LORNE LABORATORIES LTD.

GREAT BRITAIN

CE 1434

HUMAN BLOOD GROUPING REAGENTS

DIRECTIONS FOR USE

Anti-Fy^b Polyclonal: For Indirect Antiglobulin Techniques.

SUMMARY

The Fy^a and Fy^b antigens were reported in 1950 and 1951 respectively. Anti-Fy^b has been implicated in immediate and delayed Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

Anti-Fy^a	Anti-Fy^b	Phenotype	Caucasians[1]	Afro-Americans[1]
+	0	**Fy(a+b-)**	17%	9%
0	+	**Fy(a-b+)**	34%	22%
+	+	**Fy(a+b+)**	49%	1%
0	0	**Fy(a-b-)**	Rare	68

INTENDED PURPOSE

The reagent is a blood grouping reagents intended to be used to qualitatively determine the presence or absence of the Fyb antigen (FY2) on the red cells of blood donors or patients requiring a blood transfusion when tested in accordance with the recommended techniques stated in this IFU.

PRINCIPLE

The reagent contains antibodies to the Fyb antigen on human red cells and will cause indirect agglutination (clumping) of human red cells, that carry the corresponding Duffy b antigen, in the antiglobulin phase of testing. No agglutination (no clumping) generally indicates the absence of the corresponding Duffy b antigen (see **Limitations**).

REAGENTS

Lorne Human Anti-Fy^b blood grouping reagent is prepared from human serum diluted in a sodium chloride solution containing macromolecular potentiators (1.9 g%) and bovine albumin. 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. Each reagent is supplied at optimal dilution for use with all 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 - 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

Blood samples can be collected into EDTA, citrate, CPDA anticoagulants or as a clotted sample. The samples should be tested as soon as possible following collection. If a delay in testing should occur, store the samples at 2-8°C. Samples displaying gross haemolysis or microbial contamination should not be used for testing. Blood samples showing evidence of lysis may give unreliable results. It is preferable (but not essential) to wash all blood samples 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.
7. The plasma from which this reagent is manufactured is no longer delipidated, so it is normal for the reagent to have a turbid appearance.
8. 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.
9. Materials used to produce the reagents were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
10. 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 **Safety Data Sheets**, available on request.

CONTROLS AND ADVICE

1. It is recommended a positive control (ideally heterozygous cells) and a negative control 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. The reagents contain macromolecular potentiators which may cause false positive reactions with IgG sensitised cells, it is recommended that patient's cells are tested with patient's plasma to test for false positive reactions.
4. Most proteolytic enzymes destroy Fy^b reactivity.
5. 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.
6. In the **Tube Technique** one volume is approximately 50µl when using the vial dropper provided.
7. 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.
8. User must determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

Tube Technique

- Anti-human globulin i.e. Lorne AHG Elite (Cat # 435010 or 415010) or Anti-Human IgG i.e. Lorne Anti-Human IgG (Cat # 402010 or 401010).
- Coombs cell washer.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- IgG sensitised red cells i.e. Lorne Coombs Control Cells (Cat # 970010).
- Positive (ideally heterozygous) and negative control red cells.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.

Bio-Rad-ID Micro Typing Technique

- Bio-Rad ID-Cards (LISS/Coombs or Coombs Anti-IgG).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or ID-Diluent 2.
- Bio-Rad ID-Incubator equilibrated to 37ºC ± 2ºC.

Ortho BioVue Typing Technique

- Ortho BioVue System Cassettes (AHG Polyspecific or AHG Anti-IgG).
- Ortho BioVue System Centrifuge.
- Ortho BioVue System Heat Block equilibrated to 37ºC ± 2ºC.
- Ortho 0.8% Red Cell Diluent.

All Techniques

- Volumetric pipettes.

RECOMMENDED TECHNIQUES

A. Indirect Antiglobulin Technique (IAT)

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume of Lorne reagent and 1 volume of red cell suspension.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Wash red cells at least 3 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 AHG Elite or anti-Human 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
8. Confirm validity of all negative reactions with IgG sensitised red cells.

B. Bio-Rad-ID Technique (LISS/Coombs card)

1. Prepare a 0.8% suspension of red cells in ID-Cellstab or ID-Diluent 2.
2. Remove aluminium foil from as many microtubes on either LISS/Coombs or Coombs Anti-IgG ID cards as needed.
3. Place in appropriate microtube: 50µl of 0.8% red cell suspension and 25µl of Lorne reagent.
4. Incubate the ID-Card(s) for 15 minutes at 37ºC.
5. Centrifuge the ID-Card(s) in a Bio-Rad ID centrifuge.
6. Read macroscopically for agglutination.

C. Ortho BioVue Technique (AHG cassette)

1. Prepare a 0.8% suspension of red cells in 0.8% Ortho Red Cell Diluent.
2. Remove aluminium foil from as many reaction chambers on either AHG Polyspecific or AHG Anti-IgG cassettes as needed.
3. Place in appropriate reaction chamber: 50µl of red cell suspension and 40µl of Lorne reagent.
4. Incubate the cassette(s) for 15 minutes at 37ºC.
5. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
6. Read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the appropriate Duffy antigen on the red cells.
2. **Negative:** No agglutination of the red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the appropriate Duffy antigen on the 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 Technique.**
2. This reagent contains macromolecular potentiators which may cause false positive reactions with IgG sensitised cells. It is therefore recommended that the patient's cells are tested with the patient's plasma to test for false positive reactions.
3. Antibodies directed at low frequency antigens may occur as unsuspected contaminants in blood grouping antisera. In addition, certain antigens (eg. Bg, Sd^a) can be present in an exalted state on red blood cells. These phenomena may be the source of rare false positive reactions, which may occur with more than one lot of a given specificity.
4. It is not possible to claim the absence of all contaminating antibodies, as red cells carrying antigens of low frequency or exalted antigens are not always available for testing.
5. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative results and so caution should always be exercised when assigning genotypes on the basis of test results.
6. False positive agglutination may be seen when testing IgG sensitised cells.
7. False positive results may occur due to Macromolecular potentiators that are present in the reagent
8. 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 reagent was tested using the recommended test methods listed in this IFU. 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 presence of contaminating antibodies to antigens with an incidence of 1% or greater within the random population has been excluded either in tests employing the appropriate antigen-negative red cells or in tests employing the reagents previously absorbed to remove the interfering specificities.
3. Antibodies to Xg^a, Do^a, Yt^a, Co^b, Wr^a, Bg^a and V^w may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cell. This can also be said for Yt^b, M^g and V^w and other low frequency antigens which may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cells
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 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[5].

BIBLIOGRAPHY

1. Marion E.Reid & Christine Lomas-Francis, Blood Group Antigens & Antibodies, SBB Books, New York 2007; Page 183.
2. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6.
3. AABB Technical Manual, 16th edition, AABB 2008.
4. Guidelines for the Blood Transfusion Service in the United Kingdom, 6th Edition 2002. The Stationary Office.
5. 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
Anti-Fy^b Polyclonal	2 ml	317002	40
	1000 ml	317000*	20,000

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

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