MONOCLONAL BLOOD GROUPING REAGENTS.

DIRECTIONS FOR USE

Anti-D Duoclone Monoclonal:
For Tube, Bio-Rad/DiaMed-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.

PRINCIPLE

The reagent will cause direct agglutination (clumping) of test red cells that carry the D antigen and indirect agglutination of red cells that are Category D\(^n\) in the anti-D phase of testing. No agglutination generally indicates the absence of the D antigen (see Limitations).

REAGENT

Lorne Monoclonal Anti-D Duoclone blood grouping reagent is a low protein, blended reagent containing a human monoclonal IgM and IgG anti-D, diluted in a phosphate buffer containing sodium chloride (0.9 g%), bovine albumin (3 g%) and macromolecular potentiators. When typing patient samples, this reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D\(^n\)) and a high proportion of weak D (D\(^+\)) phenotypes when using the recommended techniques. The 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

see Material Safety Data Sheets, available on request.

For information on disposal of the reagent and decontamination of a spillage site indicate reagent deterioration or contamination.

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**Reagent**

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**Controls and Advice**

1. It is recommended that a positive control (ideally R1r cells) and a negative control (ideally rR cells) be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.

2. When testing 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 reagent negative control (Monoclonal D Negative Control, catalogue # 650010). Tests must be considered invalid if red cells are agglutinated using Lorne’s Monoclonal D Negative Control (catalogue # 650010).

3. Test samples for category D\(^n\) determination by the Indirect Antiglobulin, Coombs Bio-Rad/DiaMed-ID and Coombs Ortho BioVue Techniques only.

4. Weak and variant D antigens are poorly detected by gel card, microtitre plate and slide techniques. It is recommended that weak and partial variants are tested using the tube test technique.

5. The antiglobulin tube technique can only be considered valid if all negative controls remain negative.

6. In the Recommended Techniques one volume is approximately 50μl when using the vial dropper provided.

7. The use of the reagent 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 reagents are in use.

8. The user must determine suitability of reagents for use in other techniques.

**REAGENTS AND MATERIALS REQUIRED**

- Anti-human globulin e.g. Lorne AHG Elite (Cat # 435010) or Anti-Human IgG e.g. Lorne Anti-Human IgG (Cat # 402010).
- Applicator sticks.
- Automatic plate reader.
- Coombs cell washer.
- Bio-Rad/DiaMed ID-Cards (US/Coombs) and (Neutral).
- Bio-Rad/DiaMed ID-Centrefuge.
- Bio-Rad/DiaMed ID-CellStab or ID-Diluent 2.
- Bio-Rad/DiaMed ID-Incubator equilibrated to 37°C ± 2°C.
- Glass microscope slides.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- IgG sensitised red cells e.g. Lorne Coombs Control Cells (Cat # 970010).
- Microplate centrifuge.
- Ortho BioVue System Cassettes (AHA/Coombs) and (Neutral).
- Ortho BioVue System Centrfuge.
- Ortho BioVue System Heat Block equilibrated to 37°C ± 2°C.
- 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 R\(^+\)) and negative (rr) control red cells.
- Test tube centrifuge.
- Validated “U” well microplates.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.

**RECOMMENDED TECHNIQUES (NOT CATEGORY D\(^n\)) Tube Technique**

1. Prepare a 2-3% suspension of washed red cells in PBS or Isotonic saline.

2. Place in a labelled test tube: 1 volume of Lorne Duoclone reagent and 1 volume of test 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 (which can happen with D\(^+\) or weak D samples), should be incubated for 15 minutes at room temperature.

6. Following incubation, repeat steps 3 and 4.

**B. Bio-Rad/DiaMed-ID Micro Typing Technique (Neutral cards)**

1. Prepare a 0.8% suspension of washed red cells in ID-CellStab or ID-Diluent 2.

2. Remove aluminium foil from as many microtubes as needed.

3. Place in appropriate well: 1 volume of Lorne Duoclone reagent and 1 volume of test red cell suspension.

4. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.

5. Centrifuge the ID-Card(s) in a Bio-Rad/Diamed gel card centrifuge.

6. Read macroscopically for agglutination.

**C. Ortho BioVue Typing Technique (Neutral cards)**

1. Prepare a 0.8% suspension of washed 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 test red cell suspension and 25μl Lorne Duoclone reagent.

4. Centrifuge the ID-Card(s) in an Ortho BioVue System Centrfuge.

5. Read macroscopically for agglutination.

**D. Microplate Technique, using “U” wells**

1. Prepare a 2-3% suspension of washed red cells in PBS or Isotonic saline.

2. Place in the appropriate well: 1 volume of Lorne Duoclone reagent and 1 volume of test red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-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.
8. Any weak reactions should be repeated by the tube technique.

**E. Slide Technique**
1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS or Isotonic saline.
2. Place on a labelled glass slide: 1 volume of Lorne Duoclone reagent and 1 volume of red cell suspension.
3. Mix thoroughly and incubate at 37°C for 15 minutes.
4. Wash test red cells 4 times with PBS or Isotonic saline, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
5. Add 2 drops of anti-human globulin or anti-IgG to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf for a suitable alternative time and force.
7. Resuspend each cell button and read macroscopically.
8. Confirm validity of all negative reactions with IgG sensitised red cells.

**B. Bio-Rad/DiaMed-ID Micro Typing Technique (LISS/Coombss cards)**
1. Prepare 0.8% suspension of washed red cells in ID-CellStab or ID-Diluent 2.
2. Remove aluminium foil from as many reaction chambers as needed.
3. Place in appropriate microtube: 50μl of red cell suspension and 25μl of Lorne Duoclone.
4. Incubate the ID-Card(s) for 15 minutes at 37°C.
5. Centrifuge the ID-Card(s) in a Bio-Rad/Diamond gel card centrifuge.
6. Read macroscopically for agglutination.

**C. Ortho BioVue Typing Technique (AHG/Coombss cards)**
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: 50μl of red cell suspension and 25μl of Lorne Duoclone.
4. Incubate the cassette(s) for 15 minutes at 37°C.
5. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
6. Read macroscopically for agglutination.

**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 test 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 test 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. Complete washing steps without interruption and centrifuge and read tests immediately after addition of anti-human globulin because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.
3. Slide tests should be interpreted within 1 minute to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
4. 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 or cells suspended in LISS.
2. The use of solutions for making red cell suspensions other than those described in the “Recommended Techniques” sections in the document must be validated prior to use. Some solutions may give rise to false positive or false negative reactions.
3. Stored blood may give weaker reactions than fresh blood.
4. False positive agglutination may be seen when testing IgG sensitised cells.
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 reagent has been characterised by all the procedures mentioned in the Recommended Techniques.
2. Prior to release, each lot of Lorne Monoclonal Anti-D Duoclone 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 reagent 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.
5. The Quality Control of the reagent was performed using red cells that had been washed twice with PBS or Isotonic saline prior to use.
6. The reagent complies 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 reagent 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**

| LOT | Batch Number | IVD | In-vitro Diagnostic
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| Read Pack Insert | |

**LORNE LABORATORIES**

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