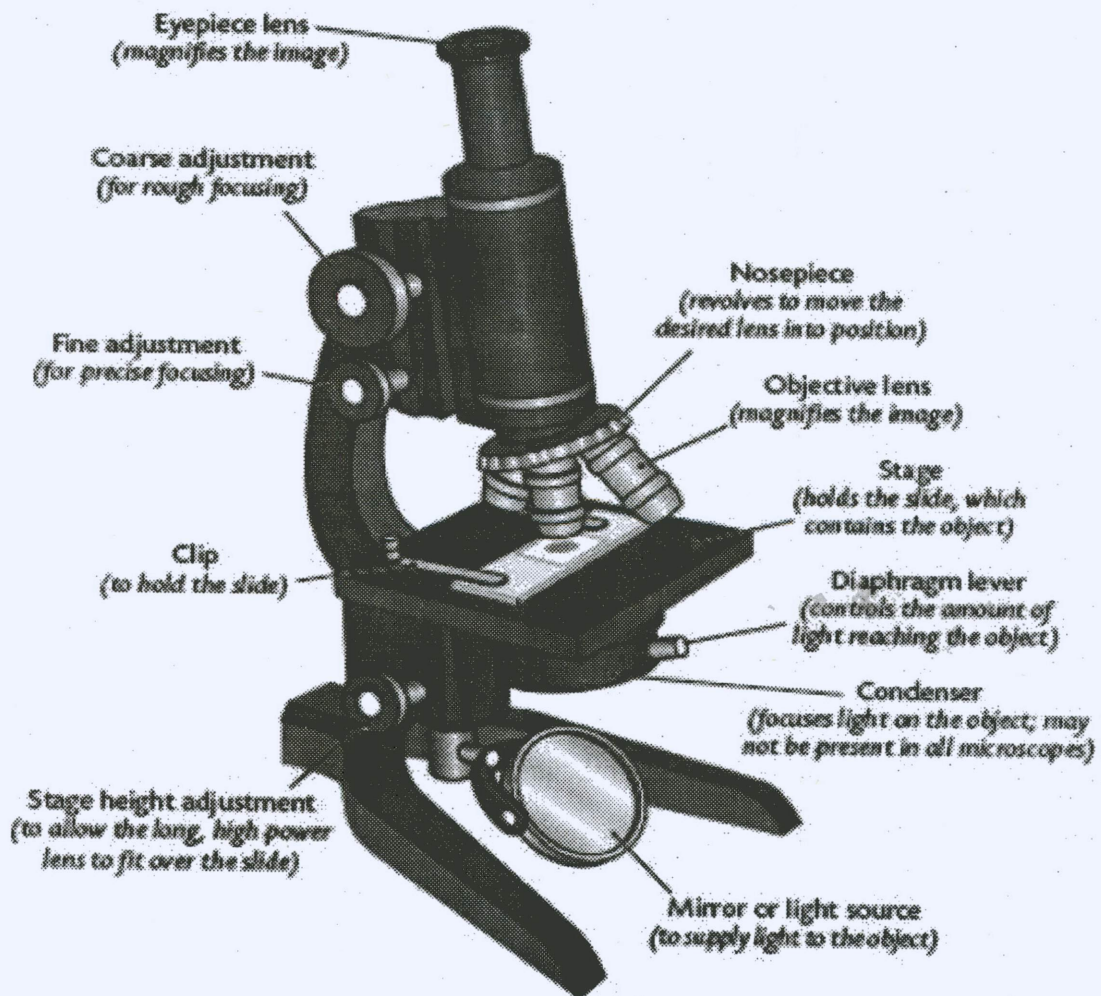
Mukthagangothri, Mysore

EXPERIMENT – I

DEMONSTRATION OF USE OF MICROSCOPE AND OBSERVATION OF VARIOUS TISSUES

a. DEMONSTRATION OF USE OF COMPOUND MICROSCOPE

The first microscopes were composed of a single lens just like a magnifying glass. They were called **simple microscopes**. Later microscopes were designed using 2 lenses. They are called **compound microscopes**. The top lens through which you look is called the **eyepiece** while the lower lens that is close to the slide is called the **objective lens** (See in the Figure). The magnification power of a compound microscope is calculated by multiplying the magnification of the 2 lenses. Since these microscopes use light to see the objects they are called **light microscopes**.



Eyepiece: The lens the viewer looks through to see the specimen. The eyepiece usually contains a 10X or 15X power lens.

Diopter Adjustment: Useful as a means to change focus on one eyepiece so as to correct for any difference in vision between your two eyes.

Body tube (Head): The body tube connects the eyepiece to the objective lenses.

Arm: The arm connects the body tube to the base of the microscope.

Coarse adjustment: Brings the specimen into general focus.

Fine adjustment: Fine tunes bring object to fine focus and increase the details of the specimen.

Nosepiece: A rotating turret that houses the objective lenses. The viewer spins the nosepiece to select different objective lenses.

Objective lenses: One of the most important parts of a compound microscope, as they are the lens closest to the specimen.

A standard microscope has three, four, or five objective lenses that range in power from 4X to 100X. When focusing the microscope, be careful that the objective lens doesn't touch the slide, as it could break the slide and destroy the specimen.

Specimen or slide: The specimen is the object being examined. Most specimens are mounted on slides, flat rectangles of thin glass.

The specimen is placed on the slide and a cover slip is placed over the specimen. This allows the slide to be easily inserted or removed from the microscope without disturbing the specimen. It also allows the specimen to be labeled, transported, and stored without damage.

Stage: The flat platform where the slide is placed.

Stage clips: Metal clips that hold the slide in place.

Stage height adjustment (Stage Control): These knobs move the stage left and right or up and down, so that specimens can be examined fully.

Aperture: The hole in the middle of the stage that allows light from the illuminator to reach the specimen.

On/off switch: This switch on the base of the microscope turns the illuminator off and on.

Illumination: The light source for a microscope. Older microscopes used mirrors to reflect light from an external source up through the bottom of the stage; however, most microscopes now use a low-voltage bulb.

Iris diaphragm: Adjusts the amount of light that reaches the specimen.

Condenser: Gathers and focuses light from the illuminator onto the specimen being viewed.

Base: The base supports the microscope and it's where illuminator is located.

Each part of the microscope works together - The light from the illuminator passes through the aperture, through the slide, and through the objective lens, where the image of the specimen is magnified and is view through eyepiece, which further magnifies the image the viewer then sees.

