NATIONAL UNIVERSITY-SUDAN

Faculty of Medical Laboratory Sciences

Student Practical Manual-

Haematology and Immunohaematology

Department

Third year, Semester (6)
Advanced Hematology (MLS-HEM-322)

Student Name: ……...

ID: ……**Batch**…….....…...

Instructions

- Wear lab coat
- Wear Gloves
- Avoid swallow any chemical
- Follow the procedures provided
- Write your results in this manual

Practical No (1)

Complete blood count CBC

Definition:

Is a blood panel requested by doctors that give information about the cells of the patient's blood. IT IS Also known as full blood count FBC

Sample and sampling:

EDTA anticoagulated blood

Included tests:

1. White blood cell count (WBC or leukocyte count)
2. WBC differential count
3. Red blood cell count (RBC or erythrocyte count)
4. Hematocrit (Hct)
5. Hemoglobin (Hb)
6. Mean corpuscular volume (MCV)
7. Mean corpuscular hemoglobin (MCH)
8. Mean corpuscular hemoglobin concentration (MCHC)
9. Red cell distribution width (RDW)
10. Platelet count
11. Mean Platelet Volume (MPV)
12. Peripheral blood picture (PBP)

- Further investigations should be done if present to confirm the diagnosis

You are provided with blood sample labeled ( ) obtained the CBC then comment on your results.

Results……
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Comment……
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Final diagnosis……

Evaluation:

Name and signature of the instructor:

Date:

Practical No (2)

Sickling test

Aim

Tests to detect the presence of Hb S depend on the decreased solubility of this Hb at low oxygen tensions.

Principle

One drop of blood is mixed with one drop of reducing agent such as sodium metabisulfite (or sodium dithionate) reagent on a slide. If the erythrocytes contain haemoglobin S, they will lose their smooth , round shape and become sickled, as the reagent removes oxygen from the cells, allowing sickling to take place.

Sample: Fresh blood in any anticoagulant.

Reagents

(A) Disodiumhydrogenphosphate(Na2HPO4)……..16.2g/l.

(B) sodium metabisulfite……. 2%

Working solution: mix 3 volumes of (A) with 2 volumes of (B) immediately before use.

Method:

1.Add 1 drop of the reagent to 1 drop of the blood in a slide.

2. Seal between the slide and a cover glass with a paraffin wax (or with nail varnish). **Making sure that no air bubble forms.**

Notice: A test on a +ve control of Hb A and Hb S must be performed at the same time.

Results:

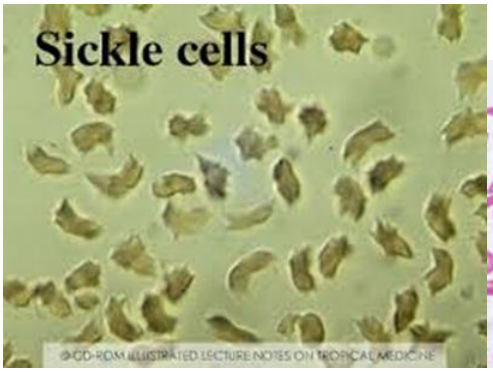
Examine the slide under the microscope using the x 40objective.

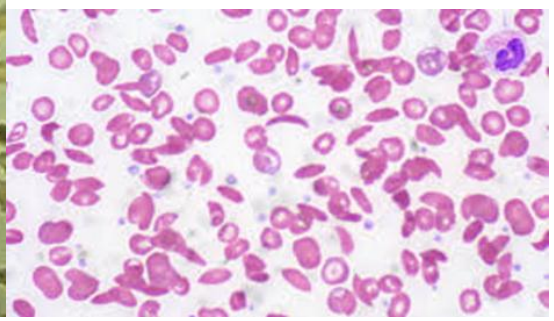
Negative result:

The erythrocytes remain round. If the test is negative, re-examine the slide after a further 30 minutes, then after 2 hours and after 24 hours.

Positive result:

The erythrocytes become sickle-shaped or banana-shaped





Quality control

False negative results may be obtained if the metabilsulphite has deteriorated or if the cover slip is not sealed properly.

A positive test does not distinguish the sickle cell trait from sickle cell disease. It is important to examine the preparation carefully and in particular near the edge of cover slip.

Practical No (3)

Solubility Test for Hemoglobin S

Aim

This test is hemoglobin S screening test is to confirm diagnosis of sickle cell anaemia

Principle

Solubility test based on the relative insolubility of hemoglobin S when combinedwith a reducing agent. When whole blood is mixed with the reducing agent,saponin lyses the erythrocytes and hemoglobin is released. If hemoglobin S is present, it will formliquid crystals and give a turbid appearance to the solution. A transparent solution is seen with otherhemoglobins that are more soluble in the reducing agent.

Reagents and Equipment:

Reagents

1. K2HPO4= 250g
2. KH2PO4= 160g
3. sodium dithionite = 5g
4. Saponin= 1 g

- Working solution:

Dissolve K2HPO4in water before adding KH2PO4 then add sodium dithionite and finally saponin , this solution is stable for 7 days.

Equipments

1. Test tubes 12x75 mm

4. Micropipette 0.02 mL

5. Micropipette tips

6. Pipettes, 2.0 mL

Quality Control

A positive control (A/S) containing 30-45% Hb S and a negative control (A/A) should be analyzedwith each patient specimen.

Specimen

Whole blood anticoagulated with EDTA, heparin, or sodium citrate is acceptable. Specimens may bestored at 4 C for up to three weeks before testing.

Procedure

1. Allow reagents and specimens to warm to room temperature prior to performing this test.

2. Pipette 2.0 mL of working solution into a labeled test tube.

3. Add 0.02 mL of whole blood to the appropriately labeled test tube.

4. Mix the contents thoroughly.

5. Incubate the tubes for 3-5 minutes in the test tube holder at room temperature.

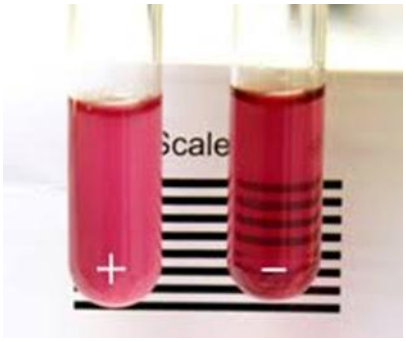
6. Read for turbidity.

