LORNE LABORATORIES LTD.
GREAT BRITAIN

LECTIN BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE

Anti-A_1 (*Dolichos biflorus*): For Tube and Slide Techniques.

SUMMARY

A_1 antigen is a subgroup of A and was discovered in 1910. Anti-A_1 is usually non-reactive at 37ºC, however examples reactive at 37ºC and predominately IgM can cause *in vivo* red blood cell destruction. About 78% of group A people are A_1 and 22% are A_2, similar proportions apply among AB people.

PRINCIPLE

The reagent will cause agglutination (clumping) of test red cells, that carry the A_1 antigen, after centrifugation. No agglutination generally indicates the absence of the A_1 antigen (see **Limitations**).

REAGENT

Lorne Anti-A_1 Lectin blood grouping reagent is prepared from an extract of *Dolichos biflorus* seeds, diluted with a sodium chloride solution containing bovine albumin. The 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

Do not freeze. 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. Reagent will remain stable for up to 7 days when subjected to temperatures not exceeding 30ºC.

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 48 hours. 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 before being tested.

PRECAUTIONS

1. The reagent is 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 reagent past the expiration date (see **Vial Label**).
4. Do not use the reagent 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 reagent has 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 reagent 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 control (ideally group A_1B cells) and a negative control (group A_2 cells) be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. In the **Recommended Techniques** one volume is approximately 40µl 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. User must determine suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Applicator sticks.
- Glass microscope slides.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Lighted Rh viewbox.
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22ºC ± 1ºC
- Positive (group A_1B) and negative (group A_2) control red cells.
- Test tube centrifuge.
- Volumetric pipettes.

RECOMMENDED TECHNIQUES

A. Tube Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume Lorne Anti-A_1 reagent and 1 volume test red cell suspension.
3. Mix thoroughly and then centrifuge all the 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. Slide Technique

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS.
2. Place on a labelled microscope slide: 1 volume Lorne Anti-A_1 and 1 volume test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth maintaining slide at room temperature.
5. Observe for macroscopic agglutination for period not to exceed 30 seconds because after 30 seconds some group A_2 and A_2B cells may show weak agglutination.
6. Any weak reactions should be repeated by the tube technique.

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 A_1 antigen on the test red cell.
2. **Negative:** No agglutination of test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of A_1 antigen on the test red cells.
3. **Discrepancies:** If the results obtained with reverse group don't correlate with forward group, further investigation is required.

STABILITY OF THE REACTIONS

1. Tube tests must be read immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes leading to false negative, or weak positive reactions.
2. Slide tests should be completed within 30 seconds to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. Anti-A_1 may react with Tn-polyagglutinable or Cad-positive cells
2. Cord blood and specimens from infants cannot be accurately typed using Anti-A_1 Lectin since the A_1 antigen is not fully developed on red blood cells until the age of six months.
3. Individuals older than six months should have their ABO blood-grouping results confirmed by testing their serum or plasma against known group A_1 and B cells before their ABO blood group can be confirmed.
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 reagent has been characterised by all the procedures mentioned in the **Recommended Technique**.
2. Prior to release, each lot of Lorne Anti-A_1 Lectin reagent is tested by the **Recommended Technique** against a panel of antigen-positive red cells to ensure suitable reactivity.
3. The Quality Control of the reagent was performed using red cells that had been washed twice with PBS prior to use.
4. The reagent complies 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 reagent 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. Widman FK. Technical Manual, 9th Edition. American Association of Blood Banks, Arlington, VA, 1985; Chapter 8
2. Race RR, Sanger R. Blood Groups in Man, 6th Edition. Blackwell Scientific, Oxford 1975; Chapter 2
3. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition. Blackwell Scientific, Oxford 1987; Chapter 7
4. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6
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.

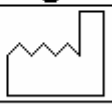
AVAILABLE REAGENT SIZES

Vial Size	Catalogue Number
5 ml	116005
1000 ml	116000

For the availability of other sizes, please contact:

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

TABLE OF SYMBOLS

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