Use and Adjustments in Compound Microscope:

1. Turn on the **illuminator**. *When using the dimmer, it is best slowly increase the light intensity as the lamp heats up quickly.*
2. Place a slide with specimen on the **stage** with the sample directly above the aperture and, if possible, fasten it to the stage with the stage clips. *Reminder: A cover slip is always needed to allow for the best quality image.*
3. Ensure the **iris diaphragm** is completely open, allowing the maximum amount of light to reach the slide and the lenses. *Caution: Do not use the iris diaphragm to control the light, it is to control resolution and contrast - use the dimmer instead.*
4. Rotate the **nosepiece** so that the **objective lens** with the lowest level of magnification is directly above the sample. *Reminder: Using lower magnifications first helps to select the part of the specimen of interest and then adjust further.*
5. Look through the **binocular-eyepiece** and adjust the iris diaphragm until the amount of light is satisfactory. More light is better than less light, but the comfort of the viewer's eyes should also be taken into account.
6. Turn the **coarse adjustment knob** until the specimen comes into broad focus. *Caution: you should not use the coarse focus with a high magnification objective for fear of the objective making contact with the slide.*
7. Turn the **fine adjustment knob** until the specimen comes into sharp focus. *Caution: should not take a long time to find focus, otherwise the high magnification objective could also hit the slide. If it is difficult to focus then restart with the lower magnification objective.*
8. The viewer should then be able to rotate the nosepiece to higher settings and bring the sample into more and more detail with a minimal amount of refocusing.

Some compound microscopes come with what is called a **rack stop**. A rack stop prevents the objective lenses from being lowered into the slide. However, some older microscopes do not have a rack stop, so it is always advisable to check and exercise care. Lowering an objective lens into a slide could easily break the slide and damage the sample. In order to move the microscope safely, one hand should be under its base for support and the other at its arm. Be sure to only switch off the microscope when the dimmer is set to the lowest intensity and always turn off the lamp before moving the microscope.

EXPERIMENT – II

PREPARATION OF BLOOD SMEAR AND OBSERVATION OF BLOOD CELLS

A **blood film** or **peripheral blood smear** is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow the various blood cells to be examined microscopically. Blood films are usually examined to investigate hematological problems (disorders of the blood) and, occasionally, to look for parasites within the blood such as malaria and filaria.

In order to take a blood sample, you have to use latex gloves and special lancets which allow you to safely pierce the skin and take the sample. Following use, the lancets and glass slides must be disposed of in to appropriately labeled Bin. All materials such as tissues, wipes, stains etc that have been in contact with blood must be disposed of safely according to the protocols of the competent organization.

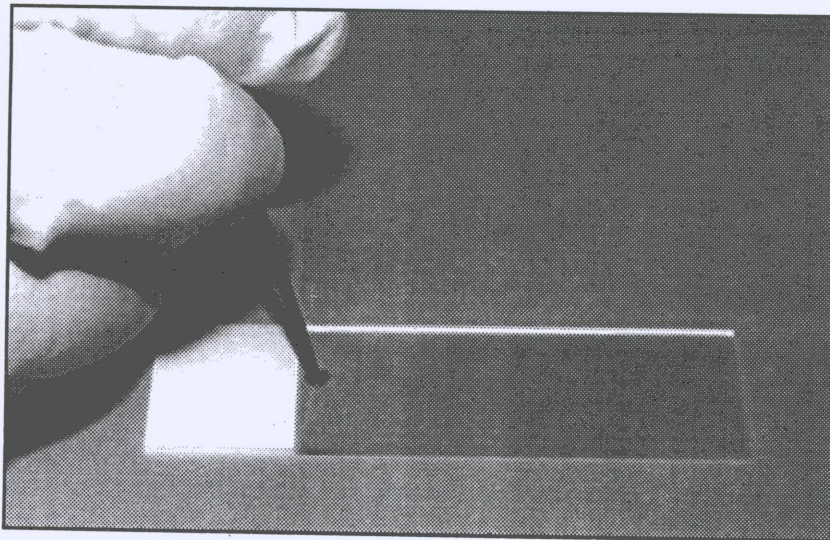
MATERIALS

- sterilized lancets or needles
- clean microscope slides and cover slips
- 95% ethyl alcohol
- distilled water

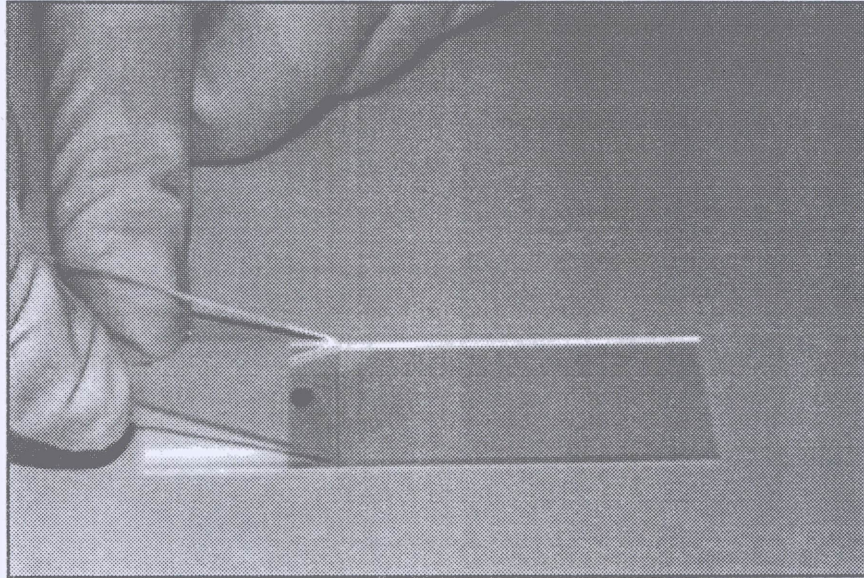
TAKING THE BLOOD

Cleanse a finger with a sterile lancet, make a puncture on a fingertip. In the meantime, keep all the materials needed ready and protected from dust, particularly the clean microscope slides.

