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B GROUPING AND SUBGROUPING

Three manual methods can be used when performing blood grouping:

- Glass slide or white porcelain tile
- Glass test tube
- Microwell plate or microplate

Newer techniques
- Column technique (sephadex gel)
- Solid phase tests

1) Slide or Tile Method

This technique may be used for emergency ABO grouping tests or for preliminary grouping particularly in an outdoor camp.
Slide or tile testing is not recommended for routine use because it is not reliable for

- weakly reactive antigens on cells
- serum grouping with low titre anti-A or anti-B

Disadvantages

- Less sensitive than the tube test
- Drying up of the reaction mixture can cause aggregation of cells , giving false positive results
- Weaker reactions are difficult to interpret.

2) Microplate Technique

Microwell plate consists of a small tray with 96 small wells each of which can hold about 200-300u1 of reagent. Microplate technology is gaining widespread popularity due to increasing workload in blood transfusion laboratories and recent availability of packaged automated system.

Three types of microplates are available
a. U-type well
b. V-type well
c. Flat-bottom

The U-type well is generally used in red cell serological work as it is easier to read the results in U- bottom plates.

grouping

Advantages of Microplate ABO grouping

1. Small volumes and low concentration of sera and red cells are used, making it cost-effective.
2. Easy handling of a microplate, which can replace 96 test tubes.
3. Batching of samples can be achieved with considerable economy in space and time.
4. If larger laboratories acquire microplate hardware items e.g. reagent dispenser, sample handler and cell washer it may further reduce the operation time.
5. Large batches of plates can be predispensed with antisera and reagent red cells before testing.
6. The technique of microplate grouping may be automated by on-line data capture in larger laboratories, which may help in
   a) reduction in reading and transcription errors
   b) saving in staff time
   c) use of bar codes for samples and microplate identification
   d) integration into a comprehensive computer system for storage of data.

3) Tube Method

Test tubes either of glass or plastic may be used. The tube technique is more sensitive than slide technique for ABO grouping.

Advantages of tube method

- It allows for fairly long incubation without drying up of the tubes contents.
- Centrifugation involved enhances the reaction allowing weaker antigens and antibodies to be detected.
- Simplicity of reading and grading of results.
- Clean and more hygienic.
- Requires smaller volume of reagents
- More sensitive than slide technique

GROUPING BY TUBE METHOD

Samples

CPDA anti coagulated Blood samples collected from any source.

Reagents required for tube method

- Working standardised Monoclonal antisera (Anti-A, Anti-B, Anti-A,B)
- Anti – A_1 (Lectin) & Anti - H (Lectin)
- Reagent cells (A cells, B cells and O cells), 3% in Normal Saline.
- Test red cells – Samples from IRCS
- Normal Saline (0.9%)

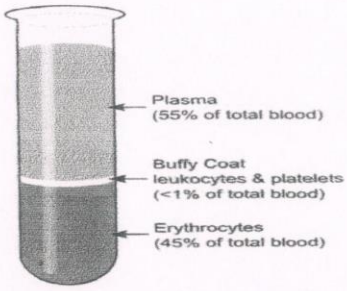
Bench Preparation (NIB/BRL/SOP/44/R1)

- Allow all reagents to come to Room Temperature
- Identify reagents RBC / blood samples to be used for reverse grouping
- Fill in proforma

Processing of blood samples

Separation of RBC & Plasma

Centrifuge at 1000 rpm for 1 min at R.T using clean pipette tip, aspirate plasma gently without disturbing settled cells and transfer to a labeled clean test tube for **reverse grouping**.



Preparation of cell Suspension (NIB/BRL/SOP/36,40,41/R1)

- Label the tubes as per S.No. of samples
- Add 1ml whole blood in respective S.No. of tubes and Normal saline (N.S) 8 ml, mix well.
- Centrifuge at 2500 rpm for 3 min at R.T
- Aspirate supernatant & discard
- Wash 3 times as above till supernatant is clear.
- Consider cell pellet as 100%
- Prepare 3%red cells suspension in normal saline

To prepare 100ul of required %suspension, mix N.S & packed RBC as below;

Preparation of % RBC suspension

% of cells	Vol. of N. S (µl)	Vol. of Washed, packed RBC (µl)
1%	99	1
2%	98	2
3%	97	3
5%	95	5
40%	60	40

Setting up tubes

Set 9 tubes for each test sample as follows;

- 3 tubes labelled - A, B, A B. (forward/cell grouping)
- 2 tubes labelled - H & A_1
- 3 tubes labelled Ac, Bc and Oc (reverse/serum grouping)
- 1 Auto control tube, Add 100ul test serum and 50ul test cells suspension of same sample and label it.

