**MONOCLONAL BLOOD GROUPING REAGENTS.**

**DIRECTIONS FOR USE**

**Anti-D Clone 1 and Clone 2 Monoclonal:** For Tube, Bio-Rad-ID, Ortho BioVue, Microplate and Slide Techniques.

**SUMMARY**
The Rh blood group system was discovered in 1940. The D antigen is the most clinically significant non-ABO red blood cell antigen and has been implicated in causing Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

**Anti-D Phenotype** | Caucasians | Afro-Americans %
--- | --- | ---
D+ | 85 | 72
D- | 15 | 28

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

**REAGENT**
Lorne Monoclonal IgM Anti-D Clone 1 and Clone 2 blood grouping reagents are low protein reagents containing a human monoclonal IgM antibody diluted with sodium chloride (0.9 g%), bovine albumin (3 g%) and macromolecular potentiators. When typing patient samples, each reagent will directly agglutinate Rh D positive cells, including majority of variants (but not DVI) and a high proportion of weak D (D-) phenotypes when using the recommended techniques. Each reagent is supplied at optimal dilution for use on patient samples with all recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see Vial Label.

**CONTROLS AND ADVICE**
- **Applicator sticks.**
- **Automatic plate reader.**
- **Bio-Rad ID-Cards (NaCl, enzyme test and cold agglutinins).**
- **Bio-Rad ID-Centrifuge.**
- **Bio-Rad ID-CellStab or ID-Diluent 2.**
- **Glass microscope slides or white card tiles.**
- **Glass test tubes (10 x 75 mm or 12 x 75 mm).**
- **Microplate centrifuge.**
- **Ortho BioVue System Centrifuges.**
- **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 (ideally R1r) and negative (rr) control red cells.**
- **Test tube centrifuge.**
- **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 of Lorne Anti-D reagent and 1 volume of test red cell suspension. 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.
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. 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 as needed.
3. Place in appropriate microtube: 50μl of red cell suspension and 25μl of Lorne Anti-D reagent.
4. Centrifuge ID-Card(s) in a Bio-Rad gel card centrifuge.
5. Read macroscopically for agglutination.

**C. Ortho BioVue Typing Technique (Neutral cards)**
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 as needed.
3. Place in appropriate reaction chamber: 50μl of red cell suspension and 25μl of Lorne Anti-D reagent.
4. Centrifuge cartridge(s) 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-D 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 or use anti-coagulated whole blood (in its own plasma).
2. Place on a labelled glass slide or card tile: 1 volume of Lorne Anti-D 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 30 seconds, with occasional further mixing during the 1-minute period, maintaining slide at room temperature.
5. Read macroscopically after 1 minute over a diffuse light and do not make 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 D 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 D 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-D is not suitable for use with enzyme treated cells, cells suspended in LISS or for use in indirect antiglobulin (IAT) techniques.
2. Stored blood may give weaker reactions than fresh blood.
3. False positive agglutination may be seen due to the presence of macromolecular potentiators in the reagent when testing IgG sensitised cells, e.g. AIHA, HDN.
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 reagents have been characterised by all the procedures mentioned in the Recommended Techniques.
2. Prior to release, each lot of Lorne Monoclonal Anti-D Clone 1 and Anti-D Clone 2 is tested by the Recommended Techniques against a panel of antigen-positive red cells to ensure suitable reactivity.
3. Anti-D grouping reagents for D grouping of patients should not react with DVI cells using the method(s) recommended for use.
4. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
5. The potency of the reagents has been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC):
   - Anti-D reference 99/836.
6. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS or isotonic saline prior to use.
7. 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*

BIBLIOGRAPHY
5. Tipper P. Sub-divisions of the Rh (D) antigen. Medical. Laboratory Science 1988, 45, 88-93.
6. Thompson KM, Hughes-Jones NC. Production and characteristics of monoclonal anti-Rh. Bailliere’s Clinical Haematology 1990; April

AVAILABLE REAGENT SIZES

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

TABLE OF SYMBOLS