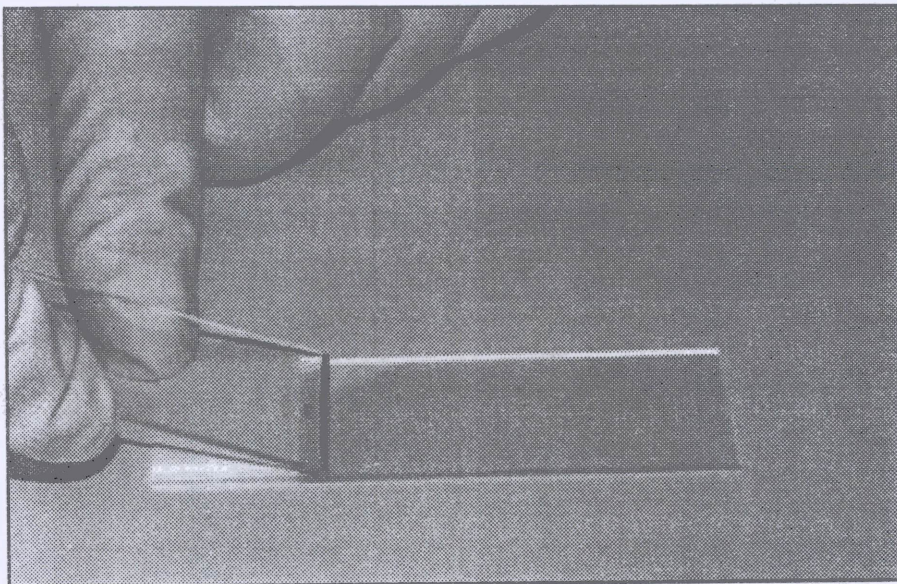
MAKING THE SMEAR



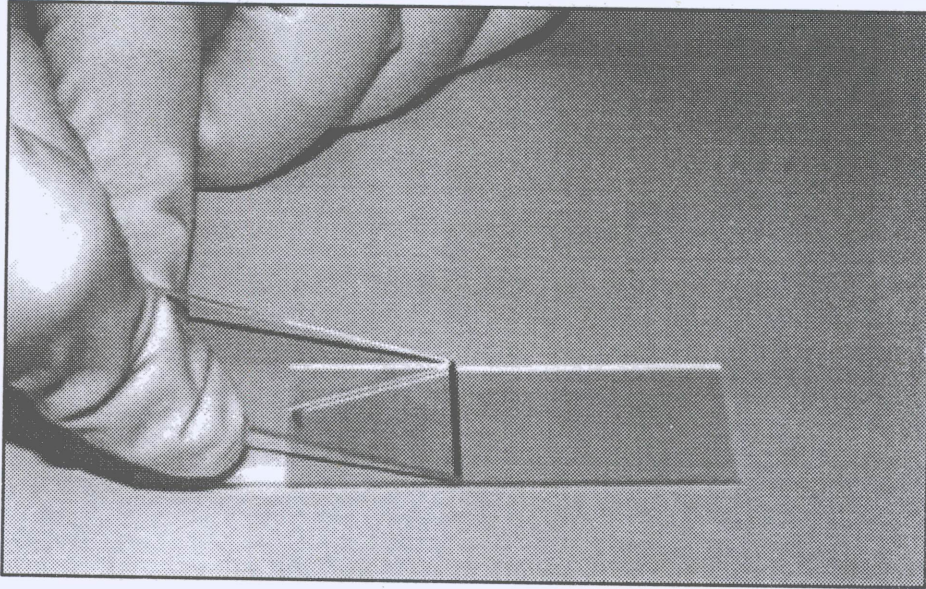
Step 1: Placing a small drop of venous blood on a glass microscope slide, using a glass capillary pipette. A wooden applicator stick can also be used for this purpose.



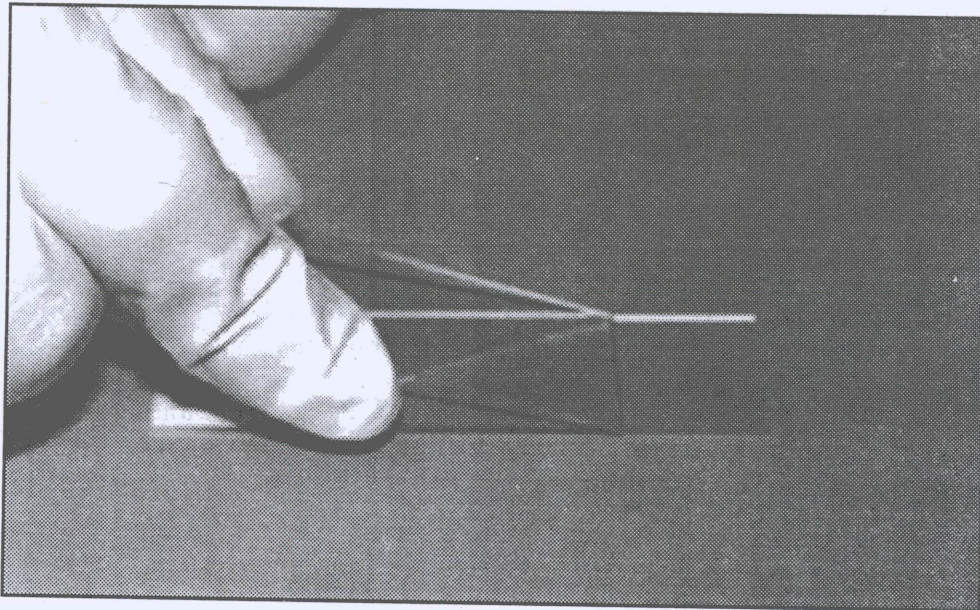
Step 2: A spreader slide has been positioned at an angle and slowly drawn toward the drop of blood.



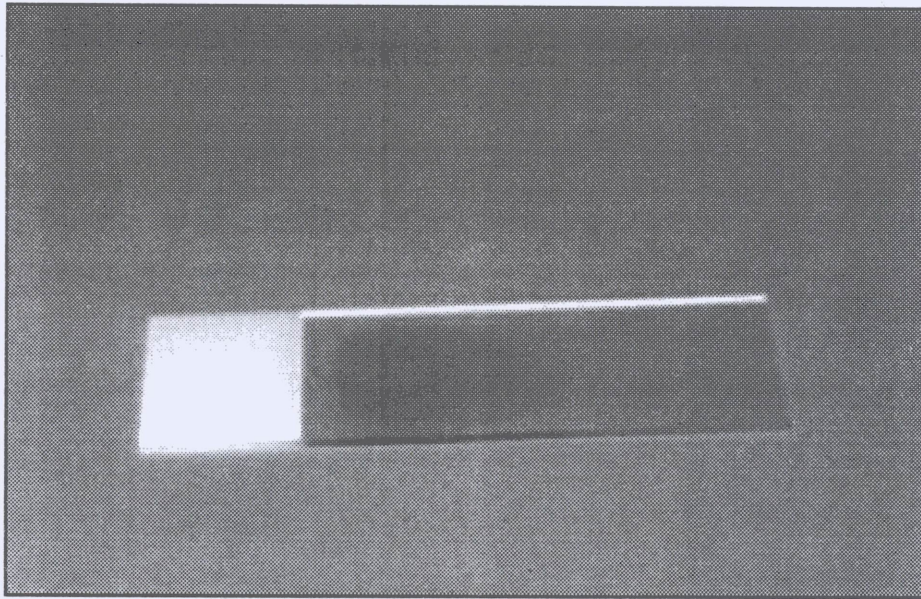
Step 3: The spreader slide has been brought in contact with the drop of blood and is being drawn away. Note layer of blood at the edge of the spreader slide.



