National University-Sudan

Faculty of Medical Laboratory Sciences

Student Practical Manual-
Haematology and Immunohaematology
Department

Second Year, Semester (3)
Serology and Immunohaematology (MLS-SERO-216)

Student Name: ……...

ID: ……**Batch**…….....…...

Instructions

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

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, , and O cells.

Normal saline.

Equipments: 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**

Test 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.

<u>Tube method (using both cells and serum):</u>

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 cells 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 your result.
7. **Results**:

   Positive reaction……..agglutination.
8. looking for agglutination & Interpret your result
9. **Results**:
10. Positive reaction……..agglutination.

    Negative reaction……o agglutination

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

.

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

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: ……..\.....\.....

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.

Never give Rh+ blood to Rh individuals especially to women of child bearing age.

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 or clotted sample.

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 resuspended the cell button and examine it for agglutination.

Interpretation:

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

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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 o 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.
6. Student's findings (measurements or observations):

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7. comments and interpretation:

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8. Evaluation (carried out by the instructor):

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9. Name and signature of the instructor:

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10. Date: ……..\.....\.....

ABO discrepancies

- ABO discrepancies occur when unexpected reactions occur in the reverse (indirect) grouping.

Blood group	(Anti-A)	(Anti-B)	Acells	Bcells	0cells
A	+ve	-ve	-ve	+ve	-ve
B	-ve	+ve	+ve	-ve	-ve
AB	+ve	+ve	-ve	-ve	-ve
O	-ve	-ve	+ve	+ve	-ve

Discrepancies

	(Anti-A)	(Anti-B)	Acells	Bcells	0cells
1. A2 blood group	**+ve**	**-ve**	**+ve**	**+ve**	**-ve**
2. Bombay blood group	**-ve**	**-ve**	**+ve**	**+ve**	**+ve**
3. Immune deficient or infant < 6 months	**- ve or +ve**	**-ve or +ve**	**-ve**	**-ve**	**-ve**
4.Pt with unexpected AB	**- ve or +ve**	**-ve or +ve**	**+ve**	**+ve**	**+ve**

Causes of discrepancies:

- Some technical errors can result in ABO discrepancies such as inadequate identification of blood specimen, cell suspension either too heavy or too light, failure to add reagents, contaminated reagents,…etc.
- It is important to make sure that any technical factors are reviewed and corrected.

1) A_2 blood group :

- This subgroup of A give reaction with A_1cells.

- 80% percent of group A individual are A_1, 20% being A_2.
- A_2 have an anti-A_1 cells antibodies in their serum.

2) Bombay blood group (O*h*):

- It is a rare blood group.
- Bombay individual is blood group O but lakes H antigen in RBCs, i.e. have no A, B, or H antigen.
- Bombay has anti-A, anti-B, and anti-H in his serum, (so react with O cells).

3) NO reaction detected in the reverse grouping:

- This is either Immune deficient, elderly patient or infant < 6 months.
- Pt with leukaemia, having immunosuppressive drugs, BM transplantation.
- The production of ABO antibodies is not detectable until 3 to 6 months of age.
- In elderly people; production of ABO antibodies is depressed.

4) Pt with unexpected antibody:

- Unexpected antibody in the pt serum other tan anti-A or anti-B may cause a discrepancy in the reverse grouping.
- A cells, B cells, and O cells possess other antigens in addition to A and A antigens, so it is possible that unexpected antibodies present in the pt. serum react with these cells.
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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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