# LORNE LABORATORIES LTD.
GREAT BRITAIN

CE 1434

## MONOCLONAL BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE

**Anti-C+D+E Monoclonal:** For Tube, Bio-Rad-ID, Ortho BioVue, Microplate and 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.

| MODIFIED WIENER HAPLOTYPE | PREVALENCE (%) | | |
|---|---|---|---|
| | WHITE | BLACK | ASIAN |
| $R_1$ | 42 | 17 | 70 |
| $R_2$ | 14 | 11 | 21 |
| $R_0$ | 4 | 44 | 3 |
| $R_Z$ | <0.01 | <0.001 | 1 |
| r | 37 | 26 | 3 |
| r' | 2 | 2 | 2 |
| r'' | 1 | <0.01 | <0.01 |
| $r^Y$ | <0.01 | <0.01 | <0.01 |

### INTENDED PURPOSE

The reagent is a blood grouping reagent intended to be used to qualitatively determine the presence or absence of the C antigen (RH2) and/or D antigen (RH1) and/or E antigen (RH3) 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 C and D and E antigens on human red cells and will cause direct agglutination (clumping) of human red cells that carry the C and/or D and/or E antigen. No agglutination (no clumping) generally indicates the absence of the corresponding Rh antigen (see **Limitations**).

### REAGENTS

Anti-C+D+E Monoclonal blood grouping reagent is a low protein reagent containing human monoclonal antibodies directed against Rhesus C, D and E antigens. 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. 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**.

| | | |
|---|---|---|
| **Anti-C+D+E Monoclonal** | RUM-1 | Hybridoma cell line secreting Human IgM |
| | MS-24 | Hybridoma cell line secreting Human IgM |
| | MS-258 | Hybridoma cell line secreting Human IgM |

### 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 in-use and transportation stability studies (at 37ºC and –25ºC) as described in document BS EN ISO 23640:2015. According to the reagents' in-use stability studies, once vials are 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.

### 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 between 2°C and 8ºC. EDTA or clotted blood may be stored up to 1 day after collection at ambient temperature and up to 21 days at refrigerated temperature (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 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, 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 ($NaN_3$). 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. 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 it is important that a reagent negative control (Mono Rh Control, Lorne catalogue number 640010) is included since the macromolecular potentiators in the reagent may cause false positive reactions with IgG coated cells.
3. Weak Rhesus antigens may be poorly detected by the gel card, microtitre plate and slide technique. It is recommended that weak Rhesus antigens are tested using the tube test technique.
4. 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.
5. In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
6. 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 determine the suitability of the reagents for use in other techniques.

### REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

#### Tube Technique
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Centrifuge capable of spinning at 1000 g for 20 seconds.
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive (ideally $R_1r$) and negative (rr) control red cells.

#### Bio-Rad-ID Micro Typing Technique
- Bio-Rad ID-Cards (NaCl, Enzyme tests and Cold Agglutinins).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or ID-Diluent 2.

#### Ortho BioVue Typing Technique
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.

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

#### Slide Technique
- Glass microscope slides or white card tiles.
- Applicator sticks.
- Timer or stopwatch

#### All Techniques
- Volumetric pipettes.

### RECOMMENDED TECHNIQUES

#### A. Tube Technique

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline 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 Technique (NaCl, enzyme test and cold agglutinins cards)

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 NaCl, Enzyme tests and 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 Technique (Neutral cassettes)

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 a 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. Microtitre plate Technique, using "U" wells

0. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline or Isotonic saline.
1. Place in the appropriate well: 1 volume Lorne Anti-Rh reagent and 1 volume red cell suspension.
2. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
3. Incubate at room temperature for 15 minutes (time dependant on user).
4. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
5. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker
6. Read macroscopically or with a validated automatic reader.
7. 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 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 mminute, 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. **Control:** The reaction can be interpreted if the results obtained with the control samples are valid. Test results of cells that are agglutinated using Monoclonal Rh Control (ref.640010) 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 immediately 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. 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.
3. 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.
4. Anti-C+D+E Monoclonal (REF:700010) is not validated to detect Rhesus antigens other than human D (RH1), C (RH2) and E (RH3) erythrocytic antigens. Moreover, Anti-C+D+E Monoclonal reagent is not validated with weak D and DVI phenotypes because the combination of Dweak and/or DVI with negative C and E antigens at the same time, is extremely rare. To detect weak D and/or DVI, the Lorne's appropriate Anti-D Monoclonal reagents shall be used according to the manufacturer's instructions for use.
5. Anti-C+D+E Monoclonal (REF:700010) is not validated to detect the Rhmod variant.
6. 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.
7. 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 precommended 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 reagent was performed using red cells with phenotypes that were verified by a UK blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.
4. The performance of the regents was confirmed against well-established CE marked comparator IVD reagent(s) in in a comparison study where the reagents were tested in parallel with all recommended methods. The overall statistical analysis results of the study are shown below:

| | Method | N | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|
| **Anti-C+D+E** | Test Tube | 1047 | 99.9% | 98.5% | 99.6% | 99.5% |
| | Ortho | 1251 | 99.9% | 99.6% | 99.9% | 99.6% |
| | Bio-Rad | 1047 | 99.8% | 99.5% | 99.9% | 99.0% |
| | Microplate | 1047 | 99.6% | 99.0% | 99.8% | 98.5% |
| | Slide | 1027 | 99.6% | 100.0% | 100.0% | 98.5% |

## 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. Issitt PD. Applied Blood Group Serology, 3rd Edition, Montgomery Scientific, Miami, 1985, Chapter 10.
2. AABB Technical Manual, 16th edition, AABB 2008.
3. Jones J, Scott ML, Voak D. Monoclonal anti-D specificity and Rh D structure: criteria for selection of monoclonal anti-D reagents for routine typing of patients and donors. Transfusion Medicine 1995. **5**, 171-184
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-C+D+E Monoclonal** | 10 ml | 700010 | 200 |

## 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 | | |


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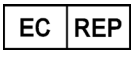


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