GB's COOMB's REAGENT

DIAGNOSTIC KIT FOR ANTI HUMAN GLOBULIN SERUM for DIRECT AND INDIRECT COOMB's TEST

GENUINE BIOSYSTEM

KIT NAME	KIT SIZE	CAT. NO
GB's COOMB's Reagent	5 ml	COS000005M
GB's COOMB's Reagent	10 ml	COS000010M

INTRODUCTION

The Coomb's Reagent is used to demonstrate the presence or absence of immunoglobulin on the surface of red blood cells. The cells that have globulin adsorbed to their surfaces are said to be sensitized, globulin may be gamma globulin (Antibody) and/or beta globulin (Components of Compliment)

Direct Agglutination test shows whether or not red cells have become sensitized in vivo. This test is useful in the investigation of eythroblastosis foetals, drug induced or auto-immune hemolytic anemias, and incompatible blood transfusions.

The Indirect antiglobulin test require that the red cells be incubated with serum to allow sensitization to occur invitro, prior to freezing them off unbound serum proteins and testing with anti-human globulin. This test is useful in antibody screening and identification. Du test, cross-matching and blood grouping by using antisera which generally requires the use of antiglobulin serum e.g. Kelll, Dutty, Lewis etc.,

METHOD PRINCIPLE

Incomplete antibodies directed against the antigens of the erythrocytes surface cause sensitization and get adsorbed on the surface of antigens but fail to bring about agglutination of these cells. Anti-Human serum will react with adsorbed antibody globulins and / or components of compliment and produces agglutination of erythrocytes.

REAGENTS	Package	
GB's COOMB's Reagent	1 x 5 ml	1 x 10 ml

WORKING REAGENT PREPARATION AND STABILITY

Unopened vial when stored at 2-8 °C is stable till the expiry date mentioned on the label of the container, (DO NOT FREEZE)

PROCEDURE:

A) DIRECT ANTIGLOBULIN TEST

1. Take 5-7 drops of anticoagulated blood under test in a test tube (10x75mm)
2. Wash the RBC's 3-4 times with normal saline to remove adsorbed plasma proteins.
3. Prepare 3-5% suspension of washed RBC's in normal saline.
4. (a) Place one drop of Anti-Human Serum in test tube labeled `Test'(T)
   (b) Place one drop of normal saline in test tube labeled `Autocontrol' -(AC)
5. Add one drop of 3-5 % suspension of RBC's to each of the above tubes and mix well.
6. Centrifuge both the tubes at 1000 RPM for 1 minute.
7. Resuspend the packed cells by gentle agitation and examine microscopically as well as macroscopically for agglutination.

RESULT:

Presence of agglutination indicates positive test result and absence indicates negative test result. Control must show absence of agglutination.

IMPORTANT:

All negative test should be confirmed by the addition of one drop of coomb's control cells to each tube, followed by centrifugation at 1000 RPM for 1 minute and examine for agglutination under microscope. Agglutination of Coomb's Control Cells indicates satisfactory performance of Anti -Human serum in the test and also confirms that the addition of Anti-Human serum in Step No.4 was not inadvertently omitted.

NOTE:

1. Sensitivity of detection of bound compliment will be increased by incubation at Room Temperature for 10 minutes followed by re-centrifugation at 1000 RPM for 1 minute.
2. Immediate centrifugation results must be taken into consideration since Anti-IgG reactions may be adversely affected by incubation.

B) INDIRECT ANTIGLOBULIN TEST

1. Prepare test erythrocytes by taking 5-7 drops of appropriate blood sample (clotted or anticoagulated) in a test tube (10 x 75 mm).
2. Wash the RBC's 3-4 times with normal saline to remove adsorbed plasma proteins.
3. Prepare 3-5% suspension of washed RBC's in normal saline.
4. Place two drops of serum under test in a test tube labelled "Test" (T)
5. Add two drops of 3-5% suspension of RBC's to the above tube.
6. Incubate the tube at 37°C for one hour and centrifuge at 1000 RPM for 1 minute.
7. Remove the supernatent and wash the packed RBC's 3-4 times with normal saline.
8. Discard the supernatent and add one drop of Anti-Human Serum to RBC's and incubate further for 25-30 minutes.
9. Centrifuge the tube at 1000 RPM for 1 minute and examine macroscopically as well as microscopically.

AUTOCONTROL TUBE:

For every test one Autocontrol Tube must be included by taking 2 drops of patient's serum along 'with 2 drops of 3-5% suspension of patient's own erythrocytes and carrying it through Step No.6 onwards.

IMPORTANT:

All negative results must be confirmed by adding to each tube 2 drops of Coomb's Control Cells followed by centrifugation at 1000 RPM for 1 minute.

Agglutination of Coomb's Control Cells indicates satisfactory performance of Anti-Human Serum in the test and also confirms that the addition of Anti-Human Serum in Step No.8 was not inadvertently omitted.

NOTE:

In antibody screening and identification tests, the addition of bovine albumin 22% to the serum-cell mixture in Step 5 may increase the sensitivity of the test for some antibodies of IgG class. For this purpose, add 2-3 drops of 22% bovine albumin solution to the serum-cell mixture on Step 5 and centrifuge the tubes at 1000 RPM for 1 minute and note the presence or absence of albumin agglutinating antibodies before proceeding for steps 6 through 9.

Within the framework of the test procedure given above various specific applications are summarized below

Application	Test Erythrocytes	Test Serum
Antibody Screening Identification	RBS's of known genotypes	Patient's Serum
Du Test Cross Matching	Patient's RBC's	Anti D (Rho) Serum
(i) Major	Donor's RBC's	Patient's RBC's
(ii) Minor	Patient's RBC's	Donor's RBC's
Blood Grouping	Patient's RBC's	Specific Blood Grouping Serum

PREPARATION OF COOMB's CONTROL CELLS:

1. Take approximately 10 - 15 drops of D (Rho) positive anticoagulated blood in a Test Tube.
2. Wash the blood cells once with excess of normal saline and prepare an approximately 5% suspension of washed RBC's in normal saline.
3. Prepare weak incomplete Anti-D by diluting Anti-D 1:64 or 1:128 with normal saline.
4. Mix equal volumes of incomplete weak Anti-D and above 5% RBC suspension in a test tube and incubate at 37°C for 60 minutes.
5. Centrifuge and discard the supernatent and wash the cells 3-4 times with excess of normal saline.
6. Decant the supernatent completely and prepare 3-5% suspension of RBC's in normal saline.

LIMITATIONS OF THE PROCEDURE:

False Negative Results: False Negative reactions may be because of one or many of the following reasons:

1. Incomplete washing of RBC's
2. Contamination of glassware by Human Serum.
3. Improper storage of erythrocytes / serum.
4. Elution of antibody during incubation / washing.
5. Inadvertent omission of addition of antiglobulin reagent.
6. Insufficient time of incubation for proper sensitization.
7. Microbial contamination of reagent leading to inactivation of Anti-Human serum.

False Positive Results: False Positive reactions may be because of one or many of the following reasons:

1. Microbial contamination of Test Cells/ Reagents.
2. Autoa gglutination of Cells (Cf: autocontrol tube)
3. Use of Indirect Antiglobulin Test for erythrocytes which are positive by Direct Antiglobulin Test.

Application Test Erythrocytes Test Serum Antibody screening identification RBS's of known genotypes Patient's serum Du test cross-matchingPatient's RBC's Anti D (Rho) serum(I) Major Donor's RBC's Patient's serum(ii) Minor Patient's RBC's Donor's RBC's Blood Grouping Patient's RBC's Specific Blood grouping serum

LITERATURE

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