

DIRECTIONS FOR USE

AHG Elite (Clear or Green): For Antiglobulin 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.

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 sensitised cells. Cells not sensitised will not be agglutinated (See Limitations).

REAGENT

Lorne AHG Elite Clear and AHG Elite 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. 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	Colour	Dye Used
AHG Elite Clear	Colourless	None
AHG Elite Green	Green	Patent Blue and Tartrazine

STORAGE

Reagent vials should be stored at 2 - 8°C 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 37°C and -25°C as described in document EN13640:2002.

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. All blood samples should be washed at least twice with PBS or isotonic saline before being tested.

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.
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.

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 e.g. Lorne Coombs Control Cells (Cat # 970010).
- Inert antibody e.g. Lorne Inert AB Serum (Cat # 110010).
- Low Ionic Strength Solution (LISS): Containing 0.03M NaCl, 0.003M Na2HPO4; NaH2PO4 buffer pH 6.7 at 22°C ± 1°C and 0.24M glycine.
- PBS solution (pH 6.8-7.2) or Isotonic saline solution (pH 6.5-7.5).
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.
- Weak anti-D e.g. Lorne Precise Weak Anti-D (Cat # 209005).

RECOMMENDED TECHNIQUES

A. Direct Antiglobulin Technique (DAT)

1. Wash 1 volume of test 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 Lorne AHG Elite 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 washed 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°C for 15 minutes.
4. Wash test red cells 4 times with PBS or isotonic saline, 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 Lorne AHG Elite 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 washed 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°C 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.
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 test materials
 - Improper storage, cell concentration, incubation time or temperature
 - Improper or excessive centrifugation

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by the procedures mentioned in the Recommended Techniques.
2. Prior to release, each lot of Lorne AHG Elite Clear and AHG Elite Green is tested by the Recommended Techniques against red cells coated with Anti-D, Anti-K and Anti-Fya to check suitable reactivity.
3. 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
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 with PBS or Isotonic saline prior to use.
8. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

DISCLAIMER

1. The user is responsible for the performance of the reagents by any method other than those mentioned in the Recommended Techniques.
2. Any deviations from the Recommended Techniques should be validated prior to use¹².

BIBLIOGRAPHY








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, Issitt PD. Anti-complement and the indirect antiglobulin test. *Transfusion* 1979; 19:688-694.
3. Howard JE, Winn LC, Gottlieb CE, Grumet FC, Garratty G, Petz LD. Clinical significance of the anti-complement components of anti-globulin antisera. *Transfusion* 1982; 22:269.
4. Howell P, Giles CM. A detailed serological study of five anti-Jka sera reacting by the antiglobulin technique. *Vox. Sang.* 1983; 45: 129-138.
5. Issitt PD, Smith TR. Evaluation of antiglobulin reagents. A seminar on performance evaluation. Washington, DC. American Association of Blood Banks. 1976; 25-73.
6. The Department of Health and Social Security. Health Services Management Antiglobulin Test. False negative results, HN (Hazard) (83) 625 Nov 1983.
7. Bruce M, Watt AH, Hare W, Blue A, Mitchell R. A serious source of error in antiglobulin testing. *Transfusion* 1986; 26: 177-181.
8. The anti-complement reactivity low ionic methods as published by FDA. Recommended Methods for Anti-Human Globulin Evaluation (revision October 1984).
9. Dynan PK. Evaluation of commercially available low ionic strength salt (LISS) solutions. *Med Lab Sci* (1981) 381: 13-20.
10. Voak D, Downie DM, Moore BPL, Ford DS, Engelbreit CP, Case J. Replicate tests for the detection and correction of errors in anti-human globulin (AHG) tests: optimum conditions and quality control. *Haematologia* 1988; 21(1): 3-16.
11. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
12. 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.

AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number
Lorne AHG Elite (Clear)	10 ml	415010
	1000 ml	415000*
Lorne AHG Elite (Green)	10 ml	435010
	1000 ml	435000*

*This size is for Further Manufacturing Use (FFMU) only and is therefore not CE marked.

TABLE OF SYMBOLS

	Batch Number		In-vitro Diagnostic
	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		



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