LORNE LABORATORIES LTD.

GREAT BRITAIN

CE 1434

MONOSPECIFIC ANTI-HUMAN GLOBULIN REAGENT (RABBIT)

DIRECTIONS FOR USE

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.

INTENDED PURPOSE

These reagents are monospecific blood grouping reagents intended to be used to qualitatively detect the presence or absence of sensitising IgG antibodies (all 4 subclasses) on human red cells when tested in accordance with the recommended techniques stated in this IFU.

PRINCIPLE

The reagents contain antibodies against human IgG antibodies on human red cells and will cause direct agglutination (clumping) of red cells that are sensitised with human IgG antibodies. No agglutination generally indicates the absence of sensitising human IgG antibodies on human red cells (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 adsorption. The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. 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**.

Reagent	Cell Line/Clone	Colour	Dye Used
Anti-Human IgG Clear	Rabbit Anti-Human IgG	Colourless	None
Anti-Human IgG Green	Rabbit Anti-Human IgG	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 EN13640:2002.

SAMPLE COLLECTION AND PREPARATION

Samples should be drawn aseptically into EDTA 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, but is not supplied sterile. 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. A positive control (weak Anti-D <0.1 IU/ml) and a negative control (an inert serum) shall 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. Before use, let the reagent warm up to room temperature. As soon as the reagent has been used, put the reagent back in storage at 2-8ºC.
4. In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
5. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
6. The user must determine the suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT SUPPLIED

- Coombs cell washer.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- IgG sensitised red cells i.e. Lorne Coombs Control Cells (Cat # 970010).
- Inert antibody i.e. Lorne Inert AB Serum (Cat # 110010).
- 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 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 37ºC ± 2ºC.
- Weak anti-D i.e. Lorne Precise Weak Anti-D (Cat # 209005).

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 37ºC 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 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 37ºC 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. Prior to release, each lot of these reagents were tested using the recommended test methods listed in this IFU against red cells coated with Anti-D, Anti-K and Anti-Fy^{a} 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 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
3. The reactivity of any Anti-IgM, Anti-IgA or Anti-light chain components that might be present has not been established.
4. 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.

DISCLAIMER

1. The end 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[6].

BIBLIOGRAPHY

1. Voak D, Downie DM, Moore BPL, and Engelfreit CP. Anti-Human Globulin reagent specification. The European and ISBT/ICSH View. Biotest Bulletin 1: 7-22 (1986).
2. The Department of Health and Social Security. Health Services Management Antiglobulin Test. False negative results, HN (Hazard) (83) 625 Nov 1983.
3. Bruce M, Watt AH, Hare W, Blue A, Mitchell R. A serious source of error in antiglobulin testing. Transfusion 1986; **26**: 177-181.
4. Voak D, Downie DM, Moore BPL, Ford DS, Engelfreit CP, Case J. Replicate tests for the detection and correction of errors in AHG (AHG) tests: optimum conditions and quality control. Haematologia 1988; **21**(1): 3-16.
5. Guidelines for the Blood Transfusion Service in the United Kingdom, 6th Edition 2002. The Stationary 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.

AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number	Tests per vial
Lorne Anti-Human IgG (Clear)	10 ml	401010	100
Lorne Anti-Human IgG (Green)	10 ml	402010	100

TABLE OF SYMBOLS

Symbol	Definition	Symbol	Definition
	Manufacturer	REF	Catalogue number
	Temperature limitation		Use by YYYY-MM-DD
IVD	In vitro diagnostic medical device		Consult instructions for use.
EC REP	Authorised Representative	LOT	Lot number
CE	CE symbol		

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