7. report as positive or negative

Results

A positive result is indicated by a turbid suspension through which the ruled lines are not visible.

Anegative result is indicated by a transparent suspension through which the ruled lines are visible



Limitations

1. This is a qualitative test and does not distinguish between hemoglobin S disease (S/S) andhemoglobin S trait (A/S). To confirm the presence of Hb S and differentiate between the twostates, a hemoglobin electrophoresis at an alkaline pH should be performed.

2. Other abnormal hemoglobin variants are known to cause sickling and will give a positivesolubility test. These variants include Hb C Harlem, Hb S Travis, and Hb C Ziguinchor. Todifferentiate these variants from HbS, a hemoglobin electrophoresis at an alkaline pH should beperformed. On occasion, a hemoglobin electrophoresis at an acid pH may be needed to completethe differentiation.

3. false positive is seen in leukocytosis , hyperproteinaemia, unstable Hb and hyper lipidemia

4. false negative results caused by:

a. Inactive or outdated reagents

b. Reagent below room temperature

b. An anemic individual (< 7.0 g/dL)

c. Recent transfusion with normal erythrocytes

d. If an infant is younger than 6 months.

Lab. Question

You are provided with blood sample labeled ( ) obtained from suspected sickler . do further investigations to confirm.

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Results……
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Comment……
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Final diagnosis……

Evaluation:

Name and signature of the instructor:

Date:

Practical No(4)

Reticulocyte count

Definition:

• Young red blood cell; still have small amounts of RNA present in them

Aim

1. Reticulocyte count help in monitoring anemic patients under treatments
2. Determine the state of increased erythropoietic activity (haemolytic anaemia)
3. Assess bone marrow activity

Principle

- The peripheral blood sample is stained with supravital stain
- These are basic dyes that have the ability to react with ribosome and nucleic acids of reticulocytes while it still alive.
- The nucleic acid-dye reaction form a blue precipitate of granules or filaments.Smears of this mixture are then prepared and examined.
- The number of reticulocytes in 1000 red blood cells is determined. This number is divided by 10 to obtain the reticulocyte count in percent.

Sample

EDTA anticoagulated blood

Requirements:

1. New methylene blue 1% or brilliant cresyl blue 1%
2. Test tubs
3. Slides
4. Water path

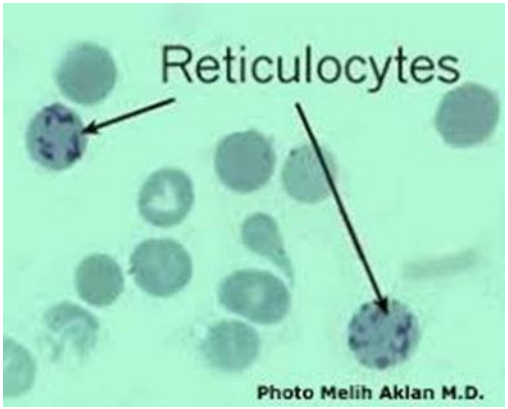
Sample preparation

- Deliver 2-3 drops of the dye solution into a 75x10 mm glass or plastic tube using Pasture pipet.
- Add twice the amount of the patients EDTA blood to the dye solution and mix.
- Keep the mixture for 37˚C for 15-20 min.
- Resuspend the Red cells by gentle mixing.
- Make film on the slide in the usual way.

Counting

- Add a drop of oil
- Count all red cells (reticulocytes + RBC) using a manual counter
- Make sure not to count WBC (large nucleated cell)
- The number of reticulocytes is counted also on outside paper to be able to calculate.
- # retics in 1000 RBCs = %

  10



Normal range

- N.R in adults= 0.2% - 2.0%
- N.R in neonates= 2.5% - 6%

Interpretation

- Increased reticulocyte count indicates active erythropoiesis (as a response of heamolytic anaemia or hemorrhage)
- reticulocyte count can be used as good indicator for successful treatment of nutritional anaemia (mainly Irion deficiency anamia and mgaloblastic anaemia)

Lab. Question

You are provided with blood film labeled ( ) and retics preparation .

1. comment on the blood film
2. obtain the retics count.
3. Suggest the possible causes of such condition

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Comment……
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Final diagnosis……

Evaluation:

Name and signature of the instructor:

Date:

Practical No(4)

Methaemoglobin reduction test

Aim

To detect the activity of the enzymes of pentose phosphate metabolic pathway. This is helpful in the diagnosis of glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Principle

Haemoglobin is oxidized in vitro to methaemoglobin (Hi) using an oxidizing agent, and then the pentose pathway is activated by methylene blue to detect the ability of the metabolic enzymes to reduce the methaemoglobin (Hi) to haemoglobin

Reagents

•Sodium nitrate (NaNo2) 180 mmol/l (oxidizing agent)

•Methylene blue 0.4 mmol/l Or Nile blue sulphate 22mg/dl (pentose phosphate pathway activator).

•Dextrose 280mmol/l

•Distilled water.

•Working reagent:5g dextrose+1.25g NaNo2 in 100 ml D.W.

•150mg methylene blue in 1litre D.W.

Sample:

Venous blood collected in EDTA, heparin or ACD anticoagulant.

- **•Method:**

In three different test tubes take: Tube	Blood	Working Reagent	Methylene blue
Test	2ml	0.1ml	0.1ml
+vecontrol	2ml	0.1ml	-
-ve control	2ml	-	-

Mix, incubate at 37˚C for 3 hours, then:

	Test	+ve Control	-ve control
Mixture	0.1ml	0.1ml	0.1ml
D.W.	10ml	10ml	10ml

Results:

Mix gently and detect the color of each tube.

1.+ve control (deficient tube): Brown color.

2.-ve control :Red color.

3.Test: Red or Brown (an intermediate color occurs in heterozygous).

Interpretation of the results:

In the +ve control tube the haemoglobin is oxidized and the pathway is not activated and so the color of methaemoglobin (brown) remains.

In the –ve control tube the haemoglonbin is not changed and so remains in red color.

Lab. Question

You are provided with blood sample labeled ( ) obtained from obtained from patient with sudden haemolytic anaemia .