Step 4: The spreader slide is further pulled out, leaving a thin layer of blood behind.



Step 5: The blood smear is nearly complete.



Step 6: End result. A glass slide with a well-formed blood film. After drying for about 10 minutes, the slide can be stained manually or placed on an automated slide stainer.

STAINING

To be able to observe and recognize the different kinds of leukocyte, you must stain them. For this purpose, normally **Gram stain** is used.

Different formulas have been used for crystal violet, safranin, and decolorizer, all of which are effective.

1. Gram's crystal violet: 1% aqueous crystal violet dye; [Hucker's crystal violet] 2 g crystal violet 90% dye content, 20 ml ethyl alcohol, 0.8 g ammonium oxalate, 80 ml distilled water
2. Gram's iodine: 1 g iodine, 2 g potassium iodide, 300 ml distilled water
3. Gram's safranin: 4 g safranin powder, 200 ml anhydrous ethanol, 800 ml distilled water
4. Gram's decolorizer: 25% acetone, 75% isopropyl alcohol
 - A heat-fixed bacterial smear is first covered completely with a few drops of a solution of crystal violet, a purple basic dye.
 - After 30-60 sec the smear is rinsed with water by squirting the slide above the smear and letting the water wash over it until the water runs clear.

- Several drops of iodine (the mordant) are applied to cover the smear and left for 60 sec., then rinsed again.
- A few drops of an isopropanol-acetone mixture or similar solvent are added at a time until the wash is colorless, then the slide is rinsed again.
- A red basic dye, aqueous safranin, is applied for 30-60 sec. followed by a rinse.
- The smear is blotted (not wiped) to remove excess water, using bibulous (absorbent) paper or a paper towel. The slide is then air dried the rest of the way.

COVER-SLIPPING

At this point, your smear is ready to be observed, but if you want to keep it for a long time, you should make the preparation permanent. To this purpose, mount the coverslip.

OBSERVATION

A magnification of 200 times is enough to observe and identify the different types of cells. If you use a higher power, you can also see the cells details better. You can examine either with dry objectives or with the oil immersion technique.

EXPERIMENT – III

ENUMERATION OF RBC AND WBCs

a. ENUMERATION OF RED BLOOD CORPUSCLES

Principle

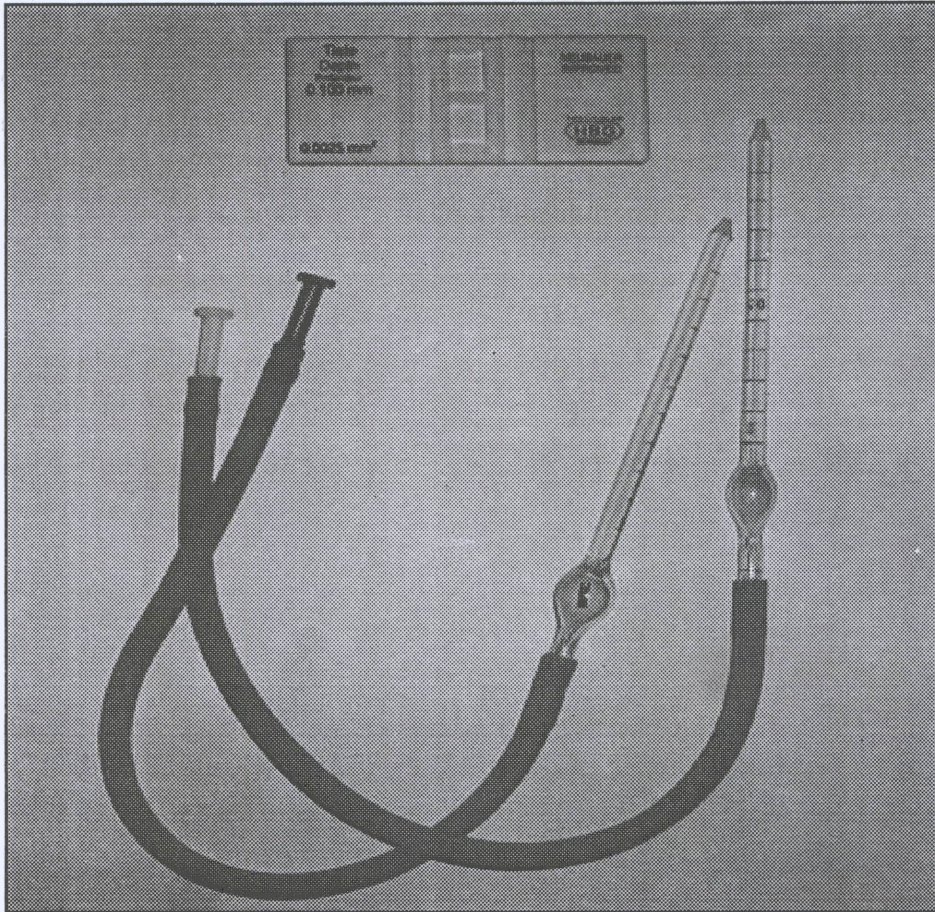
The method involves an accurate dilution of a measured quantity of blood with a fluid which is isotonic with the blood and which prevents clotting. A dilution of 1 to 200 is usually necessary. The diluted blood is placed in a counting chamber and the number of cells in a circumscribed volume is enumerated under a microscope.

Materials

- a. Red cell pipette: it is a capillary tube, graduated in 10ths, which opens into a bulb, with red glass bead. The bulb when filled to the mark above it (101) will hold 100 times the quantity of fluid contained in 10 divisions of the capillary tube.
- b. Counting chamber: A Neubauer counting chamber with ratings (HEMOCYTOMETER) is commonly used.
- c. Diluting fluid: A solution of 1% formalin (10 ml/l, 40% formaldehyde) in 31.3 g/L trisodium citrate.

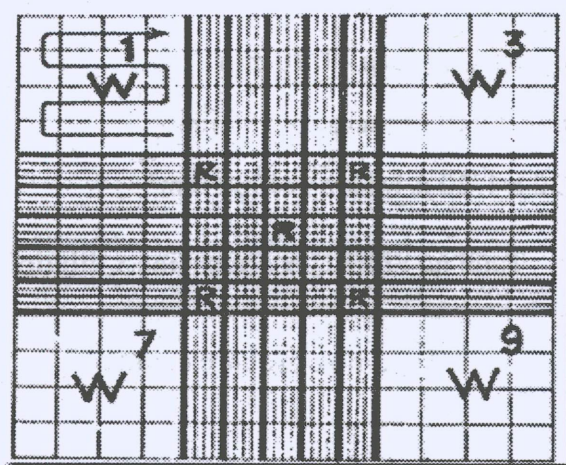
Procedure

The blood is drawn by mouth suction up to the 0.5 mark, the tip of the pipette is wiped clean and the diluents is drawn in until the solution fills the pipette and reaches the '101' mark. If the blood is being taken directly from the finger of the subject or the tail of the rat, this procedure should be done quickly to avoid clotting. The pipette is shaken by holding it loosely in one hand (after removing the attached rubber tubing) between the thumb and the forefinger at least for 3 min before loading it in the chamber. Alternatively, 20 μ l of blood can be diluted in 4ml of the diluting fluid in a test tube.

