LORNE LABORATORIES LTD.
GREAT BRITAIN

HUMAN BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE

Anti-M Polyclonal: For Tube, Bio-Rad-ID and Ortho BioVue Techniques.

SUMMARY

M antigen is part of the MNS system and was first reported in 1927. M antigen expression on red cells can show dosage. Anti-M has rarely been implicated in Haemolytic Disease of the Newborn or in Haemolytic Transfusion Reactions.

Anti-M	Anti-N	Phenotype	Caucasians %	Afro-Americans %
+	0	**M+N-**	28	26
+	+	**M+N+**	50	44
0	+	**M-N+**	22	30

PRINCIPLE

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

REAGENT

Lorne Human Anti-M blood grouping reagent is prepared from human serum diluted in 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

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°C and –25°C 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 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.
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 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.
9. Materials used to produce reagent were tested and found 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 reagent and decontamination of a spillage site see **Safety Data Sheets**, available on request.

CONTROLS AND ADVICE

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

REAGENTS AND MATERIALS REQUIRED

- Bio-Rad ID-Cards (NaCl, Enzyme test and Cold Agglutinins).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or Bio-Rad ID-Diluent 2.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5)
- Positive (ideally M+N+) and negative (N+N+) control red cells.
- Refrigerator set at 2-8ºC.
- Test tube centrifuge.
- Volumetric pipettes.

RECOMMENDED TECHNIQUES

A. Tube Technique

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 the tubes at 2-8ºC 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. Bio-Rad ID Micro Typing Technique

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 a Bio-Rad NaCl, Enzyme test and Cold Agglutinins gel card as needed.
3. Place in appropriate microtube: 50µl of red cell suspension and 25µl of Lorne reagent.
4. Incubate the ID-Card(s) for 15 minutes at 2-8ºC.
5. Centrifuge ID-Card(s) in a Bio-Rad ID centrifuge.
6. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique

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 an Ortho BioVue Neutral gel card 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 2-8ºC.
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 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 antigen on the red cells.

STABILITY OF THE REACTIONS

1. 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. 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.
2. 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.
3. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative reactions and so caution should always be exercised when assigning genotypes on the basis of test results.
4. 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 Techniques**.
2. Prior to release, each lot of Lorne Anti-M reagent is tested by the **Recommended Techniques** against a panel of antigen-positive red cells to ensure suitable reactivity.
3. 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 reagents previously absorbed to remove interfering specificities.
4. 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
5. The Quality Control of the reagent was performed using red cells that had been washed twice with PBS or Isotonic saline prior to use.
6. 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
2 ml	311002
1000 ml	311000*

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

For the availability of other sizes, please contact:

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

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