بسم الله الرحمن الرحيم

كليـــــة نبتـــــه
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Medical Laboratory Sciences Program

Department of Haematology and Immune haematology

Student Practical Log Book

Semester Four

2018-2019

Name: ……

University ID No.:……

Batch: ……

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Instructions

- Wear gloves when in contact with body fluids, such as serum, plasma, urine or whole blood.
- Wash your hands when gloves are removed or changed.
- Perform procedures carefully to minimize aerosol formation.
- Wear protective clothing such as laboratory coats or aprons when working with specimens.
- Keep your hands away from your face.
- Cover all superficial cuts before starting any work.
- Dispose of specimens and other contaminated materials according to your laboratory's biohazard control procedure
- Keep your work area disinfected, disinfect tools and other items that have been in any contaminated area.
- Do not eat or drink or apply cosmetics while in the laboratory.

Content

ABO grouping

Aim: It is one of the most important tests used in blood transfusion.

Principle: An antigen-antibody reaction, in which known antibodies are used to detect unknown antigens on RBCs surface, and known cells (antigens) are used to detect unknown antibodies in serum or other body fluids.

Reagents:

Commerial anti-A, B, and AB.

Known A, B, and O cells.

Normal saline.

Equipment : glass tubes (12x75mm), Pasteur pipettes, slides, water bath, racks and microscopes.

Sample: venous or capillary blood. May use EDTA blood or clotted sample.

Sample preparation:

Sample should first be separated to cells and serum.

Serum is ready to use.

Cells should be washed at least 3 times by N.S. and then suspended before use

Washing and suspending:

In a test tube take 3-5 drops of the packed cells, fill the tube to 2/3 by N.S., mix gently and centrifuge for 1 min, discard the supernatant saline and repeat the wash for three times at least.

To make a suspension, add 5-8 drops of saline to the washed cells to make 40-50% suspension for tube method.

The Direct ABO Blood Grouping

The direct blood grouping also called cell grouping employs known reagent anti sera to identify the antigen present or their absence on an individual's red cell.It can be performed by the slide or test tube method.

Methods:

Slide method (using suspended cells only):

1. Add 3 separate drops of 30-40% cell suspension to a clean slide.
2. Add to the first drop 1 drop of ant-A.
3. Add to the second drop 1 drop of anti-B.
4. Mix gently each alone and read after two minutes

Red cells tested with:

Anti-A	**Anti-B**	**Interpretation**
+ve	-ve	**A**
-ve	+ve	**B**
+ve	+ve	**AB**
-ve	-ve	**O**

Tube method:

1. Take two tubes, label one tube ‘anti- A’ and the second ‘ anti -B’
2. Add one drop of anti- A to the tube labeled ‘anti-A’
3. and one drop of anti- B to the tube labeled anti- B’
4. Put one drop of the 2-5% cell suspension to both tubes
5. Mix the antiserum and cells
6. Leave the tubes at RT for 5- minutes. Centrifuge for 30 seconds
7. Read the results & Interpret.

Rhesus (D) grouping

Aim: To detect the presence of Rhesus – D antigen on the red cell surface, which is the must immunogenic after A & B antigens.

Rh (D) grouping is usually performed at the same time as ABO grouping to minimize errors that may arise through repeated handling of patients sample.

A person is grouped as Rhesus (Rh) positive or negative based on the presence or absence of antigen D:

Rh positive: a person who inherits gene D and the red cell express antigen D.

Rh negative: a person who does not inherit gene D and the red cells do not express antigen

For transfusion purpose....

Rh positive blood can be given to Rh positive individuals.

Rh negative blood can be given to both Rh + & Rh- individuals.

Methods

Slides and tube methods may be used as for ABO grouping.

Reagent is anti-D (commercially available).

Sample: venous or capillary blood. May use EDTA blood

Slide method:

1. Add 3 separate drops of 2-5% cell suspension to a clean slide.
2. Add to the first drop 1 drop of anti-A.
3. Add to the second drop 1 drop of anti-B.
4. Add to the third drop 1 drop of anti-D.
5. Mix gently each alone and read after two minutes.

Tube method:

- Place 1 drop of anti-D in a clean, labeled test tube.
- Add 1 drop of a 2-5% suspension in saline of the red cells to be tested.
- Mix gently and centrifuge for the time and at the speed specified by the manufacturer.
- Gently resuspend the cell button and examine it for agglutination.

Interpretation:

Agglutination in the anti-D tube, indicates that the red blood cells under investigation are RhD+ve.

Student's findings (measurements or observations)

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Tube method (using both cells and serum)

It is essential to confirm the result of the red cell grouping by examining the patient serum for the corresponding antibodies (reverse grouping).

Any discrepancy between the results of red cell grouping and the reverse grouping should be investigated further.

The Indirect ABO Blood Grouping

The indirect blood grouping, also called serum grouping employs red cells possessing known antigen to see the type of antibodies (anti A & -B) present, or absence of these antibodies in serum.

It usually is performed by test tube method alone.

Slide reverse grouping is not reliable as serum antibodies agglutinate most cell when centrifuged, and use of test tube enhances the agglutinated reaction.

Indirect grouping method

1. Take two tubes, label one tube A- Cells' and the second 'B cells'
2. Put one drop of the serum to be tested each tube.
3. Add one drop of 2-5% A cells to the tube labeled 'A cells' and one drop of 2-5% B cells to the tube labeled 'B cells'.
4. Mix the contents of the tubes.
5. Leave the tubes at RT for 5- minutes. Centrifuge 30 seconds.
6. looking for agglutination & Interpret result

	Tube 1	**Tube 2**	**Tube 3**
serum	2 drops	2 drops	2 drops
Cells	A cells	B cells	O Cells

Results:

Positive reaction……..agglutination.

Negative reaction……no agglutination.

Blood group	Tube1 (A-cells)	Tube2 (B-cells)
A	-ve	+ve
B	+ve	-ve
O	+ve	+ve

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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Date: ……..\.....\.....

Direct & Indirect Antihuman globulin test

“Coombs´ test”

- There are two major types of blood group antibodies; IgM & IgG.
- IgM have a large pentamer structure so bind to the corresponding antigen and directly agglutinate RBCs suspended in saline.
- IgG antibodies have a monomer structure, so cannot agglutinate RBCs directly.
- The addition of AHG reagent (contain anti-IgG) to RBCs sensitized with IgG antibodies allows for agglutination for these sensitized cells.

Principle:

Antihuman globulins (AHGs) obtained from immunized nonhuman species bind to human globulins either free in serum or attached to antigens on RBCs.

Preparation of AHG:

- Human serum is injected to a laboratory animal such as rabbits. The human globulin behaves as foreign antigen. The rabbit's immune system is triggered so antibodies to human globulin are produced.

Direct AHG test (DAT)

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

1. Haemolytic disease of the newborn (HDN): maternal Ab coating fetal RBCs.
2. Haemolytic transfusion reaction (HTR): Recipient Ab coating donor RBCs.
3. Autoimmune haemolytic anaemia: auto Ab coating individual´s RBCs.

Method of DAT

1. Into a test tube (12x75), add 1 drop of 2- 3% suspension of the test RBCs.
2. Wash the cells three times with saline (ensure that all saline is completely decanted after the last wash).
3. Add 2drops of AHG reagent.
4. centrifuge, resuspended the cells, and read the result.

Indirect Antiglobulin Test (IAT)

- IAT detect in-vitro sensitization of RBCs.
- IAT used in the following situations:

1. RBC phenotype, e.g. weak D (D^{u} method).
2. Antibodies screening, identification, and titration.

Method of IAT

1. Into a test tube (12x75), add 2 drops of the test serum and 1 drop of 2-3% suspension of Screening RBCs.
2. Mix, and incubate for 30mins in 37˚C.
3. Wash the cells three times with saline (ensure that all saline is completely decanted after the last wash).
4. Add 2drops of AHG reagent.
5. Centrifuge, resuspended the cells, and read the result.

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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Date: ……..\.....\.....

Test for Weak D (D^u)

Principle: Some red cells express the D antigen so weakly that most anti-D reagents do not directly agglutinate the cells. Weak D expression can be recognized by an indirect antiglobulin (IAT) procedure after incubation of the test red blood cells with anti-D.

Reagents:

Reagent anti-D, Antihuman globulin (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 tube15 to 30 minutes at 37°C.
3. Wash the cells three times with normal saline: fill the tube to 2/3 by N.S., mix gently and centrifuge for 1 min, discard the supernatant saline and repeat the wash for three times at least.
4. Add one drop of antihuman globulin reagent.
5. Mix gently, centrifuge, and resuspended the cell button, examine the tubes for agglutination.

Interpretation:

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

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Date: ……..\.....\.....

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 Template or Surgicutt Method.

Duke Method

Requirements:

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

Procedure:

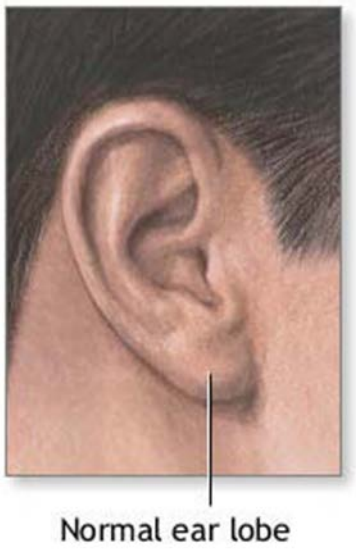
1. In Duke Method, the patient is pricked with a special needle or lancet, on the earlobe, after having been swabbed with alcohol.
2. The prick is about 3–4 mm deep.
3. Then wipes the blood every 30 seconds with a filter paper.
4. The test ceases when bleeding ceases.
5. The test causes nervousness in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

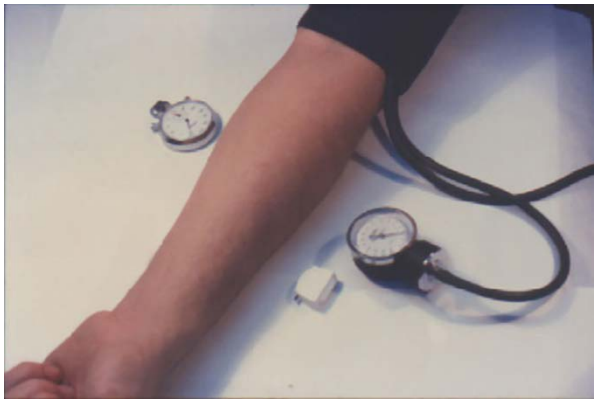
2. A sterile, disposable blood lancet is used to make a shallow incision that is 1 millimeter deep on the underside of the forearm.

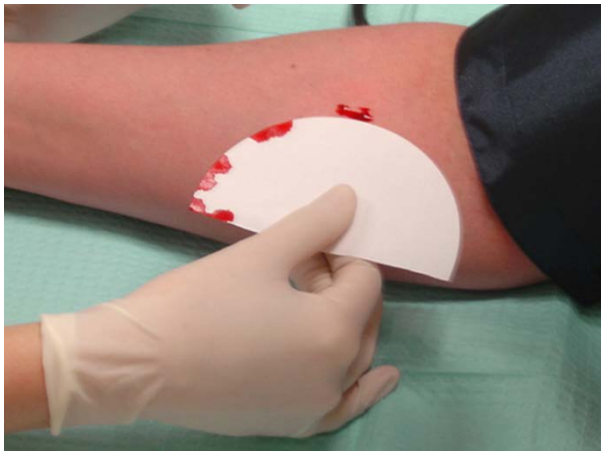
3. Every 30 seconds, filter paper is used to draw off the blood.

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.

leeding 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	ibrinogen is required for platelet-platelet interaction and the BT will, therefore, be prolonged in cases of hypofibrinogenaemia.

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, 37^{O} 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:

1. Variations are wide and the test sensitivity is limited.
2. The test is the least effective test in the diagnosis of actual haemostasis failure; so it has been replaced by APTT.
3. Poor venipuncture technique, causing hemolysis or tissue thromboplastin to mix with the blood, shortens the clotting time.

Bubbles entering the syringe when the blood sample is being obtained increase the rate of coagulation.

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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