Anti-Human IgG (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.

PRINCIPLE
When used by recommended techniques, the reagent will cause agglutination (clumping) of red cells, carrying IgG, in the antiglobulin phase of testing. No agglutination usually indicates the absence of IgG (See Limitations).

REAGENTS
Lorne Monospecific Anti-Human IgG Clear and Anti-Human IgG Green reagents contain anti-IgG derived from rabbits. All non-specific activity is removed by absorption. The reagents are supplied at optimal dilution, for use with all the recommended techniques stated below without the 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 EN13640:2002.

SAMPLE COLLECTION AND PREPARATION
Samples should be drawn aseptically into EDTA and at optimal dilution. Taking care to wash and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force. Gently resuspend red cell button and read macroscopically for agglutination.

RECOMMENDED TECHNIQUES
A. Direct Antiglobulin Technique (DAT)
1. Wash test red cells 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 Anti-IgG 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 cell button after each wash. Completely decant saline after last wash.
5. Add 2 volumes of Lorne Anti-IgG 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 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 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. Inadequate washing of red cells in the indirect antiglobulin technique may result in neutralisation of the anti-human globulin reagent.
3. A positive DAT due to complement sensitisation may not reflect in vivo complement fixation if test cells are from a refrigerated clotted sample.
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
   - Deviation from the recommended techniques
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 Anti-Human IgG Clear and Anti-Human IgG Green is tested by the Recommended Techniques against red cells coated with Anti-D, Anti-K and Anti-Fy^a to check suitable reactivity.
3. The anti-IgG potency has 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. 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 that had been washed with PBS or isotonic saline prior to use.
6. 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.

Lorne Laboratories Limited
Unit 1 Cutbush Park Industrial Estate
Danehill
Lower Earley, Reading,
Berkshire, RG6 4UT
United Kingdom
Tel: +44 (0) 118 921 2264
Fax: +44 (0) 118 986 4518
E-mail: info@lornelabs.com

TABLE OF SYMBOLS

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