LORNE LABORATORIES LTD.

GREAT BRITAIN

CE 1434

BLOOD GROUPING REAGENTS

DIRECTIONS FOR USE

Anti-C, Anti-E, Anti-c and Anti-e Monoclonal: For Tube, Bio-Rad-ID, Ortho BioVue, Microplate + Slide Techniques.

SUMMARY

Levine and Stetson discovered the Rh blood group system in 1940. Apart from D the other major Rh antigens are C, E, c and e. The D antigen is highly immunogenic; the C and e antigens are less immunogenic than E and c. The corresponding antibodies are all clinically significant since they may cause both Transfusion Reactions and Haemolytic Disease of the Newborn.

Major Rh Antigens			
C	**E**	**c**	**e**
70	30	80	98

Table 1: Frequency of each antigen in Caucasian population.

PRINCIPLE

The reagents will cause direct agglutination (clumping) of red cells that carry the corresponding Rh antigen. No agglutination generally indicates the absence of the corresponding Rh antigen (see **Limitations**).

REAGENTS

Lorne Monoclonal IgM Anti-Rh blood grouping reagents are low protein reagents containing human monoclonal antibodies diluted with sodium chloride (0.9 g%), bovine albumin (6 g%) and macromolecular potentiators. Each reagent is supplied at optimal dilution for use with all recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

Reagent	Cell Line / Clone
Anti-C	MS-24
Anti-E	MS-258
Anti-c	MS-33
Anti-e	MS-16 + MS-63

Table 2: Human IgM Cell Lines / Clones used.

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 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 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 products 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. It is recommended a positive control (ideally heterozygous) 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. When typing red cells from patients known or suspected to have auto-antibodies, protein abnormalities or a positive Direct Antiglobulin Test (DAT), it is important that a reagent negative control is tested in parallel. For the reagent negative control, only Lorne Monoclonal Rh Control, catalogue number 640010, must be used.
3. Weak Rh antigens may be poorly detected by the gel card, microtitre plate and slide technique. It is recommended that weak Rh antigens are tested using the tube test technique.
4. In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
5. The use of reagents and interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use. The user must the determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Applicator sticks.
- Automatic plate reader.
- Bio-Rad ID-Cards (NaCl, Enzyme and Cold agglutinins).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or ID-Diluent 2.
- Glass microscope slides.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Microplate centrifuge.
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.
- Plate shaker.
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive and negative control red cells:
  Monoclonal Anti-C: R_1r (positive control) and rr (negative control).
  Monoclonal Anti-E: R_2r (positive control) and rr (negative control).
  Monoclonal Anti-c: R_1r (positive control) and R_1R_1 (negative control).
  Monoclonal Anti-e: R_2r (positive control) and R_2R_2 (negative control).
- Test tube centrifuge.
- Card tiles.
- Glass microscope slides.
- Validated “U” well microplates.
- 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 Lorne Anti-Rh reagent and 1 volume red cell suspension.
3. Mix thoroughly and 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
5. Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
6. Following incubation, repeat steps 3 and 4.

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 the NaCl/Enzyme/Cold agglutinins ID-Card as needed.
3. Place in appropriate microtube: 50µl of red cell suspension and 25µl of Lorne Anti-Rh reagent.
4. Centrifuge the ID-Card(s) in a Bio-Rad ID centrifuge.
5. 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 the Neutral cassette as needed.
3. Place in appropriate reaction chamber: 50µl of red cell suspension and 40µl of Lorne Anti-Rh reagent.
4. Centrifuge cassette(s) for 5 minutes in an Ortho BioVue System Centrifuge.
5. Read macroscopically for agglutination.

D. Microplate Technique, using “U” wells

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
2. Place in the appropriate well: 1 volume Lorne Anti-Rh reagent and 1 volume 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. Resuspend 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.

E. Slide Technique

1. Prepare a 35-45% suspension of red cells in serum, plasma or PBS or Isotonic saline. If this is not possible, whole anti-coagulated blood may also be used as the sample.
2. Place on a labelled glass slide or card tile: 1 volume of Lorne Anti-Rh reagent and 1 volume of 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 for 1-minute, maintaining slide at room temperature.
5. Read macroscopically after 1 minute over a diffuse light and do not mistake fibrin strands as agglutination.
6. 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 appropriate Rh 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 Rh antigen on the red cells.
3. Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the effect of the macromolecular potentiators in the reagent on sensitised cells.

STABILITY OF THE REACTIONS

1. Read all tube and microplate tests straight after centrifugation.
2. Slide tests should be interpreted within one minute 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. Lorne Anti-Rh reagents are not suitable for use with enzyme treated cells or for use in indirect antiglobulin techniques.
2. Many Monoclonal human IgM anti-Rh antibodies have been shown to possess anti-i/I cold agglutinin activity, particularly with cord cells or enzyme treated cells. This may become apparent if tests are incubated below the recommended temperature.
3. Some red cells express variant Rh antigens and may give weaker reactions than seen with randomly selected positive control cells. Anti-C may give weaker reactions with C antigen of R_2R_Z individuals. Similarly, Anti-e may give slightly weaker reactions in absence of C antigen, e.g. R_2r, r"r and rr.
4. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative reactions. For these reasons, caution should always be exercised when assigning genotypes on the basis of test results.
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-C, Anti-E, Anti-c and Anti-e is tested by the **Recommended Techniques** 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 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 Techniques**.
2. Any deviations from the **Recommended Techniques** should be validated prior to use[9].

BIBLIOGRAPHY

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5. Tippett P. Sub-divisions of the Rh (D) antigen. Medical. Laboratory Science 1988; **45**, 88-93
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8. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
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AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number
Anti-C Monoclonal	5 ml	690005
	1000 ml	690000*
Anti-E Monoclonal	5 ml	691005
	1000 ml	691000*
Anti-c Monoclonal	5 ml	692005
	1000 ml	692000*
Anti-e Monoclonal	5 ml	693005
	1000 ml	693000*

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

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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		