BLENDING ANTI-HUMAN GLOBULIN (RABBIT)
POLYSPECIFIC ANTI-IgG AND ANTI-C3d

DIRECTIONS FOR USE

AHG Elite (Clear or Green): For Antiglobulin Techniques.

SUMMARY

In 1945, Coombs, Mounat and Race described the use of anti-human globulin serum for detecting red cell-bound non-aglutinating 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 BRC9-8. 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.

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 EN13664:2002.

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 risk of contamination.
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9. No protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
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REAGENT AND MATERIALS REQUIRED

- Coombs cell washes
- 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 Ironic 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 centrifuge 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 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 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 with the anti-human globulin, reducing its potency.
2. Inadequate washing of red cells in the indirect antiglobulin techniques may neutralise the anti-human globulin reagent.
3. Following completion of the wash phase excess residual saline may dilute the anti-human globulin, reducing its potency.
4. 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.
5. 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 use.

BIBLIOGRAPHY


AVAILABLE REAGENT SIZES

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