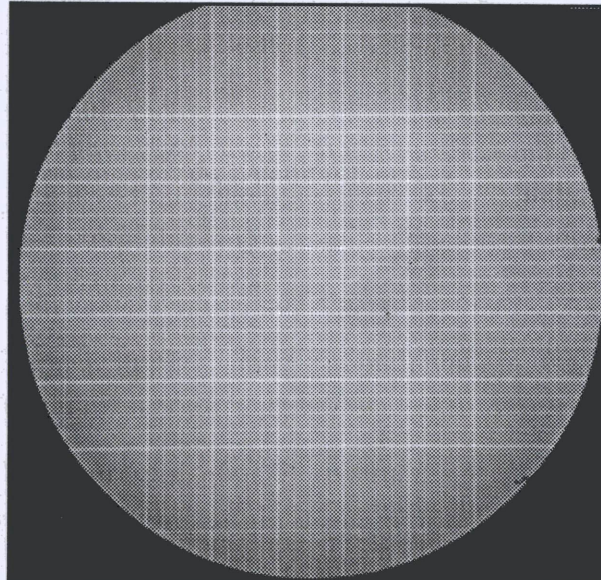


The coverslip is put on the counting chamber. A few large drops of the mixed solution are discarded from the pipette and then a small quantity of the diluted blood is put between the coverslip and the ruled platform of the counting chamber. The chamber should not overflow and there should not be any air bubble in the chamber. The solution is allowed to settle for a couple of minutes and then the counting is done under the high power of a microscope.

In the Neubauer ruling, the small squares in the centre large 1mm square are used for enumeration of erythrocytes.



The number of cells in the 4 corner groups of 16 squares are counted and also one central group including those cells which lie within the area or on the dividing lines to the left or above the section. If the dilution has been 1 to 200 (blood drawn to 0.5 mark) then the total number of cells found in the 5 groups of 16 squares is multiplied by 10,000 in order to give the number of cells in millions per mm³ of blood.



Calculation

The smallest square has an area of 0.0025 mm² and is 0.1 mm deep, being thus 0.00025 ml in volume. Since 80 such squares are counted, a volume of 0.00025 x 80 or 0.02 ml has been covered. In order to give the value per mm³, the number counted must be multiplied by 50. However, since the dilution is 1 to 200, the multiplication factor is 50 x 200 or 10,000.

Dimension of smallest RBC square

Length of smallest RBC square - 1/20 mm

Breath of smallest RBC square - 1/20 mm

Diameter of smallest RBC square - 1/10 mm

Dimension = 1/20 x 1/20 x 1/10 = 1/4000 mm³

Total no of RBC x dimension x dilution

Total count = $\frac{\text{Total no of RBC x dimension x dilution}}{\text{Total no of square counted}}$ cells / mm³

$$\text{Total count} = \frac{\text{Total no of RBC} \times 1/4000 \times 200}{80} \text{ cells / mm}^3$$

a. ENUMERATION OF WHITE BLOOD CORPUSCLES

A. TOTAL LEUCOCYTE COUNT

Principle

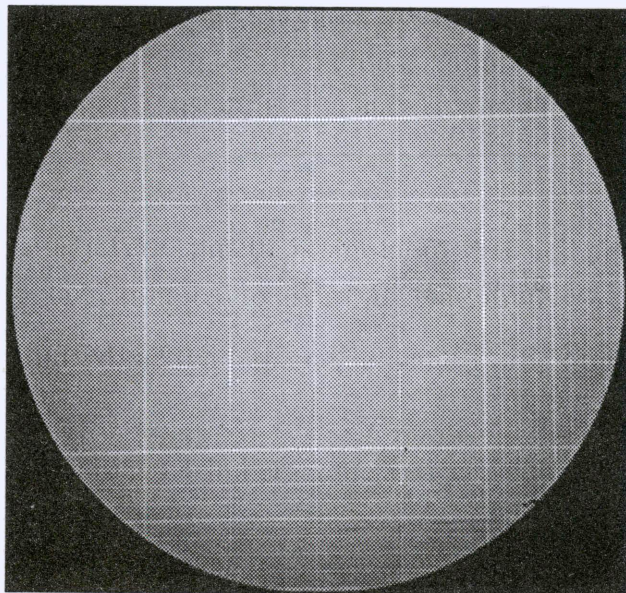
The enumeration of leucocytes is carried out according to the same principle as that of erythrocytes. Leucocytes being less numerous, a dilution of only 1 to 20 is used and the diluents is usually one which destroys the red blood corpuscles.

Materials

- WBC pipette: a WBC pipette is similar to a red cell pipette with a white colored glass bead, but calibrated to give 1 to 20 dilutions.
- Diluent fluid: Mix 2.0 ml glacial acid and 1 drop of gentian violet in 100 ml of water.
- Neubauer Counting Chamber: Same as that used for erythrocyte.

Procedure

The method of counting is similar to that for erythrocytes except that the count is made in large (1 mm) corner squares of Neubauer counting chamber.



Calculation

Dimension of smallest WBC square

Length of smallest WBC square - 1/4 mm

Breath of smallest WBC square - 1/4 mm

Diameter of smallest WBC square - 1/10 mm

Dimension = $1/20 \times 1/20 \times 1/10 = 1/160 \text{ mm}^3$

$$\text{Total count} = \frac{\text{Total no of WBC x dimension x dilution}}{\text{Total no of square counted}} \text{ cells / mm}^3$$

$$\text{Total count} = \frac{\text{Total no of WBC x } 1/160 \times 20}{\text{Total no of square counted}} \text{ cells / mm}^3$$

64

EXPERIMENT – IV

DIFFERENTIAL COUNT OF BLOOD CELLS

Materials

- a. Leishman's stain: Dissolve 0.2 g of Leishman's dye in 100 ml of acetone free methanol at 50° C for 15 min with occasional shaking. Cool and filter the solution.
- b. Buffered water: make up 50 ml of 66 mM Sorenson's phosphate buffer, adjust pH to 7.0 (Mix 3.89 ml of 9.1 g/l of KH_2PO_4 and 61.1 ml of 9.5 g/l Na_2HPO_4 or 11.9 g/l of Na_2HPO_4) to 1 L with water.

