AHG COOMB'S SERA

METHOD – IMMUNOAGGLUTINATION
PRODUCT CODE – LB12

ANAMOL
THE ORIGINAL MAKERS

INSTRUCTIONS FOR USE

INTENDED USE: Test for qualitative determination of human anti-IgG and anti-C3 on red blood cells.

SUMMARY

In 1945, Coombs, Mourant and Race described the use of anti-human globulin serum for detecting red cell-bound non-agglutinating antibodies. In 1957, Dacie et al showed that the antibodies present in antiglobulin sera were directed against certain components of complement. Antihuman globulin reagents detect non-agglutinating antibody molecules as well as molecules of complement attached to red cells following *in vivo* or *in vitro* antigen-antibody reactions.

PRINCIPLE

When used by the recommended techniques, the reagents will react with immunoglobulins and/or complement attached to the red cell surface, resulting in agglutination (clumping) of adjacent sensitized cells. Cells not sensitized will not be agglutinated (See **Limitations**).

REAGENTS

Anti-Human Globulin Elite Green reagents contain anti-IgG derived from rabbits with nonspecific activity removed by absorption and mouse monoclonal IgM anti-C3d, Clone BRIC-8. The antibodies are diluted in a buffered solution containing bovine albumin. Each reagent is supplied at optimal dilution, for use with all the recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

PRECAUTIONS

1. The reagents are intended for *in vitro* diagnostic use only.
2. If the reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagents past the expiration date (see **Vial Label**).
4. Do not use the reagents if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagents contain < 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
8. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.
9. For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

NOTES

1. It is recommended a positive control (weak Anti-D 0.1 IU/ml) and a negative control (an inert serum) be test in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. The antiglobulin techniques can only be considered valid if all negative tests react positively with IgG sensitized red cells.
3. In the techniques, here mentioned, one volume is approximately 40µl when using the vial dropper provided.
4. Use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use. User must determine the suitability of the reagents for use in other techniques.

REAGENT STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8oC on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity.

SPECIMEN COLLECTION

Samples should be drawn aseptically into EDTA to prevent *in vitro* complement binding and tested within 24 hours. If EDTA is unavailable, samples drawn into ACD, CPD or CPDA-1 are preferable to clotted ones. If only clotted samples are available, do not refrigerate them before testing. All blood samples should be washed at least twice with PBS before being tested.

MATERIALS REQUIRED

1. Glass test tubes (10 x 75 mm or 12 x 75 mm).
2. Test tube centrifuge.
3. Volumetric pipettes.
4. Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22 °C ± 1 °C.
5. IgG sensitized red cells.
6. Inert antibody Serum.
7. Weak anti-D.
8. Water bath or dry heat incubator equilibrated to 37 ^{0}C ± 2 ^{0}C.
9. Coombs cell washer.
10. Low Ionic Strength Solution (LISS).

PROCEDURE

A. DIRECT ANTIGLOBULIN TECHNIQUE (DAT)

1. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
2. Add 2 volumes of Anti-Human Globulin to each dry cell button.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination

B. NISS INDIRECT ANTIGLOBULIN TECHNIQUE (NISS IAT)

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 2 volumes of test serum and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37oC for 15 minutes.
4. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant saline after last wash.
5. Add 2 volumes of Anti-Human Globulin to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000rcf or for a suitable alternative time and force.
7. Gently resuspend red cell button and read macroscopically for agglutination

C. LISS INDIRECT AGGLUTINATION TECHNIQUE (LISS IAT)

1. Prepare a 1.5-2% suspension of washed test red cells in LISS.
2. Place in a labeled test tube: 2 volumes of test serum and 2 volumes of test red cell suspension.
3. Mix thoroughly and incubate at 37oC for 15 minutes.
4. Follow steps 4 to 7 of **NISS IAT** above.

INTERPRETATION OF RESULT

Positive: Agglutination of test red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates the presence of IgG and/or complement (C3) on the test red cells.

Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of IgG and/or complement (C3) on the test red cells.

Stability of the reactions

1. Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent.
2. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. Red cells that have a positive DAT due to a coating of IgG cannot be typed by the Indirect Antiglobulin Techniques.
2. A positive DAT due to complement sensitization may not reflect *in vivo* complement fixation if test cells are from a refrigerated clotted specimen.
3. Inadequate washing of red cells in the indirect antiglobulin techniques may neutralize the anti-human globulin reagent.
4. Following completion of the wash phase excess residual saline may dilute the anti-human globulin, reducing its potency.
5. A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO Hemolytic Disease of the Newborn or Auto Immune Hemolytic Anemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.
6. False positive or false negative results may also occur due to:
   a) Improper storage, cell concentration, incubation time or temperature.
   b) Improper or excessive centrifugation.
7. The user is responsible for the performance of the reagents by any method other than those here mentioned.
8. Any deviations from the techniques here recommended should be validated prior to use Contamination of test materials.

PERFORMANCE CHARACTERISTICS

1. The reagents have been characterized by all the procedures here described.
2. Prior to release, each lot of Anti-Human Globulin is tested, by the techniques here mentioned, against red cells coated with Anti-D, Anti-K and Anti-Fya to check suitable reactivity.
3. Potency of anti-IgG and anti-C3d have been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-AHG reference standard 96/666
4. Anti-C3d potency is demonstrated in tests employing cells coated with C3.
5. The presence of contaminating heterospecific agglutinins or antibodies to C4d has been excluded in tests employing red cells of all ABO groups and cells coated with C4d.
6. The reactivity of any Anti-IgM, Anti-IgA or Anti-light chain components that might be present has not been established.
7. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.
8. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

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SYMBOLS: Read Instruction for use | IVD In Vitro Diagnostic Use Only | Manufactured by | Expiry Date | Storage Temperature

ANAMOL LABORATORIES PVT. LTD.
61, Genesis Industrial Township, Kolgaon,
Palghar – 401 404. India.
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ISO 9001 : 2015
ISO 13485 : 2003
GMP
CE