1. Do further investigations to finalize the diagnosis.
2. What is the possible findings in the PBP

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Results……
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Comment……
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Final diagnosis……

Evaluation:

Name and signature of the instructor:

Date:

Practical No(6)

Osmotic Fragility Test

Aim

• Indicates the surface area / volume ratio. This is helpful in the diagnosis of Hereditary spherocytosis and a screening test for thalasaemia.

•Erythrocytes from patients with HS hemolyze at higher saline concentrations

than normal cells.

Principle

Spherocytic cells, for whatever cause, take up less water in the hypotonic solution before rupturing than normal red cells. Small blood volume is mixed with the excess buffer saline solution of vary concentration. The fraction of red cells lysed at each concentration is determined colorimetrically.

Reagents:

•90g NaCl+13.64g Na2HPO4+2.34g Na2HPO4 dissolved to 1litre (this is equal to100g/l NaCl (10g/dl), from which prepare10g/l NaCl).

•NaCl.

•D.W.

Sample:

Heparinized or defibrinated blood (avoid oxalate or citrate which will increate the salt concentration).

Method:

• In a small test tubes prepare serial dilutions of the saline solution (zero-D.W.,o.1, o.2, o.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9,&1.0g/dl). Prepare 5ml from each concentration according to the table below:

Conc.(g/dl)	Saline (ml)	D.W (ml)
0	-	5
0.1	0.5	4.5
0.2	1	4
0.3	1.5	3.5
0.4	2	3
0.5	2.5	2.5
0.6	3	2
0.7	3.5	1.5
0.8	4	1
0.9	4.5	0.5
1.0	5	-

- Add to each tube 0.05ml blood (may be 1 drop by pastaure pipette).

•Mix and incubate for 30 min at RT.

•After the incubation period mix again gently then centrifuge for 5min.

•Read the supernatant for each tube against the blank 1g/dl.

Calculations:

The O.D. for zero tube (D.W.) resembles 100% lysis, and then the percent lysis for all the tubes is calculated.

Plot the results (%lysis) against the saline concentration (g/l).

•From the osmotic fragility curve; find out 50% lysis, the MCF (mean cell fragility).

Normal values:

MCF = 4 – 4.5 g/l

Lab. Question

You are provided with blood sample labeled ( ) obtain the MCF then comment

Results……
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Comment……
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Final diagnosis……

Evaluation:

Name and signature of the instructor:

Date:

Practical No (7)

White blood cell count:

Manual method:

This procedure consist of diluting the blood with special diluent, transferring small volume of diluting blood onto a ruled glass platform (a hemcytometer) and counting the cells under a microscope We report the final result as the number of cells per cubic mm.

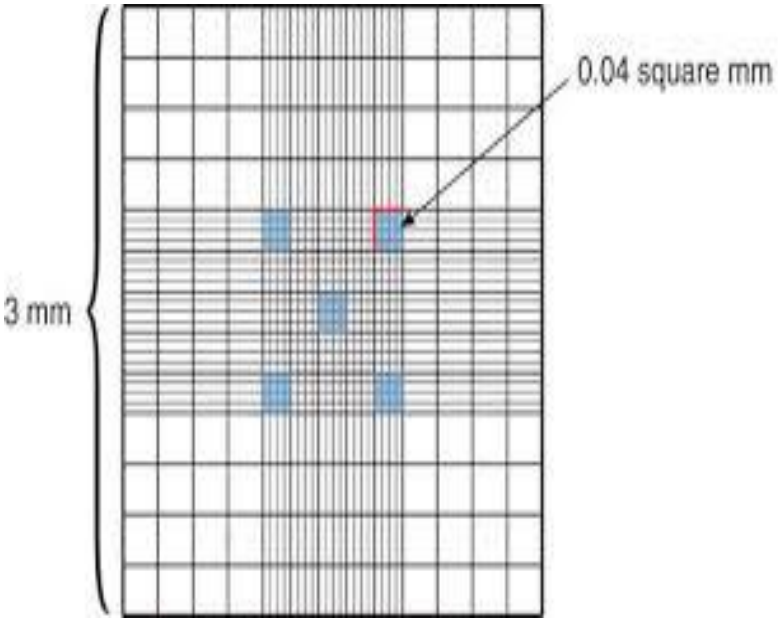
Hemocytometer:

It comprised tow identical ruled glass platforms squared by an H shaped moat.

The four corner squares (labeled W) are each subdivided into 16 squares, these are used for counting white blood cells WBC).

We use the large square in the center which is subdivided into 25 squares each one divided into 16 squares fir counting red blood cells (RBC) and platelets.

Hemocytometer contain toe raised ridges on which the cover glass rest, there is a distance 0.1 mm between the cover glass and the counting area.



Enumerating Leukocytes (WBC):

Principle:

To count WBCs, whole blood is diluted 1:20 in a weak acid (glacial acetic acid), which lyses (rupture) the red blood cells

After the hemacytometers is charged with the diluted blood, cells are counted microscopically in the four large squares labeled (W).

Cells should be counting in the tow glass platform, and the difference between them should be less than 10, and the difference between the highest and lowest counts should be less than 15

Requirements:

1. glacial acetic acid 2 %

2. counting chamber and cover glass

3. 1 ml pipette

4. 0.02 ml pipette

5. test tubes

6. microscope

7. gauze

8. Blood sample

Method:

1. Add 0.02 ml blood sample to 0.38 ml of diluting fluid
2. Mix well for at least 2 min
3. Prepare the counting chamber
4. Smoothly fill the chamber
5. Leave the chamber on bench for at least 2 min
6. Using power 10 objective, count the white cells in the specific area in the chamber

Counting formula:

Cell count (per cubic mm) = number of cells counted * dilution actor\ area count * depth (total volume)

Reference value:

$4 – 11 * 10^{9}$\L

Lab. Question

You are provided with blood sample labeled ( ) obtain the total WBCS count then comment

Results……
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Comment……
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Final diagnosis……

Evaluation:

Name and signature of the instructor:

Date:

Practical No (8)

Differential WBCS count

Lab Objectives

1. to identify the five different white blood cells
2. To determine the relative number of each type of white cell present in the blood by performing differential cell counts on relatively normal blood smear and abnormal blood smears.
3. To calculate the absolute WBCs count of each of the above blood smears..
4. To interpret the result.