I <u>Forward grouping (cell grouping)</u>

- Add 100ul each of Anti-A, Anti-B, Anti-AB, Anti-A_1,and Anti-H in respective labelled tube.
- Add 100ul of 3% test cell suspension in the five tubes labelled A, B, AB, A_1 & H.

II <u>Reverse grouping (serum grouping)</u>

- Add 100ul each of the test serum in tubes labelled Ac, Bc and Oc.
- Add 50ul each of reagent A cells, B cells and O cells in the above tubes respectively.
- Mix the contents of all the 9 tubes by shaking the tube rack carefully and centrifuge at 1000 rpm for 1 minute.
- Dislodge cell button by gently shaking the tubes and read against well-lit background
- Grade and record agglutination reactions.

grouping

INTERPRETATION OF RESULTS

Grouping

Cell grouping			Serum grouping			Result
Anti-A	Anti-B	Anti-AB	Ac	Bc	Oc	
+	-	+	-	+	-	A
-	+	+	+	-	-	B
-	-	-	+	+	-	O
+	+	+	-	-	-	AB
-	-	-	+	+	+	Oh or any other irregular antibody

III Sub-grouping

Group	A_1 (Lectin)		Anti – H (Lectin)
	A_1/ A_1B	A_2/A_2B	
A	+	-	**Should give intensity of reaction in order**
AB	+	-	
B	**NA**		**$O > A_2 > A_2B > B > A_1 > A_1B$**
O			
O^h Bombay Group			**Neg**

Grading of Agglutination

Defining the Strength of Reaction (Grading of Agglutination)

To record the difference in the strength of reaction, it is necessary to have a

Grid ... Manual ABO and Rh Blood grouping

system of grading or scoring the reactions, as depicted in figure-1

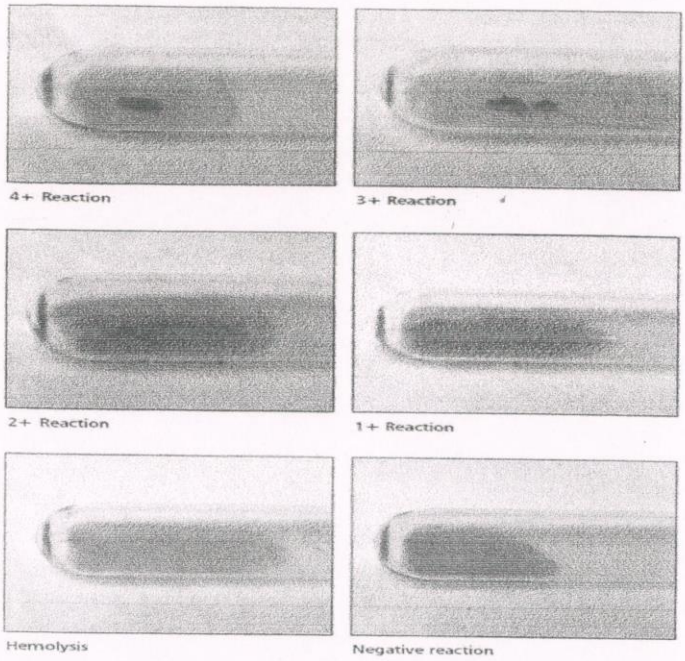


Figure.1 Grading of agglutination by tube method

Grades	Description
4+	1 big clump
3+	2 or 3 clumps
2+	many small clumps with clear supernatant
1+	many small clumps with turbid supernatant
w	granular suspension
Zero or -	smooth suspension
H	partial or complete hemolysis (positive reaction)