MONOCLONAL BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE
Anti-D Duoclone Monoclonal Standard Grade
For Tube, Bio-Rad-ID, Ortho BioVue Microplate and Slide Techniques

LORNE LABORATORIES

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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 %
+	Rh D +ve	85	72
0	Rh D -ve	15	28

PRINCIPLE

The reagent will cause direct agglutination (clumping) of test red cells that carry the D antigen and indirect agglutination of test red cells that are Category D^{VI} in the antiglobulin 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 weak D (D^u) phenotypes and indirectly agglutinate DVI phenotypes when using the recommended techniques. The reagent is supplied at optimal dilution without need for further dilution or addition. For lot reference number and expiry date see Vial Label.

Product	Cell Line/Clone	Type of Antibody
Anti-D Duoclone (RH1)	RUM-1	Hybridoma cell line secreting Human IgM
	MS-26	Hybridoma cell line secreting Human IgG

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 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 reagent is not intended for in vitro diagnostic use.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagent past the expiration date (see Vial Label).
4. Do not use the reagent 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 reagent has 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 reagent contains <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 reagent 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.

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 known positive and known a negative control must be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results. The positive control is a sample possessing the antigen corresponding to the antibody in the reagent used, whereas the negative control is a sample devoid of the antigen corresponding to the antibody in the reagent used.
2. When typing red coated with antibody or other proteins (such as in HDN, AIHA), it is important to test the red cells using a reagent negative control (such as Lorne's Monoclonal D Negative Control, catalogue number 650010). Red cells coated with antibody or abnormal proteins can be agglutinated when suspended in reagents containing chemical potentiators.
3. Test samples for category D^{VI} determination by the Indirect Antiglobulin, Coombs Bio-Rad-ID and Coombs Ortho BioVue Techniques only.
4. Weak and variant D antigens are poorly detected by gel card, microtiter plate and slide techniques. It is recommended weak and partial variants are tested using the tube test technique.
5. The antiglobulin tube technique can only be considered valid if all negative tests react positively with IgG sensitized red cells.
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.
9. For serious incidents in other countries, please report it to the Manufacturer and, if applicable, to your National Competent Authority.

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 ID-Cards (LISS/Coombs) and (NaCl, enzyme test and cold agglutinins).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or ID-Diluent 2.
- Bio-Rad ID-Incubator equilibrated to 37ºC ± 2ºC.
- Glass microscope slides or white card tiles.
- 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 (AHG/Coombs) and (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho BioVue System Heat Block equilibrated to 37ºC ± 2ºC.
- 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_1r) and negative (rr) control red cells.
- Test tube centrifuge.
- Validated "U" well microplates.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.

RECOMMENDED TECHNIQUES (NOT CATEGORY D^{VI})

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 Duoclone reagent and 1 volume of 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^u or weak D samples), should be incubated for 15 minutes at room temperature.
6. Following incubation, repeat steps 3 and 4.

B. Bio-Rad-ID Micro Typing 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 as needed.
3. Place in appropriate microtube: 50µl red cell suspension and 25µl Lorne Duoclone reagent.
4. Centrifuge the 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 40µl of Lorne Duoclone reagent.
4. 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 red cells in PBS or Isotonic saline.
2. Place in the appropriate well: 1 volume of Lorne Duoclone reagent and 1 volume of 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 Duoclone reagent and 1 volume of test 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 mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

RECOMMENDED TECHNIQUES (TO DETECT CATEGORY DVI)

A. Indirect Antiglobulin Technique (IAT)
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 Duoclone and 1 volume of red cell suspension.
3. Mix thoroughly and incubate at 37ºC 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-ID Micro Typing Technique (LISS/Coombs cards)
1. Prepare 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 Duoclone.
4. Incubate the ID-Card(s) for 15 minutes at 37ºC.
5. Centrifuge the ID-Card(s) in a Bio-Rad gel card centrifuge.
6. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique (AHG/Coombs 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 40µl of Lorne Duoclone.
4. Incubate the cassette(s) for 15 minutes at 37ºC.
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 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. 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 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.
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. Stored blood may give weaker reactions than fresh blood.
3. False positive agglutination may be seen when testing IgG sensitised cells.
4. False positive or false negative results may also occur due to:
   - Contamination of test materials
   - Improper storage, cell concentration, incubation time or temperature

SPECIFIC PERFORMANCE CHARACTERISTICS
1. The reagent has been characterized 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.
   - Deviation from the recommended techniques
   - Improper or excessive centrifugation

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[7].

BIBLIOGRAPHY
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3. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition, Oxford, Blackwell Scientific Publications, 1987, Chapter 7.
4. Tippett P. Sub-divisions of the Rh (D) antigen. Medical. Laboratory Science 1988; 45, 88-93
5. 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
6. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
7. 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
10 ml	740010E
1000 ml	740000E
5000 ml	740000EX5

TABLE OF SYMBOLS

Symbol	Definition	Symbol	Definition
	Manufacturer		Catalogue number
	Temperature limitation		Use by YYYY-MM-DD
	Consult instructions for use.		Lot number

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