Aim

Increases in any of the normal leukocyte types or the presence of immature leukocytes or erythrocytes in peripheral blood are important diagnostically in a wide variety of inflammatory disorders and leukemia.

Principle

A stained smear is examined in order to determine the percentage of each type of leukocyte and assess the erythrocyte and platelet morphology.

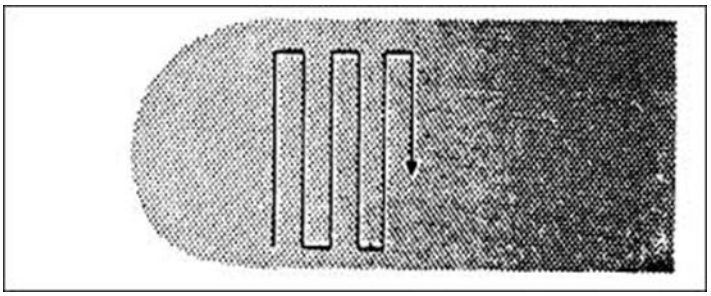
SPECIMEN: EDTA-anticoagulated blood. Smears should be made within 1 hour of blood collection.

Reagents, Supplies And Equipment

- Manual cell counter designed for differential counts
- Microscope,
- immersion oil

Procedure

- Using the 100 X oil objective, place a drop of oil on the slide and examine the smear for RBCS and platelets morphology and number.
- Find a thin area where red cells are not or little overlapping. (It is the appropriate area for differential counts.
- Begin the count the number of each white cell until 100 white cells have been counted in the thin area of the slide as shown below.



- Results are expressed as a percentage of the total leukocytes counted.

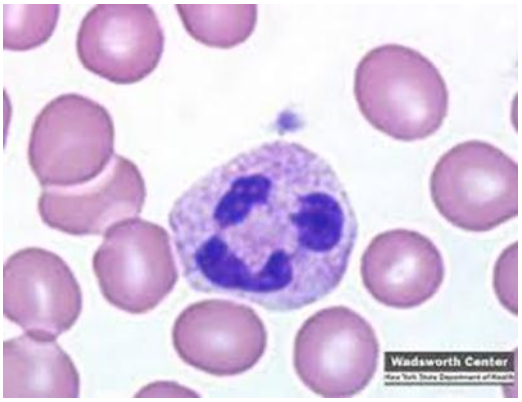
Absolute WBCS count

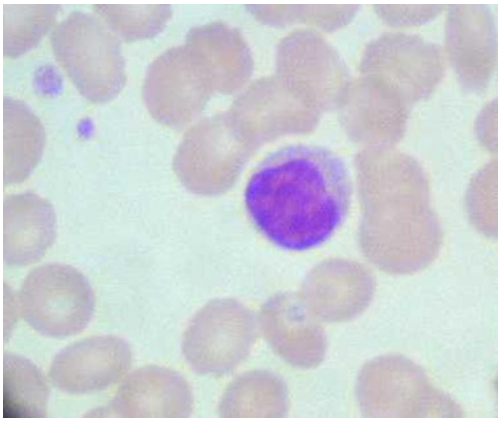
It is also helpful to know the actual number of each white cell type per µL of blood. the absolute count and is calculated as follows:

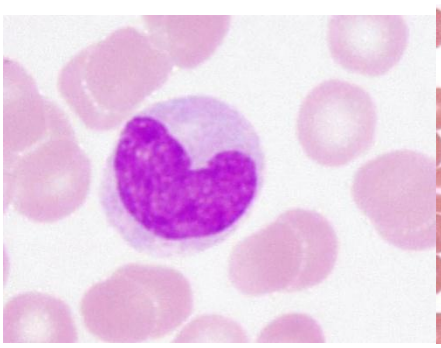
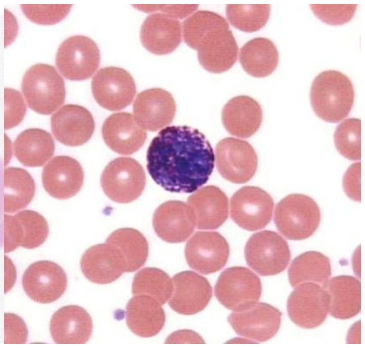
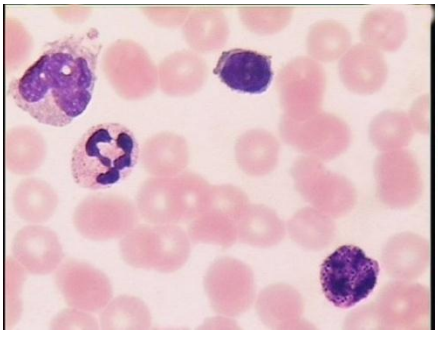
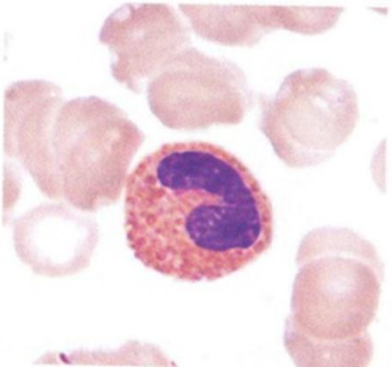
Absolute number of cells/µl = % of cell type in differential x white cell count/100

Normal ranges

- Neutrophils - 2.0–7.0×10 9/l (40–70%)
- Lymphocytes - 1.0–3.0×10 9/l (20–40%)
- Monocytes - 0.2–1.0×10 9/l (2–10%)
- Eosinophils - 0.02–0.5×10 9/l (1–6%)
- Basophils - 0.01–0.1×10 9/l (0–1%)





Lab. Question

You are provided with blood films labeled ( ) and( )

1. obtain the differential WBCS count then comment
2. calculate the absolute count
3. give the possible causes

Results……
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Comment……
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Final diagnosis……

Evaluation:

Name and signature of the instructor:

Date:

Practical No (9)