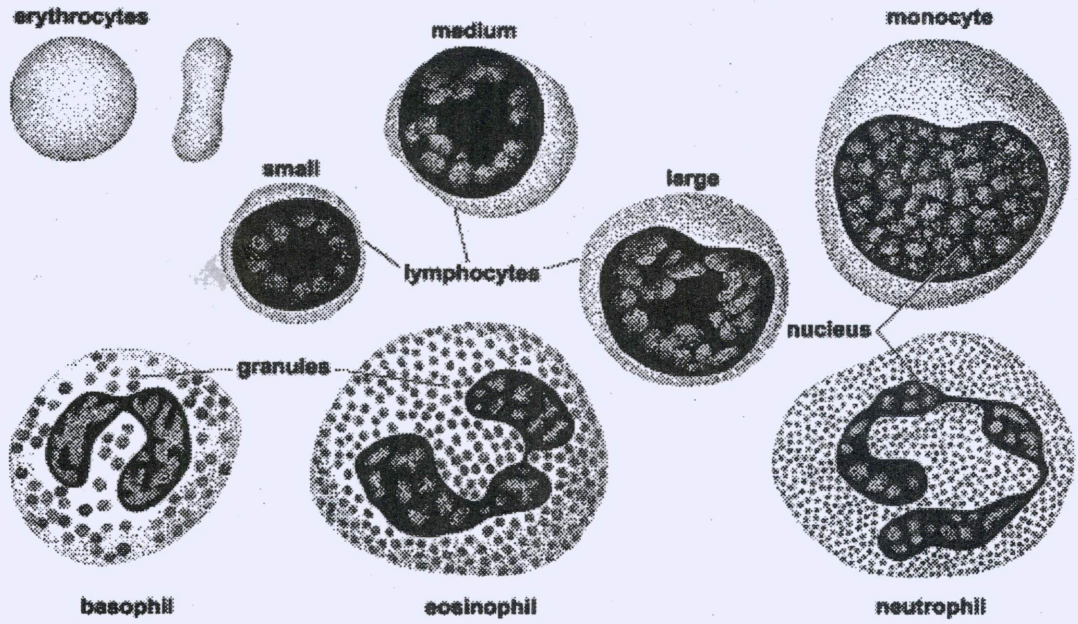
Preparation of blood smear

Place a drop of blood collected either directly in EDTA (within 3 hour of collection) on a clean and dry glass slide and prepares a film following the instructions given in earlier section. Note that the smear should be at least 3-4 cm in length and film should be thin such that there is only few overlap of RBC.

Procedure

Place the slide flat on the two glass rods over a sink, cover the slide with the stain and wait for 2-3 min. Dilute the stain by addition of buffered water and allow to stand for a period of 5-7 min. Drain off the stain and wash in water. Air dry and view it under microscope.

Scan the smear under low power by choosing an ideal portion of the smear for counting. Start counting under high power oil immersion objectives from the edge of the smear, moving on towards the centre. Identify the leucocytes as they appear and record. Shift the slide laterally and then move towards the edge. Repeat these movements till a total of 100 cells are counted. Express the values of different morphological types as percentage.



Normal count/100 squares

EXPERIMENT – V

ESTIMATION OF HEAMOGLOBIN – CYANOMETHMOGLOBIN METHOD

CYANOMETHEMOGLOBIN METHOD

Principle

In solution the ferrous ions (Fe^{2+}) of the hemoglobin's (Hb) are oxidized to the ferric state (Fe^{3+}) by potassium ferric cyanide to form methemoglobin. Methemoglobin then reacts with the cyanide ions (CN) cyanide to form cyanmethemoglobin, which has the absorbance at 540 nm.

Reagents

- Cyanmethemoglobin solution (Drapkin's solution): Dissolve 0.05 g potassium cyanide, 0.200g potassium ferric cyanide, and 0.140 g dihydrogen potassium phosphate in 1L of distilled water. Add 1 ml of triton X-100 and mix. Stable for at least 6 months.
- Hemoglobin standard: Lyophilised human methemoglobin, each vial is equivalent to hemoglobin concentration of 18 g/dl whole blood when reconstituted in 50 ml of Drapkin's solution. Stable for at least 6 months when stored at 2-6°C.

Procedure

Transfer 0.02ml of blood using a calibrated hemoglobin pipette, into tube containing 5.0 ml of Drapkin's reagent. Rinse the pipette several times with the reagent. Allow the diluted hemoglobin solution to stand at least 5 min to achieve full color development. Measure the absorbance at 530-550 nm of the test sample and standard hemoglobin against a reagent blank.

Calculation

$$\text{Hemoglobin unknown (g/dl)} = \frac{\text{Unknown sample} \times \text{Con. of Hb standard (g/dl)}}{\text{Known hemoglobin content}}$$

EXPERIMENT – VI

DETERMINATION OF BLOOD GROUP AND RH FACTORS, BLEEDING TIME, CLOTTING TIME AND ERYTHROCYTE SEDIMENTATION RATE

a. TO DETERMINE THE BLOOD GROUP AND RH FACTOR

Principle

The agglutinations with corresponding sera (agglutins) undergo agglutination. RBC agglutinate in presence of agglutins and this is sensitive to the RH specific sera

Materials

- Microscope, glass slide, pricking needles and cotton swabs
- Alcohol, unit serum-A, B and D

Procedure

- Clean two glass slides with sodium citrate or acetic acid and dry.
- Clean the finger with a disinfectant or sterilizer and prick the finger tip with the pricking needle.
- Mark A and B on the edges of the first slide and D on the second slide.
- Place a drop of blood besides each marking on the prepared slides.
- Add a drop of serum to the samples at A, B and D with the respective serum A, B and D.
- Mix the blood and serum with separate needles. Leave for 5 min and observe under microscope for agglutination.

Results

The sample that show agglutination with a particular sera will be the blood group and RH Factor + ve or - ve.

a. BLEEDING TIME

Introduction

Blood has a natural capacity to clot, which is asserted by a variety of physiological factors. When an artery, capillary or vein is punctured blood starts to flow and after a while it stops. The time required for this to happen is termed as bleeding time.

Normal bleeding time ranges between 2-5 mins with an average being 3.25 minutes.

Materials Required

Sterile lancet, cotton swabs, filter paper, stopwatch.

Procedure

Clean the finger tip or ear lobule and puncture using a syringe. Note the time using stop watch when the first drop of blood appears.

