

MODULE

Hematology and Blood
Bank Technique



Notes

22

ANTI GLOBULIN TEST

22.1 INTRODUCTION

The anti globulin test (Coombs test) was introduced by Coombs, Mourant and Race in 1945. The test was developed to detect antibodies which can bind the antigen on the surface of RBCs but cannot agglutinate them (incomplete antibodies).



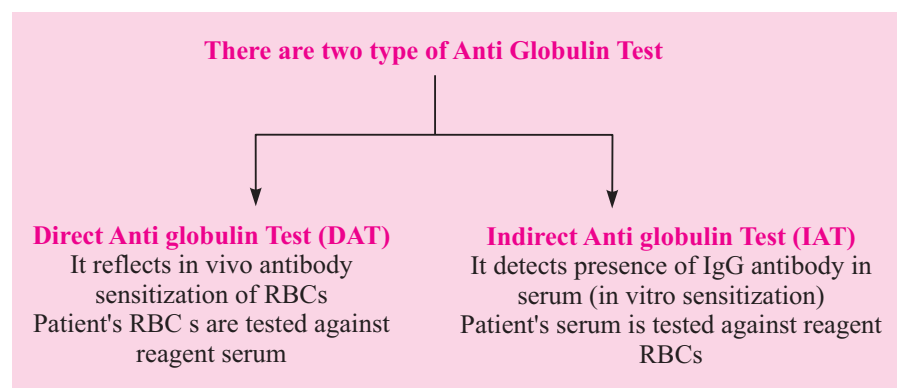
OBJECTIVE

After reading this lesson, you will be able to:

- describe the principle of Anti-Globulin test
- explain the procedure of Direct and Indirect Anti Globulin test
- describe the uses of Direct and Indirect Anti globulin test

22.2 PRINCIPLE

The test is based on the principle that antihuman globulin (AHG) antibodies combine with RBCs coated with human immunoglobulin or complement (in vivo or in vitro). The AHG acts as a bridge and causes agglutination of the RBCs.



22.3 DIRECT ANTI GLOBULIN TEST (DAT)

To identify if a patient's RBCs are coated with immunoglobulin, complement or both, AHG with reactivity to human immunoglobulin and/or complement is added to the patient's RBCs. If cross linking and subsequent agglutination is present, direct Coomb's test is positive.

Specimen: EDTA blood

Materials

1. Test tubes
2. AHG reagent
3. Positive control cells (IgG coated)
4. Centrifuge and microscope

Procedure

1. Make 5% cell suspension of patient blood by washing 3 times with normal saline.
2. After last washing decant the supernatant completely.
3. Take 1 drop of patient's cell suspension in a test tube.
4. Add 2 drops of A.H.G reagent with the patient's cell suspension in the test tube.
5. Mix well and centrifuge the mixture for at 1500 RPM for 1 minute.
6. Gently shake the tube and examine with naked eye and under microscope to see the agglutination.
7. If the test result is negative, add a drop of control cells.
8. Mix well and centrifuge the mixture for at 1500 RPM for 1 minute and look for agglutination. If no agglutination is seen, the result is invalid.

Result

Presence of agglutination means a positive DAT. This indicates the presence of human immune globulin or complement bound to RBCs.

Absence of agglutination means a negative DAT.

Uses of DAT

1. Presence of autoantibodies against RBC as in the case of warm autoimmune hemolytic anemia (AHA).
2. To detect hemolytic transfusion reactions when incompatible blood is transfused, the donor cells get coated with recipient's antibodies and the DAT is positive.

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Anti Globulin Test

- To detect hemolytic disease of Newborn: detects the presence of maternal antibodies attached to fetal RBCs.
- Antibodies induced by drugs.

22.4 INDIRECT ANTI GLOBULIN TEST (IAT)

Reagent RBCs (Coomb's control cells) are incubated in the presence of patient's serum. On adding AHG reagent, IgG coated red cells agglutinate