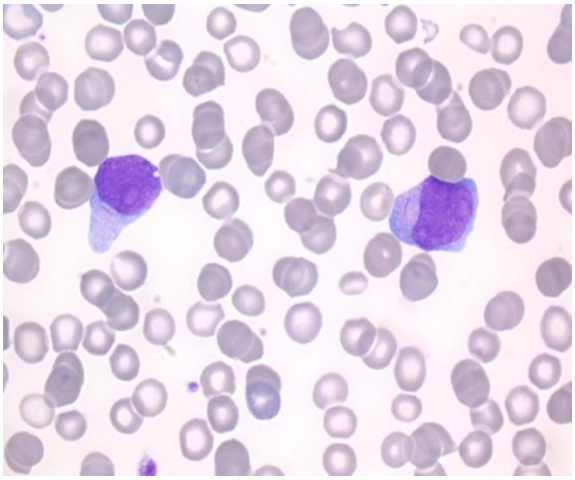
Acute leukaemia

Diagnosis of acute leukaemia includes:

1. CBC
2. Bone marrow examination
3. Cytochemical
4. Flow cytometry (immunophenotyping)
5. PCR
6. Cytogenatics

Complete blood count

- Hb is low
- Low RBCs and reticulocyte count
- Red cell indices (MCV, MCH, MCHC) are normal
- WBCs: normal, decreased or increased
- WBCs: to 200,000/cmm or more
- Platelets are low
- PBP:Normocytic normochromic red cells , variable number of blast cells and thrombocytopenia



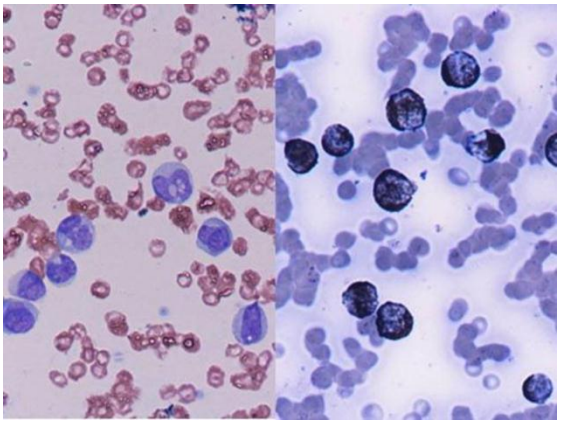
Bone marrow examination

- hypercellular with >20% leukaemic blasts cells
- Normally the blast cells are 5% or less

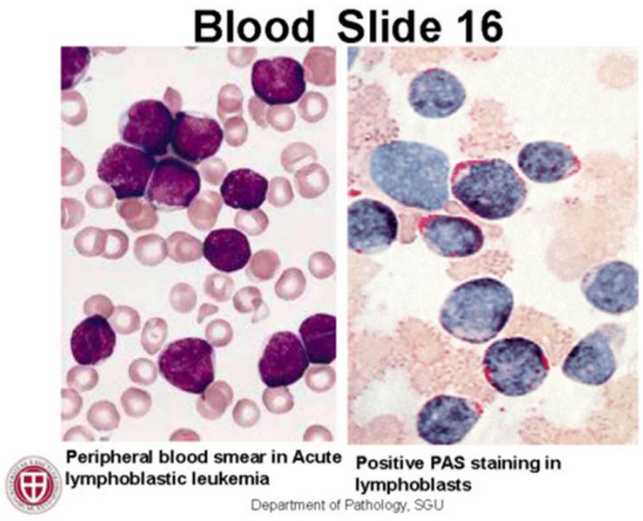
Cytochemistry:

Cells are exposed to chemical stains that react with only some types of leukemia cells. These stains cause color changes that can be seen under a microscope. These stains include:

- Sudan black B: positive in AML, negative in ALL
- Myeloperoxidase:positive in AML, negative in ALL
- PAS: positive in ALL



Sudan black B in acute myeloid leukaemia



Immunohistochemistry:

- A sample of cells is treated with special antibodies.
- The cells are then passed in front of a laser beam.
- If the cells attached to antibodies the laser will make them give off light, which can be measured and analyzed by a computer.

Lab. Question

You are provided with blood films labeled ( )

1. comment then suggest the diagnosis
2. mention the further investigations to finalize the diagnosis

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Evaluation:

Name and signature of the instructor:

Date:

Practical No (10)

Laboratory diagnosis of chronic myeloid leukemia (CML):

Complete blood count:

1. Hb: slight decreased
2. TWBCs: marked increased (50 – 300 * 10^9\L
3. Platelet count: increased

Peripheral blood picture:

RBCs: Normocytic normochromic

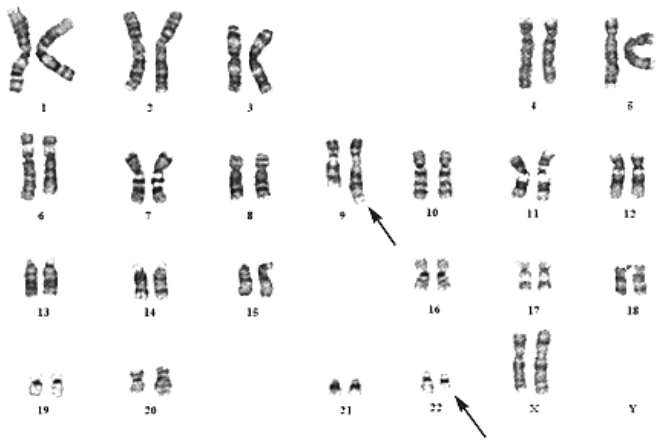
WBCs: full spectrum of myeloid stages (Blast, Promyelocytes, Myeloytes, Metamyelocytes) with mature cells.

