ANTI HUMAN GLOBULIN (AHG) COOMBS

CAT NO	DESCRIPTION	PACK SIZE
BGRAH10	ANTI HUMAN GLOBULIN	10x10ml

Intended Use:

Anti-human 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. 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 sensitised cells. Cells not sensitised will not be agglutinated.

Appearance, Preparation and Stability:

The reagent contains anti-IgG derived from rabbits with non-specific 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. The reagent is green in colour due to the dyes Patent Blue and Tartazine that are added to it. Reagent vials should be stored at 2-8°C. Prolonged storage at temperature 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 as soon as possible. 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. For Indirect Antiglobulin Tests and compatibility tests, serum which is not more than 48 hours old should be used.

Materials required but not provided:

Coombs Cell washer, Glass test tubes, IgG sensitised red cells, Inert antibody, LISS, Volumetric pipettes, Water bath or dry heat incubator, weak anti-D.

Disposal of reagents and dealing with spillages:

Details are contained in the MSDS. Available on request.

Precautions:

1. The reagents are intended for In vitro diagnostic use only.
2. If a reagent vial is cracked or leaking discard the contents immediately.
3. Do not use the reagents past the expiry date
4. Do not use 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 0.2um 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. Materials used to produce the products were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
9. 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.

Quality Controls:

1. It is recommended a positive control (weak anti D, 0.1 U/ml) and a negative control (an inert serum) be tested 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 sensitised red cells.
3. In the recommended techniques one volume is approximately 50ul when using the vial dropper provided.
4. Use of 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.

Recommended Techniques:

Direct Antiglobulin Technique:

i) Prepare a 5% suspension of red cells in Isotonic Saline. Add 50 µl of the cell suspension to a test tube and wash the red cells 3 times with Isotonic Saline, taking care to decant saline between washes and resuspend the cell button after each wash. Completely decant saline after last wash.
ii) Add 2 volumes of Anti Human globulin reagent to each dry cell button.
iii) Mix thoroughly and centrifuge all tubes for 20 seconds at 3400 rpm or a suitable alternative time and force.
iv) Gently resuspend red cell button and read macroscopically for agglutination.

To All negative antiglobulin tests one drop of Coombs control cells can be added an observed for agglutination.

Indirect Anti-gloublin Techniques:

i) Prepare a 2-3% suspension of washed red cells in PBS.
ii) Place in a labelled test tube: 2 volumes of test serum and 1 volume of test red cell suspension.
iii) Mix thoroughly and incubate at 37oC for 15 minutes.
iv) Wash test red cells 4 times with PBC taking care to decant saline between washes and resuspend each red cell button after each wash.
v) Add 2 volumes of AHG reagent to each dry cell button.
vi) Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
vii) Gently resuspend red cell button and read macroscopically for agglutination.

LISS Indirect Antiglobulin technique:

i) Prepare a 1.5 – 2% suspension of washed test red cells in LISS.
ii) Place in a labelled test tube: 2 volumes of test serum and 2 volumes of test red cell suspension.
iii) Follow steps iv – vii of the Indirect Anti-gloublin technique.

CROSS MATCHING PROCEDURE:

i) For each cross match to be conducted, label two test tubes, one for Albumin labelled as A and the other for Saline labelled as B.
ii) Prepare a 5% suspension of red cells to be tested in isotonic saline.
iii) Pipette 2 drops of the recipient serum into each of the tubes A and B.
iv) Pipette 1 drop of the donor Red cells into each of the tubes A and B.
v) To the Tube A add two drops of AMS Bovine Serum Albumin.
vi) Mix well and centrifuge both tubes at 3400 rpm for 20 seconds.
vii) Observe for haemolysis. Resuspend the cell button and check for agglutination under a microscope.
viii) Incubate the Tube B (Saline) at Room temperature and the Tube A (BSA) at 37oC for 15 minutes.
ix) Observe for haemolysis. Resuspend the cell button and observe for agglutination under a microscope.
x) Wash the Tube A cells with Isotonic Saline 3 times. Remove the saline completely and add two drops of the AMS AHG reagent. Mix well.
xi) Centrifuge for 20 seconds at 3400 rpm.
xii) Resuspend the cell button gently and observe for agglutination under a microscope.

Interpretation of test results:

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

Stability of reactions:

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

Limitations:

- Red cells that have a positive DAT due to a coating of IgG cannot be typed by Indirect Antiglobulin technique.
- A positive DAT due to complement sensitisation may not reflect in vivo complement fixation if test cells are from a refrigerated clotted specimen.
- Inadequate washing of red cells in the indirect antiglobulin techniques may neutralise the anti-human globulin reagent.
- Following completion of the wash phase excess residual saline may dilute the anti-human globulin, reducing its potency.
- A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO haemolytic disease of the newborn or Auto immunue haemolytic anaemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.
- False positive or false negative results may also occur due to:
- Contamination of test materials
- Improper storage, cell concentration, incubation time or temperature

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- Improper or excessive centrifugation.

Note:

- The user is responsible for the performance of the reagents by any method other than those mentioned in the Recommended Techniques
- Any deviations from the 'Recommended techniques' should be validated prior to use.

Performance Characteristics:

1. The reagents have been characterised by the procedures mentioned in the Recommended Techniques
2. Prior to release of each lot, the reagents are tested against red cells coated with Anti-D, Anti K and Anti Fy to check suitable reactivity.
3. The anti-IgG and anti-C3d potencies have been tested against the following minimum potency standard obtained from NIBSC: Anti AHG reference standard 98/666.
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 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 bee established.
7. The quality control of the reagents was performed using red cells that had been washed 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.

References:

1. Coombs RRA, Mourant AE, Race RR. A new test for the detection of weak and incomplete Rh antibodies. Brit J Exp Pathol, 1945; 26-255.
2. Wright MS, Issit PD. Anti-Complement and the indirect antiglobulin test. Transfusion, 1979; 19:688-694.
3. Howard JE, Winn LC, Gottleib CE, Grumet FC, Garratty G, Petz LD. Clinical Significance of the anti-complement components of anti-gloublin anti sera. Transfusion 1982; 22-269.
4. Guidelines for the Blood Transfusion service in the United Kingdom. H.M.S.O Current edition
5. British committee for Standards in haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and cross matching. Transfusion Medicine 1995, 5, 145-150.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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