Specimen: Patient's Serum

Materials

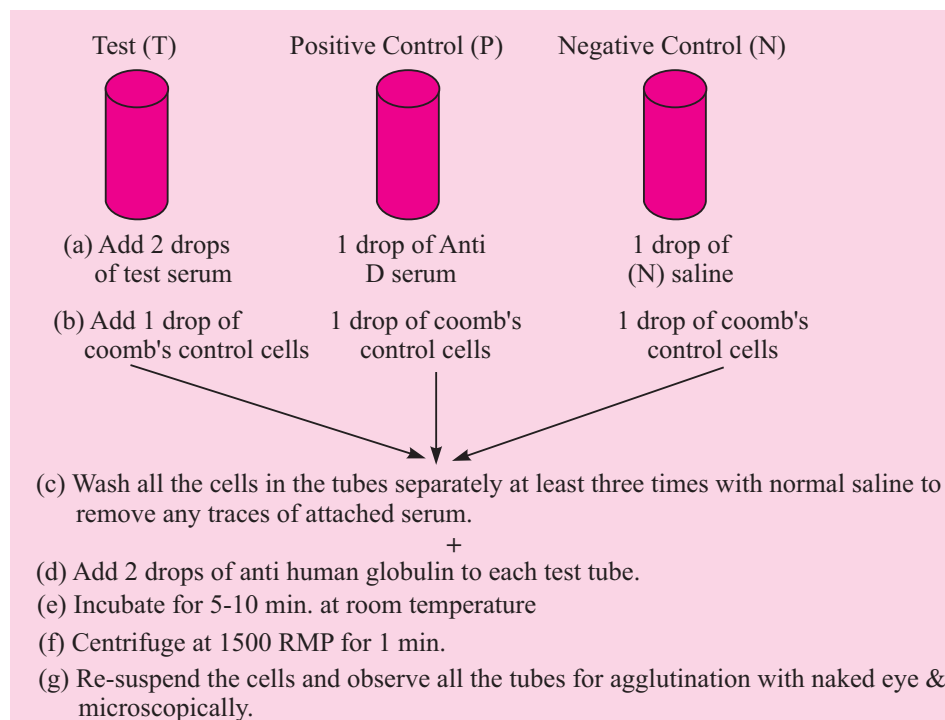
In addition to the material used in DAT;

- O positive red blood cells (Coomb's control cells)
- Anti D serum
- Normal saline

O positive red blood cells are prepared as follows:

- A pool of 'O' positive blood group cells is taken from 3 separate donors.
- These cells are saline washed at least 3 times & a 5% saline suspension of the washed cells is made.

Procedure : Take three test tubes labeled as test, positive control & negative control.



Anti Globulin Test

Test	Positive Control	Neg. Control	Result
Agglutination	Agglutination	No Agglutination	Positive
No Agglutination	Agglutination	No Agglutination	Negative

If positive control shows no agglutination or the negative control shows agglutination it means the test has not been performed correctly and needs to be repeated.

Interpretation

A positive test means patient's serum contains Anti D antibodies.

A negative test means patients serum does not contain anti D antibodies

Uses of IAT

(a) Cross matching

To detect the presence of recipient antibodies bound to donor RBCs.

(b) Typing of erythrocyte antigen

(c) Detecting presence of Anti D antibodies in a Rh negative woman married to an Rh positive man.

Sensitivity of IAT can be increased by

1. Optimizing the temperature at 37°C
2. Increasing the ratio of serum to red cells
3. Addition of LISS (low ionic strength saline), albumin and enzymes



INTEXT QUESTIONS 22.1

1. Antiglobulin test is commonly known as
2. test detects antibodies bound to RBCs
3. test detects unbound antibodies
4. Common used of Direct Antiglobulin tests are &
5. Common used of Indirect Antiglobulin tests are &

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WHAT HAVE YOU LEARNT

- Antiglobulin test is commonly known as coomb's test
- Antiglobulin test is used to detect incomplete RH antibodies
- It is based on the principle that antihuman globulin antibodies combine with RBCs coated with human immunoglobulin or complement
- Direct and Indirect antiglobulin tests are the two types of antiglobulin tests
- Direct antiglobulin tests detects antibodies bound to RBCs
- Indirect antiglobulin test detects the presence of unbound antibodies in serum
- DAT is used to detect hemolytic transfusion reactions and also to detect hemolytic diseases of newborn
- Indirect anitglobulin test is used in cross matchin and to detect the presence of Anti D antibodies in Rh negative woman married to Rh positive man.



TERMINAL QUESTIONS

1. List the types of Antiglobulin test
2. Enlist the uses of Direct Antiglobulin test
3. Enlist the uses of Indirect Antiglobulin test



ANSWERS TO INTEXT QUESTIONS

22.1

1. Coomb's test
2. Direct Antiglobulin
3. Indirect Antiglobulin
4. Hemolytic transfusion reaction & Hemolytic diseases of Newborn
5. Cross matching & Detecting presence of Anti D antibodies