PLT: Thrombocytosis

Bone marrow aspiration:

1. Hypercellular: (E:G ratio 1:10)
2. Myeloctic hyperplasia
3. Increased megakaryocytes
4. Variable fibrosis

1. Cytogenetic to determine the Philadelphia chromosome :



Lab. Question

You are provided with blood films labeled ( ) comment then suggest the diagnosis

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Evaluation:

Name and signature of the instructor:

Date:

Practical No (11)

Laboratory diagnosis CLL:

Complete blood count:

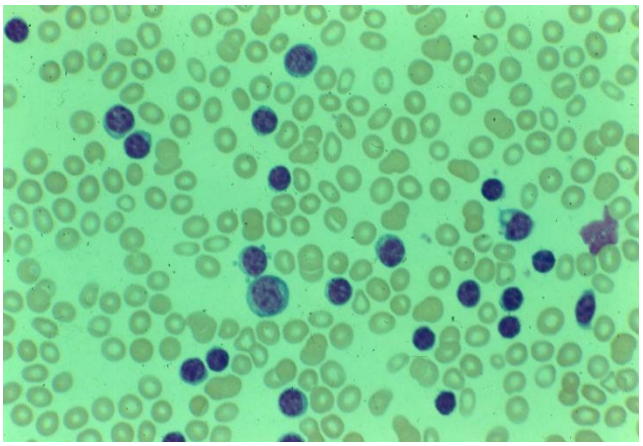
1. Hb, TRBCs, and PCV are low
2. Increased TWBCs and persistent lymphocytosis (have more than 10.000 lymphocytes\mm^3
3. Platelet: decreased

Peripheral blood film:

RBCs: Normocytic normochromic

WBCs: persistent lymphocytosis and smudge cells

PLT: Thrombocytopenia



Lab. Question

You are provided with blood films labeled ( )

2. comment then suggest the diagnosis
3. mention the further investigations to finalize the diagnosis

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Evaluation:

Name and signature of the instructor:

Date:

Tests for blood hamostaisis:

Tests of the Vascular Platelet Phase of Haemostasis:

Tests of the Coagulation Cascade:

Clotting Time (CT) or Coagulation time

Activated Partial Thromboplastin Time (APTT).

Prothrombin Time (PT).

Thrombin time (TT)

Tests of Fibrinolysis and the Mechanisms That Control Hemostasis:

Fibrin Degradation Products (FDP)

Practical No (12)

Bleeding Time (BT)

Aim

The bleeding time test is a useful tool to test for platelet plug formation and capillary integrity. Occasionally, the bleeding time test will be ordered on a patient scheduled for surgery.

Four procedures are currently in use for determining the bleeding time:

1. The Duke method.
2. The Ivy Method.
3. The Mielke Method.
4. The Simplate or Surgicutt Method.

Duke Method

Requirements:

1. Sterile lancet,
2. Cotton
3. rectified spirit
4. filter paper
5. Stop watch.

Procedure:

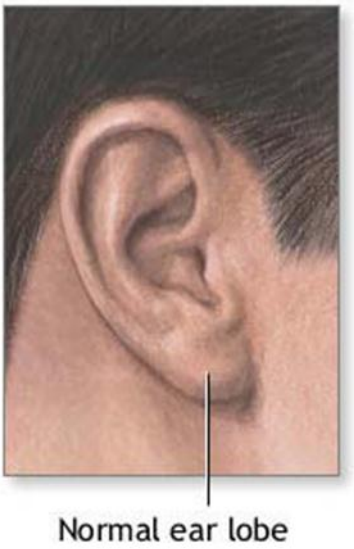
1. In Dukemethod, the patient is pricked with a special needle or lancet, on the earlobe, after having been swabbed with alcohol.
2. The prick is about 3–4 mm deep.
3. Then wipes the blood every 30 seconds with a filter paper.
4. The test ceases when bleeding ceases.
5. The test causes nervousness in the patient.

Normal range: The usual time is about 1–3 minutes.

Limitations

a. No repeat testing is allowed due to space.

b. This test method is the easiest to perform, but is the least standardized and has the less precision and accuracy.



Ivy Method

1. In the Ivy method, a blood pressure cuff is placed on the upper arm and inflated to 40 mmHg to control capillary tone and to improve the sensitivity and reproducibility– this will maintain constant pressure within the capillaries and help standardize the procedure- .

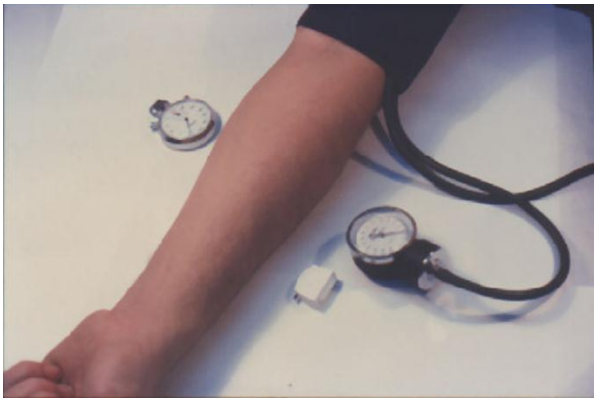
2. A sterile, disposable blood lancet is used to make a shallow incision that is 1 millimeter deep on the underside of the forearm.

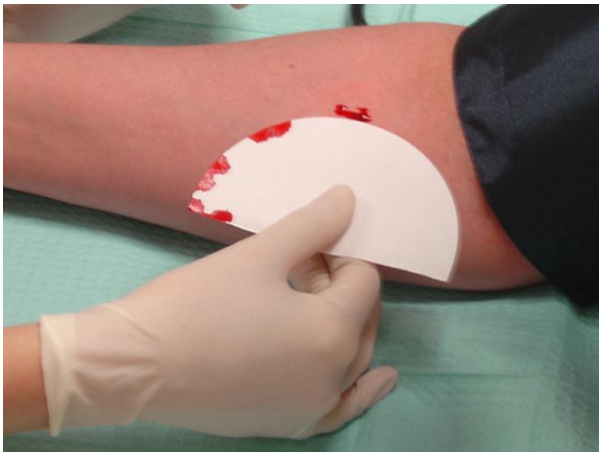
3. Every 30 seconds, filter paper is used to draw off the blood.

4. The time from when the incision is made until all bleeding has stopped is measured.

5. The test is finished when bleeding has stopped completely.

Normal value: 2 – 7 minutes.





Interpretation of bleeding time

- A prolonged bleeding time may be a result from decreased number of thrombocytes, abnormal platelet function or impaired blood vessels.
- The greatest source of variation in this test is largely due to difficulty in performing a standardized puncture. This usually leads to erroneously low results.

Bleeding Time Abnormalities:	
Collagen disorders	e.g. Ehlers Danlos syndrome
Thrombocytopaenia	A platelet count of <50 x 10/L is generally considered to prolong the BT.
Qualitative platelet disorders	• Inherited and acquired platelet disorder including the use of anti-platelet drugs such as aspirin and clopidogrel will prolong the BT. • Paraproteinaemias can also lead to defective platelet function and may, therefore, prolong the BT. • Other acquired disorders of platelet function such myelodysplastic syndromes (MDS) and myeloproliferative disorders (MPD) will also prolonged the BT.
Von Willebrand Disease (VWD)	A deficiency of Von Willebrand Factor (VWF) may prolong the BT but not in all cases.
Hypofibrinogenaemia	Fibrinogen is required for platelet-platelet interaction and the BT will, therefore, be prolonged in cases of hypofibrinogenaemia.

