LORNE LABORATORIES LTD.
GREAT BRITAIN

MONOCLONAL BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE

Anti-M Monoclonal: For Tube, Bio-Rad-ID, Ortho BioVue and Microtitre plate Techniques.

SUMMARY

The 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[1]	Afro-Americans[1]
+	0	**M+N-**	28%	25.4%
+	+	**M+N+**	50%	48.4%
0	+	**M-N+**	22%	26.7%

INTENDED PURPOSE

The reagent is a blood grouping reagent intended to be used to qualitatively determine the presence or absence of the M antigen 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 M antigen on human red cells and causes direct agglutination (clumping) of human red cells that carry the M antigen. No agglutination (no clumping) generally indicates the absence of the M antigen (see **Limitations**).

REAGENTS

Lorne Monoclonal Anti-M blood grouping reagent is a reagent containing a murine monoclonal IgG antibody (clone # LM110/140), diluted in a buffer containing sodium chloride (0.6 g%), bovine albumin (4.0 g%) and a preservative. The reagent does 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 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 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 unbuffered saline solution 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 reagents 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 reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

CONTROLS AND ADVICE

1. A positive control (ideally heterozygous) and a negative control shall be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. When typing red cells from a patient who is diagnosed with a disease that causes the red cells to become coated with antibody or other proteins (such as HDN, AIHA), it is important to test the patient's red cells using Lorne's Negative Control (catalogue # 650010). Tests must be considered invalid if red cells are agglutinated using Lorne's Negative Control.
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. 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 reagent is in use.
6. The end user must determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

Tube Technique
- Centrifuge capable of spinning at 1000 g for 20 seconds.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Positive (ideally M+N+) and negative (N+N+) control red cells.

Bio-Rad-ID Micro Typing Technique
- Bio-Rad ID-Cards (NaCl, Enzyme tests and Cold Agglutinins).
- Bio-Rad ID-Centrifuge.

Ortho BioVue Typing Technique
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.

Microtitre plate Technique
- Validated "U" well microtitre plates.
- Microtitre plate centrifuge.
- Plate shaker.

All Techniques
- Volumetric pipettes.
- Unbuffered saline solution.

RECOMMENDED TECHNIQUES

A. Tube Technique

1. Prepare a 2-3% suspension of red cells in unbuffered saline (**see Limitations**).
2. Place in a labelled test tube: 1 volume of Lorne reagent and 1 volume of red cell suspension.
3. 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. Ortho BioVue Typing Technique (Neutral cards)

1. Prepare a 0.8% suspension of red cells in unbuffered saline (**see Limitations**).
2. Remove aluminium foil from as many reaction chambers on Neutral cassettes as needed.
3. Place in appropriate reaction chamber: 50µl of red cell suspension and 40µl of Lorne reagent.
4. Centrifuge cassette(s) in an Ortho BioVue Centrifuge.
5. Read macroscopically for agglutination.

C. Bio-Rad ID Micro Typing Technique

1. Prepare a 0.8% suspension of red cells in unbuffered saline (**see Limitations**).
2. Remove aluminium foil from as many microtubes on NaCl, Enzyme tests and Cold Agglutinins ID-Card(s) as needed.
3. Place in appropriate microtube: 50µl of red cell suspension and 25µl of Lorne reagent.
4. Centrifuge the ID-Card(s) in a Bio-Rad ID centrifuge.
5. Read macroscopically for agglutination.

D. Microtitre plate Technique, using "U" wells

1. Prepare a 2-3% suspension of red cells in unbuffered saline (**see Limitations**).
2. Place in the appropriate well: 1 volume Lorne reagent and 1 volume of red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
4. Incubate at room temperature for 15 minutes (time dependant on user).

5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Re-suspend the cell buttons using carefully controlled agitation on a microplate shaker
7. Read macroscopically or with a validated automatic reader.
8. Any weak reactions should be repeated by the tube technique.

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 M 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 M antigen on the red cells.

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. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. This reagent reacts optimally with M antigens at pH 8.5. Even though the reagent contains an ideal buffer for this pH, the following points should be adhered to:
   - Suspensions of red cells in buffered media (e.g. Alsevers) should be washed at least 3 times in unbuffered saline before use.
   - The use of buffered media for washing or making red cells suspensions may give spurious test results and should be avoided.
   - Unbuffered saline with a pH of less than 6 should not be used for washing or making red cells suspensions.
2. Cells modified by proteolytic enzymes must not be used as the MN antigens may have been destroyed.
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 phenotypes 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. Prior to release, each lot of this 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. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
3. 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 unbuffered 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[5].

BIBLIOGRAPHY

1. Marion E.Reid & Christine Lomas-Francis, Blood Group Antigens & Antibodies, SBB Books, New York 2007; Page 190.
2. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 14.
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.

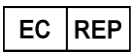
AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number	Tests per vial
Anti-M Monoclonal	2 ml	772002	40

TABLE OF SYMBOLS

Symbol	Definition	Symbol	Definition
	Manufacturer		Catalogue number
	Temperature limitation		Use by YYYY-MM-DD
	In vitro diagnostic medical device		Consult instructions for use.
	Authorised Representative		Lot number
	CE symbol with verification by a Notified Body		

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



Advena Ltd. Tower Business Centre, 2nd Flr., Tower Street, Swatar, BKR 4013, Malta