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BHAT Bio-Scan®

Anti HUMAN Globulin

For Professional Use

For Direct and Indirect Anti globulin Test IVD

READ THE PACK INSERT BEFORE USE PROVIDED ALONG WITH THE KIT

CATALOGUE No.: A-Hg

INTENDED USE: (COOMB's Antisera) Anti-human serum is an important diagnostic aid in determining the presence or absence of red blood cell antibody or component of human complement on red blood cells. Accordingly (COOMB's antisera) Anti-Human serum is used for compatibility test, antibody detection, antibody identification, testing for RHO (D) antigen (DU tests) and umbilical cord red blood cell testing. (COOMB's Anti sera) Anti- human serum may be used in the direct antiglobulin test and in the indirect antiglobulin test to detect antibodies and/or complement on red blood cells.

INTRODUCTION

Generally antibodies involved in transfusion reactions are of two types, namely the complete and the incomplete, whereas the complete antibodies agglutinate red cells in saline medium, the incomplete type of antibody sensitizes red cell without agglutination. Usually IgM class of antibodies and IgG_1, and IgG_3, type of IgG antibodies fix complement. Sellysis, *in vitro*, is mediated through the complement system and the complement component C_3b is further acted upon to produce C_3d.

In the direct antiglobulin tests, Anti human globulin regent is used to detect antibodies adsorbed to the red blood cells *in vitro* Anti human globulin reagent is useful for compatibility testing, antibody detection, antibody identification, umbilical cord red blood testing and detection of the D^2 variant of the human red blood cell antigen D (Rho).

REAGENT

Anti human globulin is balanced ready to use blend of highly purified immunoglobulin. It contains Anti human IgG antibodies and antibodies reactive with human complement components C_3b and C_3d.These anti complement antibodies are IgM class monoclonals and they impart the necessary sensitivity to the reagent.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and titre.

REAGENT STORAGE AND STABILITY

a) Store the reagent at 2-8ºC. DO NOT FREEZE. b) The Shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE

Normal human red blood cells, in presence of antibody directed towards the antigen they possess, may fail to agglutinate and become sensitized. This may be due to the particular nature of the antigen and antibody involved. Anti human globulin or components of human complement involved and cause agglutination of the red blood cells.

NOTE

(1). *In vitro* diagnostic reagent for laboratory and professional use only. Not for medicinal use. (2). The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. (3). Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded. (4). Reagents are not from human sources, hence contamination due to HBsAg and HIV is practically excluded.

SAMPLE COLLECTION AND STORAGE

No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed samples.

For Direct Antiglobulin Test : Blood drawn into EDTA is preferred but oxalated, citrated or clotted whole blood may be used. The blood samples should be tested as soon

as possible after collection and should not be stored.

For indirect Antiglobulin Test : Serum not more than 48 hours old , should be used. Donor units may be tested may be tested upto the end of their dating.

Preparation of Coombs Control Cells

(1). Dilute Anti-D (IgG)/Anti-D (polyclonal) reagent 1:50 in isotonic saline. (2). Prepare a 5% suspension of group 'O' Rho D positive cells in isotonic saline. (3). Mix equal volumes of diluted Anti-D reagent (as in 1 above) and 5% suspension of 'O' Rho D positive cells (as in 2 above) and incubate at 37ºC for 15 minutes. (4). Decant and wash thoroughly with isotonic saline at least thrice. (5). Resuspend in isotonic saline to make a 5% suspension of coombs control cells.

Additional Material Required

For Direct Antiglobulin Test: Test tubes (10x75mm), Pasteur pipettes, Centrifuge, Isotonic saline, Coombs control cells, Optical aid, for Indirect Antiglobulin Test and Compatibility Test: Test tube (10x75 mm), Pasteur pipettes, Bovine Serum Albumin, Centrifuge, Incubator (37ºC), Isotonice salince, Coombs control cells, Optical aid.

Procedure

Bring reagent to room temperature before testing.

Direct Antiglobulin Test

(1). Prepare a 5% suspension of the red cells to be tested in isotonic saline. (2). Pipette one drop of the cell suspension into a test tube. (3). Fill the tube with fresh isotonic saline and centrifuge for 30 seconds at 3400 rpm (1000g). (4). Decant and repeat this washing atleast thrice. (5). Add two drops of Anti human globulin reagent and mix well, (6). Centrifuge for one minute at 1000 rpm (125g) or for 20 seconds at 3400 rpm (1000g). (7). Very gently, resuspend the cell button observing for agglutination macroscopically. (8). To all negative antiglobulin tests add one drop of coombs control cells and observe for agglutination.