Practical No (13)

Clotting Time CT:

Aim :

Clotting time was used as a screening test to measure all stages in the intrinsic coagulation system and to monitor heparin therapy.

Principle:

Clotting Time is the time required for blood to form a clot in vitro.

It based on that whole blood will form a solid clot when exposed to a foreign surface such as a glass tube.

Methods:

1. Capillary Method.
2. Slide Method.
3. Tube Method

Tube Method (Lee-White method)

Reagent & equipment

1. Water bath, 37O C.
2. Glass test tube (10 x 75 mm)
3. Stopwatch.
4. Plastic syringe.

Specimen : 4 ml of fresh whole blood .

1. Label 3 glass test tubes with patient name and number them, 1, 2, and 3.

2. Perform a clean, Untraumatic venipucture using a 20-gauge needle and drawn 4 mL of blood.
3. Start the stopwatch as soon as the blood enters the syringe.
4. Remove the needle from the syringe, and fill each of the three tubes with 1 ml blood.
5. The last 1 ml of blood may be discarded.
6. Place the three test tubes in a 37°C water bath.
7. At exactly 3 min., Remove the first tube form water bath and tilt gently to a 45° angle to see whether the blood has clotted.
8. If Blood not clotted return it to the water bath and examine it at 30 second intervals.
9. After the blood in the first tube has clotted, examine the second tube immediately.
10. Then examine the 3rd one.
11. Record the time it took the blood in the 3rd test tube to clot.
12. Then one tube should remain in the 37°C water bath to be checked for clot retraction. Also, this same tube may be allowed to remain in the water bath overnight and checked the next day for clot lysis.

Normal Range:

5 – 10 Minutes

Interpretation:

Conditions accompanied by increased Clotting Time:

1. Factors V, VII, VIII, IX, XI, XII Deficiencies.
2. Hemorrhagic disease of Newborn
3. Vitamin K deficiency.
4. Heparin Therapy.
5. Presence of Circulating antibodies (inhibitors)
6. Afibinogenemia

Limitations:

7. Variations are wide and the test sensitivity is limited.
8. The test is the least effective test in the diagnosis of actual haemostasis failure; so it has been replaced by APTT.
9. Poor venipucture technique, causing hemolysis or tissue thromboplastin to mix with the blood, shortens the clotting time.
10. Bubbles entering the syringe when the blood sample is being obtained increase the rate of coagulation.

Lab. Question

Obtain the bleeding time by duke's method and clotting time for your colleague then comment.

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Evaluation:

Name and signature of the instructor:

Date:

Practical No (14)

Prothrombin time (PT):

Principle:

The PT test measures the clotting time of plasma in the presence of optimal concentration of tissue extract thromboplastin, and indicate the overall efficiency of the extrinsic and common clotting factors.

Reagents:

1. Patient and control plasma (platelet poor plasma)
2. Thromboplastin reagent with calcium (CaCl2 0.025 mol\L)

Preparation of platelet poor plasma (PPP):

1. Platelet rich plasma (PRP) is obtained by centrifuging blood at room temperature (20 C) for 10 – 15 min at 2000 rpm. The PRP is carefully removed without contamination with red cells or buffy coat;
2. Remover plasma is centrifuge at 2ooo rpm to obtain PPP

Method:

1. 0.1 ml of patient and control into a glass test tube placed in a water path
2. 0.2 ml of thromboplastin reagent with CaCl2 and start the stopwatch
3. Mix the content of the tube and record the end point

Expression of the result:

1. As the mean of the duplicate reading in second
2. As the ratio of the mean patients plasma time to the mean of normal control plasma time
3. As an international normalized ratio (INR)

Normal range:

11 – 16 secs.

Interprétation:

1. Oral anticoagulant
2. Liver diseases especially obstructive
3. Vitamine K deficiency
4. Disseminated intravascular coagulation (DIC)

Activated partial thromboplastin time (APTT) :

Also known as the partial thromboplastin time with kaolin (PTTK). And the kaolin cephalin clotting time (KCCT).

Principle:

APTT test measures the clotting time of plasma after the addition of the activation of contact factors but without added tissue thromboplastin. And indicate the overall efficiency of intrinsic pathway.

Reagents:

1. Platelet poor plasma (PPP)
2. Kaolin: 5g in barbitone buffer saline PH 7.4
3. Phospholipid
4. CaCL2 (0.025 mol\L)

Method:

1. Mix equal volume of the phospholipid reagent with kaolin suspension and leave it in the water path
2. 0.1 ml of patient and control PPP in glass test tube
3. Add 0.1 ml of phospholipid reagent with kaolin
4. Incubate 2 – 3 min at water path 37 C
5. Add 0.1 ml of CaCL2 reagent and start the stopwatch
6. Mix the content of the tube and record the end point

Normal range:

26 – 40 sec.

Interpretation:

1. DIC
2. Liver diseases
3. Hemophilia
4. Administration and contamination of heparin and other anticoagulant
5. Circulating antibodies and inhibitors

Lab. Question

You are provided with PPP sample labeled ( ) obtain the PT and APTT then comment

Results……

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Comment……

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Final diagnosis……

Evaluation:

Name and signature of the instructor:

Date:

Practical No (15)

Direct ABO and Rh D grouping

Aim

1. Essential for blood transfusion
2. Organ transplantation
3. In pregnancy to prevent HDN

Principle

A suspension of red cells of specimen is reacted with known reagent antisera anti A, anti B and anti D. agglutination indicates the present of corresponding antigen

Reagent

Antisera (anti A, anti B and anti D)

Method

- **Tube method**

1. Take 3 small test tubes and label them as A, B and D.
2. Add one drop of anti A in the tube marked A, one drop of anti B in tube marked B and one drop of anti D in tube marked D.
3. Then add one drop of the cell suspension to each tube
4. Examine for agglutination