Blot the drop on a filter paper and continue to blot every 30 seconds till the blood stops coming. Count number of blood spots on blotting paper and multiply by 30 seconds for calculating the bleeding time.

Bleeding Time = No. of spots x 30 seconds

a. CLOTTING TIME

Introduction

When the blood is shed it loses fluidity in a few minutes and sets into a semi solid jelly. This phenomenon is called coagulation or clotting of blood. Clotting time is determined to assess the integrity of hemostatic mechanism.

When the process of coagulation studied under microscope it is seen that minute granules appear at first. These granules join together to form needle like structures which again unite to form long threads passing across the whole bulk of blood. These threads cross one another and form a sort of network, into the meshes of which the red and white cells get entangled.

Time required normally for the blood to clot is 3-8 minutes with an average of 5 minutes.

Method

Clotting time is determined by two methods-

- a. Slide method
- b. Capillary method

Materials required

Sterile lancet, cotton swab, round head pin or needle, glass slide, capillary tube, stopwatch.

Procedure

a. Slide method

Clean the finger tip and prick with a sterile needle. 2-3 drops of blood is collected on the cleaned glass slide. Dip the tip of the pin or needle into the blood on the slide and pull. Repeat this procedure at an interval of 30 seconds until a string or thread is formed at the tip of the pin or needle, record the time taken, for the appearance of the thread. Report as clotting time.

b. Capillary method

Prick the finger tip and fill the blood into a capillary tube of a length of 5-6 cms. The tube is broken at small lengths at an interval of 30 seconds until the broken piece hangs to the main capillary tube with a string of blood clot. This indicates the formation of fibrin. Record the time taken for the thread formation. Report as clotting time.

a. ERYTHROCYTE SEDIMENTATION RATE

Principle

RBCs from freshly drawn blood sediment when allowed to stand undisturbed, this characteristic to RBC and rate at which they sediment is measured as Erythrocyte sedimentation rate (ESR).

Material

Westergren ESR tube: It is a glass tube of 300 x 2.5 mm dimensions and graduated in mm from above downwards till 200 mm of the tube.

Procedure

Blood (1.6 ml) is collected into 0.4 ml of 3.1 % trisodium citrate; alternatively EDTA anticoagulated blood can also be diluted with trisodium citrate (1:4). This has to be carried out within 2 hour of collection of blood. A delay of 6 hour is permitted, if stored at 4°C. Soon after the dilution, draw it into the Westergren tube up to the 200 mm mark and place absolutely vertical and leave undisturbed for 1 hour. Note the height of the plasma column and read to the nearest mm at the end of 1 hour. This is reported as ESR in mm.

Normal Range

Men - 0 to 5 mm

Women - 0 to 7 mm

EXPERIMENT – VII

URINE - QUALITATIVE ANALYSIS

Urine examination remains one of the simplest no-invasive techniques hence widely used in clinical investigators. The results of urine investigation depend upon using specimen appropriately collected. For most of the urine analysis early morning midstream specimen is preferred sample. The specimen obtained after proper precautions to avoid contamination is essential for bacteriological examination of urine. For several metabolic investigation and measurement of some excreted products, a 24hrs urinary collection may be essential.

a. URINE SUGAR

Principle

The copper sulphate in Benedict's solution reacts with reducing sugars, giving colour change.

Aim: To test the presence of sugar

Specimen: Random Urine

Procedure

In the test tube with 2 ml of Benedict's reagent, add 5-6 drops of the urine sample to be tested and mix well. Place the test tube in a boiling water bath for 5 minutes and observe change in color or precipitate formed. Cool the solution. Colour change that normally occurs are blue to green, yellow, orange or red depending upon the amount of sugar present in the test sample.

Report

Formation of a green, red, or yellow precipitate indicates presence of reducing sugars

b. URINE ALBUMIN – Test – 1 Heating Method

Principle

Proteins in urine are coagulated by heat and the degree of coagulation is directly proportional to the amount of proteins present. Coagulation can be further enhanced when drops of acetic acid are added.

Aim: to test the presence of albumin

Specimen: Random Urine

Procedure

1. Pipette out 5 ml of urine into a test tube
2. Heat the sample for 5 minute, add glacial acetic acid when hot, mix well
3. Note the presence of precipitate.

Report

Highly turbid +++; Moderately turbid ++; Very light +.

TEST - 2: BIURET TEST

Aim: to test the presence of albumin

Reagent: Biuret reagent

Specimen: Random Urine

Procedure

1. Pipette out 5 ml of Biuret reagent into a test tube.
2. Add 8 drops of urine sample into it and mix well with a stirrer.
3. Place in a boiling water bath for 5 minutes.
4. Allow to cool at room temperature.
5. Note for the presence of precipitate in the final solution.

