LORNE LABORATORIES LTD.

GREAT BRITAIN

MONOCLONAL BLOOD GROUPING REAGENTS

DIRECTIONS FOR USE

Anti-Le^a Monoclonal: For Tube, DiaMed/BioRad-ID and Ortho BioVue Techniques .

SUMMARY

The Lewis system antigens are not an integral part of the red cell membrane and are produced by tissue cells and found primarily in plasma and watery secretions. Red cells acquire Lewis antigens by absorption from surrounding plasma. The amount of Lewis antigen expressed on a cell can vary with the cell's ABO phenotype. Anti-Le^a has not been associated with Haemolytic Disease of the Newborn, but examples of Anti-Le^a have caused Haemolytic Transfusion Reactions.

Anti-Le^a	Anti-Le^b	Phenotype	Caucasians %	Afro-Americans %
+	0	**Le(a+b-)**	22	23
0	+	**Le(a-b+)**	72	55
0	0	**Le(a-b-)**	6	22
+	+	**Le(a+b+)**	Rare	Rare

PRINCIPLE

The reagent will cause agglutination (clumping) of test red cells, that carry the Lea antigen, after centrifugation. No agglutination generally indicates the absence of the corresponding Lewis antigen (see **Limitations**).

REAGENTS

Lorne Monoclonal Anti-Le^a grouping reagent contains human monoclonal IgM antibodies, diluted in a phosphate buffer containing sodium chloride, EDTA, bovine albumin and a macromolecular potentiator. Anti-Le^a is made with Clone P3N20V3. The reagent is 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 - 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 37^{o}C and –25^{o}C as described in document EN13640:2002.

SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed then store specimens at 2-8ºC. EDTA and citrate samples should be typed within 7 days after collection. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS or Isotonic saline or Isotonic saline 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 contains <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. 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 reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

CONTROLS AND ADVICE

1. It is recommended a positive and negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. In the **Tube Technique** one volume is approximately 50µl when using the vial dropper provided.
3. The use of the reagent 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 reagent is in use.
4. The user must the determine suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Test tube centrifuge.
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- DiaMed NaCl, Enzyme and Cold agglutinins ID-Cards.
- DiaMed ID-Centrifuge.
- DiaMed ID-CellStab or ID-Diluent 2.
- Ortho BioVue System Neutral Cassettes.
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.
- Le(a+) positive and Le(a–) negative control cells.
- Volumetric pipettes.

RECOMMENDED TECHNIQUE

A. Tube Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume of Lorne Lewis reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at room temperature for 15 minutes.
4. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination

B. DiaMed/BioRad-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in ID-Cellstab or ID-Diluent 2.
2. Remove aluminium foil from as many microtubes on the NaCl/Enzyme/Cold agglutinins ID-Card as needed.
3. Place in appropriate microtube: 50µl of 0.8% test red cell suspension and 25µl of Lorne reagent.
4. Incubate the ID-Card(s) for 15 minutes at room temperature.
5. Centrifuge the ID-Card(s) in a Diamed ID centrifuge.
6. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique

i. Prepare a 0.8% suspension of washed test red cells in 0.8% Ortho Red Cell Diluent.
ii. Remove aluminium foil from as many reaction chambers on the neutral cassette as needed.
iii. Place in appropriate reaction chamber: 50µl of test red cell suspension and 40µl of Lorne reagent.
iv. Incubate the cassette(s) for 15 minutes at room temperature.
v. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
vi. Read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the Lea antigen on the test red cells.
2. **Negative:** No agglutination of the test red cells constitutes a negative result and within accepted limitations of the test procedure, indicates absence of the Lea antigen on the test red cells.

STABILITY OF THE REACTIONS

1. The tube tests should be read immediately after centrifugation. Delays may result in dissociation of antigen-antibody complexes leading to false negative, or weak positive reactions.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. Lorne Lewis reagents must only be used with washed red cells suspended in physiological saline because Lewis antigens are present in plasma. Cells suspended in plasma/serum **cannot** be used since the soluble antigen present may neutralise the test reagent, giving false negative results.
2. Red cells of most new-borns will type Le(a-b-) with monoclonal or human anti-Lewis reagents, although some specimens will produce weak positive reactions in direct antiglobulin tests with mouse monoclonal Anti-Le^a.
3. The Lewis phenotypes of children under six years of age cannot be accurately determined. Red cell Lewis antigens are weaker during pregnancy.
4. Stored blood may give weaker reactions than fresh blood
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 all the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Lorne Monoclonal Anti-Le^{a} is tested by the **Recommended Technique** against a panel of antigen-positive red cells to ensure suitable reactivity.
3. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
4. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS or Isotonic saline or Isotonic saline or Isotonic saline prior to use.
5. 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 Technique**.
2. Any deviations from the **Recommended Technique** should be validated prior to use[6].

BIBLIOGRAPHY

1. Kholer G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. Nature 1975; **256**, 495-497
2. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition, Blackwell Scientific, Oxford 1987; Chapter 7.
3. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6
4. BSCH Blood Transfusion Task Force. Guidelines for microplate techniques in liquid-phase blood grouping and antibody screening, Clinical Laboratory Haematology 1990; **12**, 437-460.
5. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
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
Anti-Le^{a} Monoclonal	2 ml	632002
	1000 ml	632000*

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

For the availability of other sizes, please contact:

Lorne Laboratories Limited
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TABLE OF SYMBOLS

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