MONOCLONAL BLOOD GROUPING REAGENTS.

DIRECTIONS FOR USE
Anti-A, Anti-B and Anti-A,B Monoclonal:
For Tube, DiaMed-ID, Ortho BioVue, Microplate and Slide Techniques.

SUMMARY
In 1900, Landsteiner discovered the serum of some people would agglutinate the red cells of others. Four common phenotypes are now recognised: O, A, B and AB. Subgroups of A and B have since been identified.

<table>
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<th>Forward Group</th>
<th>Revert Group</th>
<th>ABO Phenotype</th>
<th>Caucasians</th>
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<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>A</td>
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<tr>
<td>A</td>
<td>AB</td>
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<td>10</td>
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<tr>
<td>B</td>
<td>AB</td>
<td>O</td>
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PRINCIPLE
The reagents will cause direct agglutination (clumping) of test red cells that carry the corresponding ABO antigen. No agglutination generally indicates absence of the corresponding ABO antigen (see Limitations).

REAGENT
Lorne Monoclonal IgM ABO blood grouping reagents contain mouse monoclonal antibodies diluted in a phosphate buffer containing sodium chloride, EDTA and bovine albumin. Each reagent is supplied at optimal dilution for use with all the recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see Vial Label.

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.
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 bioburden. Once a vial has been opened the contents should remain viable by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
7. The user must determine the suitability of the reagents for use in other techniques.

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 EN13340:2002.

SAMPLE COLLECTION AND PREPARATION
Blood samples drawn with or without an anticoagulant may be used for antigen typing. If testing is delayed, then store specimens at 2-8ºC. EDTA and citrate samples should be typed within 7 days after collection. Samples collected into tubes should be typed within 7 days after collection. Samples collected into EDTA and citrate should be stored at 2-8ºC. EDTA and citrate samples should be typed within 7 days after collection. PBS solution (pH 6.8-7.2) or Isotonic saline solution (pH 6.5-7.5).

CONTROLS AND ADVICE
1. It is recommended a positive control 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. Since these reagents do not contain macromolecular potentiators, it is very unlikely that false positive reactions are caused with IgG coated cells. The reagents will cause direct agglutination (clumping) of test red cells that carry the corresponding ABO antigen. No agglutination generally indicates absence of the corresponding ABO antigen (see Limitations).
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.
4. Read macroscopically or with a validated automatic reader. Centrifuge the centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
5. Read macroscopically for agglutination.

RECOMMENDED TECHNIQUES
A. Tube Technique
1. Prepare a 2-3% suspension of washed test red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume of Lorne Anti-ABO reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at room temperature for 1 minute.
4. Centrifuge all tubes for 10 seconds at 1000 rcf for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination.
6. Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
7. Centrifuge the centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
8. Read macroscopically for agglutination.

B. DiaMed-ID Micro Typing Technique
1. Prepare a 0.8% suspension of washed test red cells in PBS or Isotonic saline.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 5μl of test red cell suspension and 25μl of Lorne Anti-ABO reagent.
4. Centrifuge the centrifuge cassette(s) in the DiaMed gel card centrifuge.
5. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique
1. Prepare a 0.8% suspension of washed test 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: 5μl of test red cell suspension and 40μl of Lorne Anti-ABO reagent.
4. Centrifuge the centrifuge cassette(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 washed test red cells in PBS or Isotonic saline.
2. Place in the appropriate well: 1 volume Lorne Anti-ABO reagent and 1 volume of test 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 dependent 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. Any weak reactions should be repeated by the tube technique.

E. Slide Technique
1. Prepare a 33-45% suspension of test red cells in serum, plasma or PBS or Isotonic saline.
2. Place on a labelled glass slide: 1 volume of Lorne Anti-ABO reagent and 1 volume of test red cell suspension.
3. Use a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.
4. Read macroscopically after 2 minutes over a diffuse light and do not mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination. Incubate at room temperature for 15 minutes (time dependent 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. Read macroscopically or with a validated automatic reader. Any weak reactions should be repeated by the tube technique.
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 test red cells constitutes a positive test result and within accepted limitations of the test procedure, indicates the presence of the appropriate ABO antigen on the test red cells.
2. Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the appropriate ABO antigen on the test red cells.
3. Discrepancies: If the results obtained with reverse group don’t correlate with forward group, further investigation is required.

STABILITY OF THE REACTIONS
1. Read all tube and microplate tests straight after centrifugation.
2. Slide tests should be interpreted within two minutes 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. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. When using Monoclonal Anti-A,B, blood specimens of weak A or B subgroups (e.g. Ax) may give rise to false negative or weak reactions when tested using slides, microtitre plates or gel cards. It is advisable to re-test weak subgroups using the tube technique.
3. Lorne monoclonal Anti-A and monoclonal Anti-B are not validated to detect Ax and A3 or Bx and B3 antigens resp and we therefore do not claim reactivity of the monoclonal Anti-A or Anti-B reagent against these weak A and B sub-groups.
4. Stored blood may give weaker reactions than fresh blood.
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 • Cord samples contaminated with Wharton’s jelly

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-A, Anti-B and Anti-A,B 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 potency of the reagents has been tested against the following minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-A reference standard 03/188 And / Or Anti-B reference standard 03/161.
5. Lorne Anti-B does not react with “Acquired-B” red cells.
6. Lorne Monoclonal ABO reagents do not detect crypt antigens such as T, Tn or Cad.
7. The Quality Control of the reagents was performed using red cells that had been washed at least twice with PBS or Isotonic saline prior to use.
8. 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

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

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