Report



ಕರ್ನಾಟಕ ರಾಜ್ಯ ಮುಕ್ತ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ

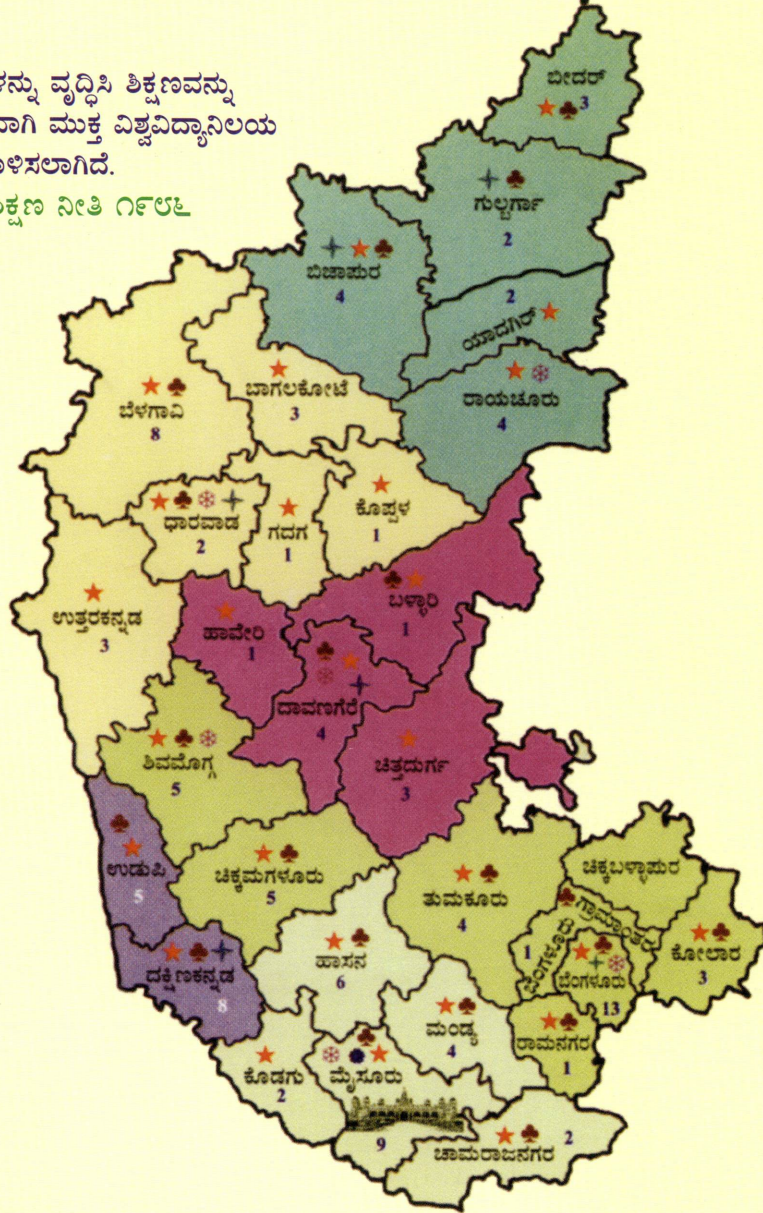
ಮಾನಸಗಂಗೋತ್ರಿ, ಮೈಸೂರು-570 006

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ರಾಷ್ಟ್ರೀಯ ಶಿಕ್ಷಣ ನೀತಿ ೧೯೮೬

♣ ಪ್ರಾದೇಶಿಕ ಕೇಂದ್ರಗಳು

- ಬೆಂಗಳೂರು
- ದಾವಣಗೆರೆ
- ಗುಲ್ಬರ್ಗಾ
- ಧಾರವಾಡ
- ಶಿವಮೊಗ್ಗ
- ಮಂಗಳೂರು
- ತುಮಕೂರು
- ಹಾಸನ
- ಚಾಮರಾಜನಗರ
- ಬಳ್ಳಾರಿ
- ಮಂಡ್ಯ
- ಕೋಲಾರ
- ಬಿಜಾಪುರ
- ಬೆಳಗಾಂ
- ರಾಮನಗರ
- ಬೆಂಗಳೂರು (ಮತ್ತೊಂದು)
- ಚಿಕ್ಕಮಗಳೂರು
- ಉಡುಪಿ
- ಕಾರವಾರ
- ಬೀದರ್
- ಮೈಸೂರು

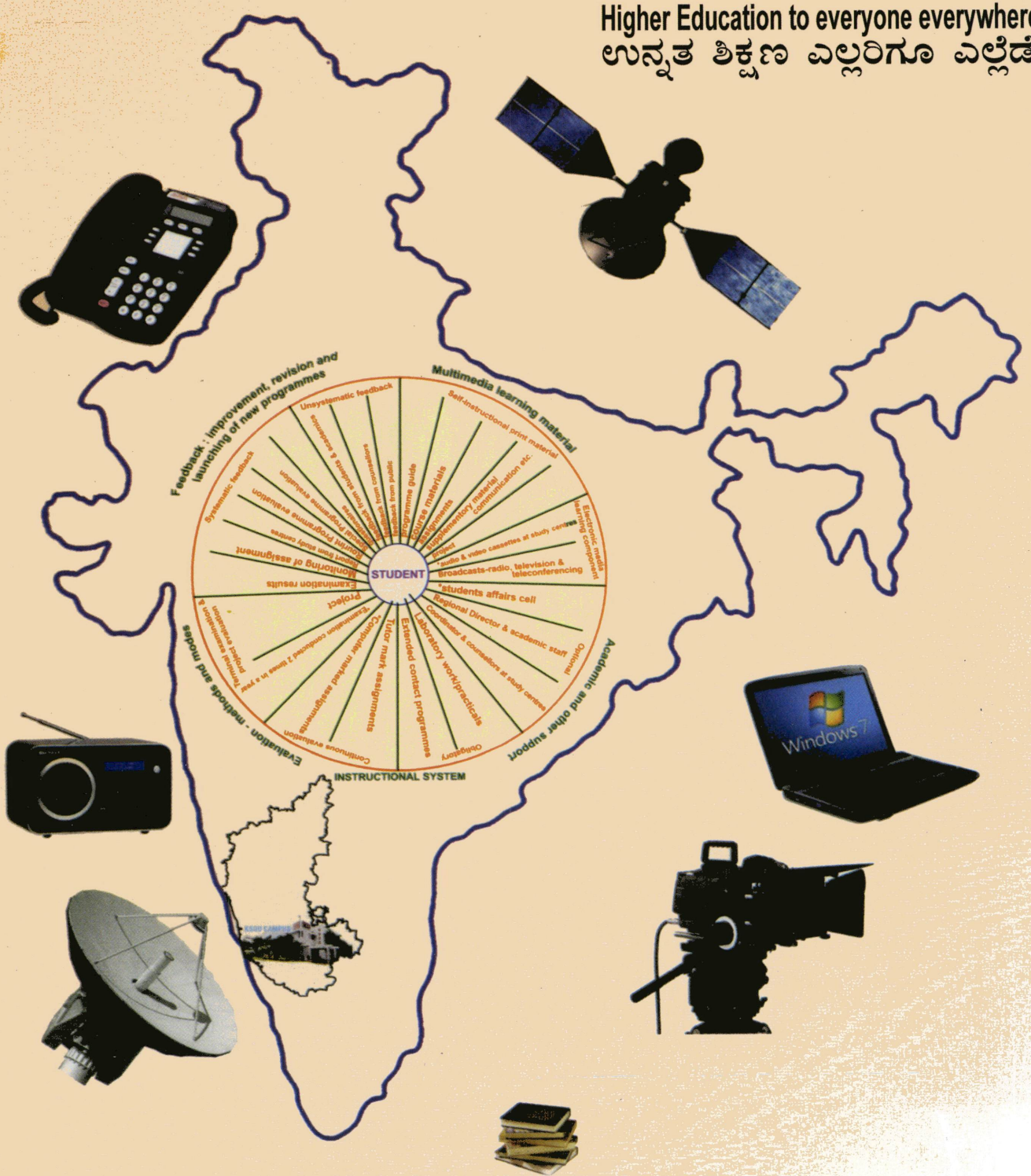


- ♣ ಮುಖ್ಯ ಕಛೇರಿಗಳು
- ★ ಒಟ್ಟು ಅಧ್ಯಯನ ಕೇಂದ್ರಗಳು : ೧೨೩
- ♣ ಪ್ರಾದೇಶಿಕ ಕೇಂದ್ರಗಳು : ೨೧
- ✿ ಬಿ ಎಡ್ ಅಧ್ಯಯನ ಕೇಂದ್ರಗಳು : ೧೦
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