Blood Grouping Reagents
Anti-Human Globulin (Coombs) Reagent
Polyspecific

REF IVD

CATALOGUE NUMBER
Green: BG-AHG5, BG-AHG10, BG-AHG1L
Clear: BG-AHGC5, BG-AHGC10, BG-AHGC1L

INTENDED USE
These reagents are suitable for use by the slide and tube techniques and are designed for use by operators trained in serological techniques

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. 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.

INTENDED USE
These reagents are polyspecific blood grouping reagents intended to be used to qualitatively detect the presence or absence of sensitizing IgG antibodies (all 4 subclasses) and compliment factors C3d and C3b on human red cells when tested in accordance with the recommended techniques stated in the IFU.

REAGENTS
Rapid Labs Polyspecific Anti-Human Globulin Green reagents contain 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. The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitization or an allergic reaction by the user. 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.

Reagent	Cell line/Clone	Colour	Dye used
AHGElite Clear	Rabbit Anti-Human IgG BRIC-8 (Anti-C3d)	Colourless	None
AHG Elite Green	Rabbit Anti-Human IgG BIRC (Anti-C3d)	Green	Patent blue And Tartrazine

STORAGE
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. This reagent has undergone transportation stability studies at 37ºC and –25ºC as described in document BS EN ISO 23640:2015.

SAMPLE COLLECTION AND PREPARATION
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

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 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 0.2 µm 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.

DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES
For information on disposal of the reagents and decontamination of a spillage site see Material Safety Data Sheets, available on request.

CONTROLS AND ADVICE
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 sensitised red cells.
3. In the Recommended Techniques one volume is approximately 50µl 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.

REAGENTS AND MATERIALS REQUIRED

- Coombs cell washer.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- IgG sensitised red cells .
- Inert antibody.
- Low Ionic Strength Solution (LISS): Containing 0.03M NaCl, 0.003M Na_2HPO_4: NaH_2PO_4 buffer pH 6.7 at 22ºC ± 1ºC and 0.24M glycine.
- PBS (pH 6.8-7.2) or isotonic saline solution (pH 6.5-7.5)
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.
- Weak anti-D.

RECOMMENDED TECHNIQUES

A. Direct Antiglobulin Technique (DAT)

1. Wash 1 volume of red cells (2-3% suspension in PBS or Isotonic saline) 4 times with PBS or isotonic saline, 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 Rapid Labs Anti-Human Globulin to each dry cell button.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination.

B. Indirect Antiglobulin Technique (NISS IAT)

1. Prepare a 2-3% suspension of test red cells in PBS or Isotonic saline
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 37 ºC 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 Rapid Labs Anti-Human Globulin to each dry cell button
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
7. Gently resuspend red cell button and read macroscopically for agglutination.

C. LISS Indirect Antiglobulin Technique (LISS IAT)

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

INTERPRETATION OF TEST RESULTS
1. **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.

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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) 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. 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

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 sensitisation 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 neutralise 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 Haemolytic Disease of the Newborn or Auto Immune Haemolytic Anaemia. 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:
   - Contamination of testmaterials
   - Improper storage, cell concentration, incubation time or temperature
   - Improper or excessive centrifugation

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Prior to release, each lot of Rapid Labs Anti-Human Globulin Clear and Anti-Human Globulin Green is tested by the Recommended Techniques against red cells coated with weak Anti-D, Anti-K and Anti-Fya to check suitable reactivity. The tests complied with the test requirements as stated in the current version/issue of the 'Guidelines for the Blood Transfusion Services in the United Kingdom.'
2. The anti-IgG and anti-C3d potencies 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
3. Anti-C3d potency is demonstrated in tests employing cells coated with C3.
4. The reactivity of any Anti-IgM, Anti-IgA or Anti-light chain components that might be present has not been established.
5. The Quality Control of the reagents was performed using red cells with phenotypes that were verified by a UK blood transfusion Centre and had been washed with PBS or Isotonic saline prior to use.

STABILITY OF THE REACTIONS

1. Washing steps should be completed 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**.

BIBLIOGRAPHY

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5. Guidelines for the Blood Transfusion Service in the United Kingdom. 6th Edition 2002. The Stationery Office. 6. 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

Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalogue Number	LOT	Lot Number	N	Tests per kit
	Store between 2-8°C		Do not re-use		
	Manufacturer		Date of manufacture		

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