- **Slide method**

1. Add three separate drops of capillary blood or red cell suspension on clean slide
2. Add anti A to the first drop , anti B to the second then anti D to the third one
3. Read the reaction

Anti A	Anti B	Anti D	Blood group
+	-	+	A+ve
+	-	-	A-ve
-	+	+	B+ve
-	+	-	B-ve
+	+	+	AB+ve
+	+	-	AB-ve
-	-	+	O+ve
-	-	-	O-ve

Source of errors:

1. Failure to use correct reagent
2. Used of expired reagent
3. Bacterial contamination
4. Failure to identify haemolysis as positive reaction
5. Clerical error

Practical No (16)

The Indirect ABO Blood Grouping

Theindirectbloodgrouping,alsocalledserumgroupingemploysredcellspossessingknowna ntigentoseethetypeofantibodies(antiA&-B)present,orabsenceoftheseantibodiesinserum.Itusuallyisperformedbytesttubemethoda lone.Slidereversegroupingisnotreliableasserumantibodiesagglutinatemostcell when centrifuged, and use of test tube enhances the agglutinated reaction.

Indirect grouping method

1. Take two tubes, label one tube A-Cells ’and ‘ B cells’
2. Put one drop of the serum to be tested each tube.
3. Addonedropof2-5%Acellstothetubelabeled‘Acells’andonedropof2-5%Bcellstothetubelabeled‘Bcells’.
4. Mix the contents of the tubes.
5. LeavethetubesatRTfor5-minutes.Centrifuge30seconds.
6. Looking for agglutination & Interpret result

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5
Cellss uspension	1drop	1drop	-	-	-
serum	-	-	2 drops	2 drops	2 drops
Antisera	anti-A	anti-B	-	-	-
Cells	-	-	A Cells	B cells	O cells

Results:

Positivereaction……..agglutination.

Blood group	Tube1 (Anti-A)	Tube 2 (Anti-B)	Tube3 (A-cells)	Tube4 (B-cells)
A	+ve	-ve	-ve	+ve
B	-ve	+ve	+ve	-ve
AB	+ve	+ve	-ve	-ve
O	-ve	-ve	+ve	+ve

Lab. Question

You are provided with patient red cell suspension labeled (x) and (Y) , patient serum labeled (A)and (B)obtain the direct and indirect blood grouping

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Comment……
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Evaluation:

Name and signature of the instructor:

Date:

Practical No (17)

Test for Weak D(D^u)

Principle: Some red cells express the D antigen so weakly that most anti-Dreagentsdonotdirectlyagglutinatethecells.WeakDexpressioncan be recognized by anindirectantiglobulin(IAT)procedureafterincubationofthetestredblood cells with anti-D.

Reagents:

Reagent anti-D, Anti-humanglobulin (coomb's reagent).

Procedure:

1. If the original, direct test with anti-D was performed by tube testing ,the same tube may be used for the weak D test.
2. After recording the original anti-D tube test is negative ,Mix and incubate the tube15to30minutes at 37°C.
3. Wash the cells three times with normal saline :fill the tube to 2/3by N.S., mix gentlyandcentrifugefor1 min ,discard the supernatant saline and repeat the wash for three times at least.
4. Add one drop of antihumanglobulin reagent.
5. Mix gently ,centrifuge, and suspend the cell button ,examine the tubes for agglutination.

Interpretation:

Absence of agglutination in the tube with anti-Dis a negative result ,indicating that the cells do not express Dan should be classified as Rh-D-ve.

Lab. Question

You are provided with patient red cell suspension labeled (x) and (Y) obtain the Rh D grouping

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Evaluation:

Name and signature of the instructor:

Date:

Practical No (18)

Direct &Indirect Anti-human globulin test

"Coombs´test"

- There are two major types of blood group antibodies ;IgM & IgG.
- IgMhavealargepentamerstructuresobindtothecorrespondingantigenandd irectlyagglutinateRBCssuspendedinsaline.
- IgGantibodieshaveamonomerstructure,socannotagglutinateRBCsdirectly.
- TheadditionoAHGreagent(containanti-IgGtoRBCssensitizedwithIgGantibodiesallowsforagglutinationforthes esensitizedcells.

Principle:

Antihumanglobulins(AHGs)obtainedfromimmunizednonhumanspeciesbindt ohumanglobulinseitherfreeinserumorattachedtoantigensonRBCs.

Preparation of AHG:

- Humanserumisinjectedtoalaboratoryanimalsuchasrabbits.Thehumanglob ulinbehavesasforeignantigen.Therabbit'simmunesystemistriggered so antibodies to human globulin are produced.

DirectAHGtest(DAT) :

- DAT detect in –vivo sensitization of RBCs.
- Clinical conditions that can result In- vivo coating of RBCs are:

1. Haemolytic disease of the new born(HDN):maternal Ab coating fetal RBCs.
2. Haemolytic transfusion reaction(HTR):

recpientAbcoatingdonorRBCs.

3. Autoimmunehaemolyticanaemia:autoAbcoatingindividual´sRBCs.

MethodofDAT

1. Intoatesttube(12x75),add1dropof2-3%suspensionofthetestRBCs.
2. Washthecellsthreetimeswithsaline(ensurethatallsalineiscompletelydecantedafterthelastwash).
3. Add2dropsofAHGreagent.
4. centrifuge,resuspendthecells,andreadthe result.

IndirectAntiglobulinTest(IAT)

- IATdetectin-vitrosensitizationofRBCs.
- IATusedinthefollowingsituations:

1. RBCphenotype,e.g.weakD(D**u**method).
2. Antibodiesscreening,identification,andtitration.

Method of IAT

1. Intoatesttube(12x75),add2dropsofthetestserumand1dropof2-3%suspensionofOscreeningRBCs.
2. Mix,andincubatefor30minsin37˚C.
3. Washthecellsthreetimeswithsaline(ensurethatallsalineiscompletelydecantedafterthelastwash).
4. Add2dropsofAHGreagent.
5. Centrifuge,resuspendthecells,andreadtheresult.

Lab. Question

You are provided with patient1 red cell suspension labeled (x) and patient2 serum (Y)

1. obtain the DAT for PT1 and IAT for PT2
2. Mention the possible causes of such conditions

Results……

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Comment……

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Evaluation:

Name and signature of the instructor:

Date: