MLS PROCEDURE

Haematology
- Immuno-Haematology or Blood Group Serology
- Preparation of standard cells for Blood Group Serology
- Blood Grouping

BLOOD GROUPING

We have two types of blood grouping
1.) Cell Grouping
2.) Serum Grouping

Principle of Blood Grouping:
The antigen ~~(antibody)~~ and the antibody when mixed in the ~~right~~ environment, temperature & ratio and incubated for a recommended period (room temp for ABO) under proper chemical environment (ionic ~~strength~~ & pH) will exhibit a proper reaction within the frame work of law of mass action.

Positive reaction is observed or denoted by
1.) Agglutination — Also called Transfusion Reaction
2.) Haemolysis

→ In agglutination, we see clumping (cells coming together)
→ In haemolysis we see that the liquid is just colored (red). Normally this means rupture of the cell membrane.

Sample	Anti A	B	AB	O
1	+	−	+	−
2	−	+	−	+
3	−	−	+	+

} This is the scale for cell grouping

And negative reaction is observed or denoted by absence of neither agglutination or Haemolysis.

1.) CELL GROUPING : In this, You are given;

(a) Known Antisera - e.g (Anti A, B, AB & D. If Rhesus; Anti-D(d), Anti-D)

(b) Cell is Unknown - e.g A, B, AB & O

Whole Blood

Sample	Anti A	B	AB	O (H)	Result
1	+	−	+	−	A
2	−	−	−	+	O
3	−	+	+	−	B
4	+	+	+	−	AB

} Cell Grouping

*learn

2.) SERUM GROUPING : In this, we use;

(a) Known cells i.e A, B, AB, O & D^+ or −

(b) Unknown Antisera (serum)

Anti-serum

Sample	A	B	AB	O	Result
1	+	−	−	+	Group B
2	−	+	−	+	A
3	−	−	−	−	AB
4	+	+	+	−	O

} Serum Grouping

NOTE → Blood grouping is a process of determining ones blood group.

When rinsing a pipette, Rinse 8-times.

16/10/21

NOTE ⇒ The Serum grouping is also called "Reverse Grouping"

* Methods of Grouping
1) Tile/Slide grouping
2) Tube method of grouping
3) Gel ~ ~ ~
4) Microplate ~ ~ ~

1.) TILE/SLIDE METHOD OF GROUPING

the grouping tile has Holes

—Procedure:

(a) Use opaque tile or slide
(b) Make a 2% cell suspension of test sample
(c) i.) Put one volume (a drop) of antisera (Anti A, B and AB) each on the tile/slide with the help of pasteur pipette.

ii.) In a second roll put one drop of A cell, B cell and C cell. With the aid of a pipette. Wash pipette after taking each sample to prevent carryover effect. When rinsing a pipette, rinse 8 (eight) times.

iii) Put one drop of 2% cell suspension of test sample in the first roll nearer to antisera A, B & AB with pipette, wash pipette each time before re-use. Wash with distilled water first and then normal saline.

SOP ⟷ Standard operating Procedure

iv.) Put 1 (one) drop of serum from first sample in the second roll nearer to the cell (A, B, O cell). Wash pipette each time before re-use. Wash with distilled wash and then with normal saline.

* v.) Mix the anti sera with cell suspension in the first roll, using different spreader (stick) for each mixing & spread this mixture on your prefered slide.
Take due precaution/Procedure such as no over-lapping or mixing of different samples.

vi.) Same procedure has to be followed in the second roll.

vii.) Rock & rotate the tile and leave the ~~test~~ on the table atleast 2mins for reaction to take place at room temperature. Then rock again and look for reaction

viii.) Record interpretation of result immediately.

Antigen is found in the blood
Antibody is found in the serum

2) TUBE TECHNIQUE/METHOD OF GROUPING

The tube technique can be done in two methods;

a) Spine-tube Method

b) Sedimentation

PROCEDURE — (a) Spin-tube Method

(1) Un-Known test sample collected/received should be centrifuged to separate the cell and serum separate the cells, wash it with normal saline at least 3-time

(2) 2-5% saline suspension of red cell are made with normal saline (N10 Sodium Chloride).

(3) Pooled A, B and O cells are to be prepared not older than 6 hours

(4) Anti sera are to be serially arranged

(5) Tubes are labelled properly and put into racks.

Tubes meant for cell grouping should have separate label mark & put into holes. It should be labelled separately and Separated from tubes meant for serum grouping.

(6) Prepare Papain 16 solution

(7) For individual case, arrange in a horizontal roll of the rack 5-tubes for cell grouping and 4-tubes for serum grouping.

(8) In the tubes, marked for cell groupings, add anti-sera (Anti A, B, AB, A_1 & H)

one drop each.

(9) In the tubes marked for serum, add a drop of the pooled A cells, B cells, AB cells, O cells/papain.

(10) Add a drop of the tube to 5% of the cell suspension. Do the same for serum grouping.

(11) Spin the tubes likely for 30 secs and examine for reaction.

(12) Leave your tubes for about 5-10 mins on the bench at room temperature for incubation

(13) Spin/centrifuge at 1500 RMP.

(14) Examine for reaction, agglutination or haemolysis.

(b) For Sedimentation Method

— PROCEDURES

(1) All tubes are taped gently after adding the anti-sera, etc.

(2) Incubate at room temperature for about 1 hour or 1 and half hour.

(3) Observe result (agglutination, haemolysis & Negative reaction i.e No agglutination).

① ② ③

3) MICROPLATE Technique

This is a recent introduction and advancement in blood grouping procedure particularly in respect of ABO and rhesus grouping. And it is very useful in places where lab. scale work is done in laboratory. Antiglobulin test has been performed satisfactorily in some laboratory.

The Microplate are injection molded polystirene plate approximately 12X8 cm in size containing 96 wells arranged in 8 rolls from Alphabet A to H vertically, X12 horizontally arranged wells. The wells may be shaped U or V. Since electrostatic property vary with different brand of plates, preferrably one brand of plate maybe chosen for you.

The electrostatic effect may be eliminated by the addition of Bufine albumin or twin-20. Plates maybe rinsed and wetted in twin 20 shaking and dried overnight at 37% before use.

PROCEDURE:

Diluent / Material required for use

1. 0.02 albumin in saline

(2) Test samples to be collected in EDTA to avoid ABO haemolysing. Red cells used for serum grouping also are best diluted EDTA, microlitre plates used can be employed in;

(a) Saline Method
(b) Enzyme Method
(c) Coombs (Direct & Indirect Coombs); Precisely, Indirect Coombs.

COOMBs is also known as ANTIGLOBULINS or ANTI-ANTI BODY. However, Commonest method in use is Saline Method.

PROCEDURE

(1) Decide whether U or V plate are to be used.
(2) Select plates with low electrostatic property.
(3) Red cells preferably from EDTA sample are washed in 0.02% albumin Saline solution
(4) Resuspend the cell in either 0.02% albumin saline solution or LIS (Low Isotonic solution) to make 2-5% suspension.
(5) for serum grouping, the red cells are diluted with EDTA or Saline (0.02 albumin saline)
(6) For ABO Rhesus grouping in U plates, 2-5%

• EDTA - Ethylene Diamethyl Tetra-acetic Acid

suspension is acceptable But in V plates, diluted cell suspension of 0.1% to 0.2% are used

(7) Shake for half a minute i.e 30 seconds.

(8) Incubate for 30 minutes to 2 hours at room temperature

(9) After incubation, centrifuge the plate on plate centrifuge at 100grams for 40seconds and examine for agglutination either after agitation or tilting or bending the plate

In former, automatic reader is employed which is fitted with microcomputers and gives out printed results & stores it. Reading is done at 570nm wavelength.

In Later, the whole plate is tilted at an angle of 60 - 70° and kept in same position until the negative reaction begins to treal but the positive result remains in descrete button to the bottom of the well. Magnifying hand-lens may be used to interprete the result

23/11/21

Grouping of Infant Blood

There is the basic difference in grouping of adult and infant blood;

1.) The ABO antigen are not fully developed at birth.

2.) Antibody are usually absent at birth until a certain age, only dell grouping is done.

The antigen A and B can be detected Conj before birth, but at birth to distinguish the subgroups is difficult at birth. eg A can be A_1, A_2, A_3, Ax ~~if~~ Cause plant lectins for A_1 is better reagent for this purpose. The strength of A_1 antigen rises gradually and picks at 3 years.

Similarly B antigen also gradually rises and becomes steady by 3 years. And H antigen is also expressed on fetal red cell but not as strong as A,B antigen but at birth it is fully well developed.

Fully developed antigen in new born are;

1.) Rhesus
2.) K
3.) Fy
4.) JK
5.) MNS
6.) DI
7.) DO, etc

ANTIBODY

In most infants the anti A and B are ... of IGM type

I means Concentration of antibody
and can first be detected from 3-6 months.
At 3 months, the antibody level is only 25% high as compared in adults. It gradually increase at 6 month. and becomes steady at 6-10 years.
At 3months, the median anti A titre is 1/4 (1 in 4).
At 6-9months, it increases to 1/16 (1 in 16) and reaches maximum at around 8yrs.
So the antibody of the IGM type is not significantly higher So most of the antibodies detected at the cord(placenta) is IGG and probably from the maternal origin.

A

Technology for Gel Method of Blood Grouping
Gel Technology for blood grouping:
A recent advancement in blood grouping procedures where ready-made monoclonars cell of anti A, B and D are provided in Gel-matrix within the micro-tubules
These monoclonars cells are provided in Gel-matrix contained within microtubules. Control micro-tubes is for negative control and there are two other micro-tubule that contain neutral gel. They are meant for reverse grouping with A1 cells & B cells.

Challenges/Problems that Arise While Doing A, B, O Blood Group

There are about 3 challenges/Problems that arise while doing the ABO blood group;

1) Test Sample - From the Test Sample
2) Technical Competence Problem
3) Human Error

1. Test Sample ⇒ It consists of two important component (i.e the patient's sample);
   1. Red Cells
   2. Serum (Plasma)

1) Red Cells ⇒ Red cell may give rise to what is called "Rouleaux Formation" due to weakening or loss of antigen, poly-agglutination, Erythrocyte coated with antibody in vivo (inside the human body), In vivo contamination of red samples, Chimera or in condition/situation whereby more than one cell population is found.

Problems of Serum

Problem with serum is mainly antibody related e.g
① Unexpected antibody Loss or Absence
② Auto-antibody

PUO → Pyresia of Unknown Origin

(3) Antibody with Haemolysin property,
(4) Serum may also give rise to problem if there is change in biochemical nature

(2) Technical Problem ⇒ this may arise due to the material (equipment, Glassware & Reagent.

(3) Human Error ⇒ this is as a result of incorrect labelling and incorrect interpretation of results

* How to Solve these Problems of Errors Encountered in ABO Blood Group

1) Check the Label of the sample for its correctness,
2) whether it is contaminated or not.
3) How old is the sample
2) Check the Reagents (antisera, pooled RBC), Reject if the sample is not stored properly.
3) Check the Centrifuge, the thermostatic equipment & Glass-wares must be clean.
4) Follow the SOP (Standard Operating Principle)
Repeat test procedure
Patient's disease history is required

Solving Problems

1) Antigen in the Red Cells – If weak or absent (the agglutination is weak or absent) in cell grouping, the serum grouping may show be exhibited by acquired antigen (B antigen or T, Tn, Tk or activation by Tb).

PREPARATION OF STANDARD BLOOD CELLS FOR GROUPING

Saline

Isotonic saline — 0·85% is used

Normal saline — 0·95% is used or 9·5g/L or 0·95g/100(dl)

Suspension of washed red cells having known & unknown antigen on their surface are required for various tests in immunohaematology test.

The washing of cells is necessary to remove plasma which may interfere with the reaction of various cells.

Antibody ← Plasma Serum

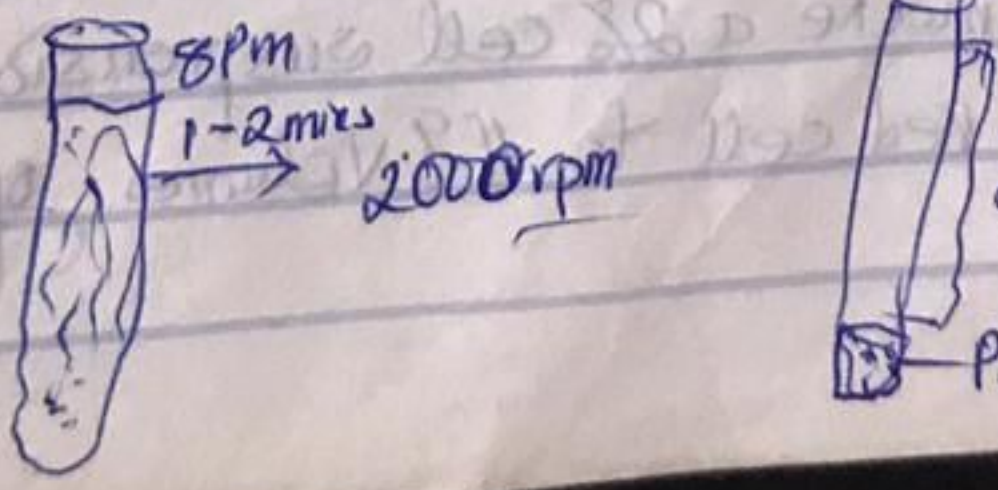
ABO Blood group system; — Group A, B, AB & O
We have more than 250 blood group system; two are;
① ABO
② Rhesus, etc.

In addition, blood group substance in plasma may neutralize the anti-serum (antibody), leading to a false-Positive result. It is also necessary to use red cell suspension of appropriate strength for optimum reaction with antibody molecules.

The red cells are suspended in an isotonic solution of sodium chloride (0.85% of Isotonic saline or 8.5g/L or 0.15mol 0.15 mole/Litre), ph adjusted to 7 (Buffer Saline is used to avoid the ph from fluctuating).

Techniques of Preparing the Standard Blood Cells

1) Place 0.2 – 0.5mL of blood in a test-tube
2) Fill the tube with saline (3quarter full).
3) Centrifuge or spin at 2000 Revolution (rpm) for 1–2 minute to obtain packed-Red-cells. If is done 3 time to get an ideal washing. ×3



4.) Remove the supernatant by pouring off the saline in one quick continous movement. OR By using a pasteur pipette.

5.) Tap the tube to re-suspend the cells in the residual fluid.

6) Repeat the procedure with the same cell at least trice (3x)

(ii) The last wash should show clear supernatant with no sign of haemolysis.

① 2% → 1vol + 49vol
1ml 49ml → 1+49 = 50

50 × 2 = 100

In 100ml we have 2% of RBC + 98% of Normal saline (NaCl)

② 3% ⇒ 3Vol + 97Vol = 100

③ 5% ⇒ Same way 5 + 95

④ 10% ⇒ 10 — 90 ⑤ 20% ⇒ 20 — 80 — etc

7.) @ To make a 2% cell suspension, add 1 vol of packed red cell to 49 volumes of saline, then mix properly.

To make 5% cell suspension, add 1vol. of the packed cell to 19vol. of your saline.

5% ⇒ 1vol + 19vol = 20

$\frac{1}{20} \times 100 = 5\%$

To make a 10% cell suspension, add 1 Vol. of packed cells to 9 Vol. of saline.

10% ⇒ 1 Vol (Blood) + 9 Vol (saline) = 10

OR

1*10vol + 9 × 10 vol

= 10 + 90 = 100

$\frac{1}{10} \times 100 = 10\%$

$\frac{1}{100} \times 100 = 100\%$

20% ⇒ 1vol + 4 Vol = 5

$\frac{1}{5} \times 100 = 20\%$

Comr Uzom Solomon