Initial Phase

(1). Label two test tubes as A (for albumin) and B (for saline), depending upon the number of donors to be cross matched, as many pairs of such labelled tubes would be required. (2). Prepare a 5% suspension of the red cells to be tested in isotonic saline. (3). Pipette drops of recipient serum in both the labelled test tubes. (4). Pipette one drop of donor red in both the labelled test tubes and mix cell. (5). Only to the albumin tube (A) add two drops of Bovine Serum Albumin reagent and mix well. (6). Centrifuge both the tubes for one minute at 1000 rpm (125g) or for 20 seconds at 3400 rpm (1000 g). (7). First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically. (8). Proceed to incubation phase.

Antiglobulin Phase

(1). Only the albumin tubes (A) are tested in the antiglobulin phase. (2). Wash the mixture of red blood cells and serum thoroughly with lsotonic saline for three times. Decant completely after the last wash. (3) Place two drops of Anti human globulin reagent into the last tubes containing the sedimented cells and mix well. (4). Centrifuge for one minute at 1000 rpm (125 g) or for 20 seconds at 3400 rpm (1000 g). (5). Very gently resuspend the cell button and observe for agglutination macroscopically.

Interpretation of Results

Direct Antiglobulin Test

Agglutination of the red blood cells is a positive test result and indicates the presence of human IgG complement component on the red blood cells. No agglutination is a negative test result and indicates the absence of human IgG complement component on the red blood cells.

Indirect Antiglobulin Test

In all phases of the compatibility test, if no agglutination or haemolysis is observed then the patient and the donor may be considered compatible, if haemolysis or agglutination at any point till the completion of the antiglobulin phase is observed.

Remarks

(1). If plasma is used in the indirect antiglobulin test the complement dependent antibodies may not detected due to the absence of calcium. (2). To all negative test results, after the antiglobulin test phase, one drop of Coombs control cells should be added. if Coombs control cells do not agglutinate then the compatibility test must be repeated. (3). In the indirect antiglobulin test procedure an auto control tube (individuals cell in his own serum) should be run. (4). Red blood cells showing a positive direct antiglobulin test cannot be used for the indirect antiglobulin test. (5). It is recommended that Anti-IgG activity of the Anti human globulin reagent be tested from time to time preferably on a daily using Coombs control cells as a positive control. (6). All glassware used in the test should be scrupulosity clean dry and free from contamination with human serum. (7). Contaminated Bovine serum albumin, saline or glassware may inactivate anti human globulin reagent. (8). Use of various drugs and certain diseases (such as megaloblastic anaemia) are known to be associated with a positive direct antiglobulin test. (9). Cord cell obtained from a newborn exhibiting hemolytic disease of the newborn, especially due to ABO incompatibility may give false negative results. (10). Anti human globulin reagent does not certain Anti-C, and is free from Anti-T activity. (11). As under centrifugation or overcentrifugation could lead to erroneus results, it is recommence that each laboratory calibrate its own equipments and the time required for achieving the desired results. (12). The label minimum titer claim is based on antibody (incomplete) and complement coated red cells for Anti Human reagent. This is based on titration procedure as recommended by the manufacture. Any deviation in test procedure would result in variable results.

Warranty

This product is designed to perform as described on the label and package insert. The manufacture disclaims any implied warranty of use and sale for any other purpose.

Bibliography

(1). Kohler C, & Millstein C, (1975), Continuous cultures of fused cells secreting antibody of predefined specificity, Nature, 256, 495-497. (2). Lee H.H., Rouger P., Germain C., Mailer A & Salmon C. (1983). The production and standardisation of monoclonal antibodies as AB blood group typing reagents, Symposium of international Association of Biological Standardisation on monoclonal antibodies. (3). Human Blood Groups, by Geoff Daniels, 1st Ed, Blackwell Science, Oxford 1995. (4). HMSO, Guidelines for the Blood Transfusion Services., 2nd Ed., 1994. (5). Data on File : Global invitro LLP.