MONOCLONAL BLOOD GROUPING REAGENTS.

LORNE LABORATORIES

CE 0843

DIRECTIONS FOR USE

Anti-D Clone 1 and Clone 2 Monoclonal:

For Tube, 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.

Anti-D	Phenotype	Caucasians %	Afro-Americans %
+	Rh D +ve	85	72
0	Rh D -ve	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 D^{VI}) and a high proportion of weak D (D^{u}) 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.

Product	Cell Line / Clone
Anti-D Clone 1	RUM-1
Anti-D Clone 2	MS-201

WEAKENED EXPRESSION OF THE RhD ANTIGEN

The collective term D^{u} is widely used to describe red cells which have a weaker expression of the D antigen than normal. The term weak D denotes individuals with a reduced number of complete D antigen sites per red cell. The term partial D denotes individuals with missing D antigen epitopes. D^{VI} cells is a partial D category which misses most D epitopes. Both Clone 1 and Clone 2 reagents will detect most examples of partial and weak D red cells by direct agglutination, but will not detect D^{VI} cells.

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 EN13640:2002.

SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with or without 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 ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS or Isotonic saline before being tested. Samples showing evidence of lysis may give unreliable results.

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. 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. 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. It is recommended a positive control (ideally R$_1$r cells), a negative control (ideally rr cells) and a reagent negative control (such as Lorne Negative Control, catalogue # 650010) 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 is included since the macromolecular potentiators in the reagent may cause false positive reactions with IgG coated cells, e.g. in cases of AIHA or HDN. Lorne Negative Control for Monoclonal Anti-D Reagents (Cat. # 650010) is recommended.
3. Weak and partial D antigen variants are poorly detected by the gel card, microtitre plate and slide technique. It is recommended that weak and partial D variants are tested using the tube test technique.
4. In the Recommended Techniques one volume is approximately 50μl when using the vial dropper provided.
5. The use of the reagents 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.
6. The user must the determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Applicator sticks.
- Automatic plate reader.
- DiaMed ID-Cards (Neutral).
- DiaMed ID-Centrifuge.
- DiaMed ID-CellStab.
- Glass microscope slides.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Microplate centrifuge.
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- 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$_1$r) 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 washed test 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.
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 negative or questionable result (as can happen with weak D samples), should be incubated for 15 minutes at room temperature.
6. Following incubation, repeat steps 3 and 4.

B. DiaMed-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in ID-CellStab.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50μl of test red cell suspension and 25μl of Lorne Anti-D reagent.
4. Centrifuge ID-Card(s) in a Diamed gel card centrifuge.
5. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique (Neutral 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 test red cell suspension and 40μl of Lorne Anti-D 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 washed test red cells in PBS or Isotonic saline.
2. Place in the appropriate well: 1 volume Lorne Anti-D reagent and 1 volume 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 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 test red cells in serum, plasma or PBS or Isotonic saline.

2. Place on a labelled glass slide: 1 volume of Lorne Anti-D 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 2-minute period, maintaining slide at room temperature.
5. Read macroscopically after 2 minutes 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 test 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 test 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. 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. 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 Clone1 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 D^{VI} 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[9].

BIBLIOGRAPHY

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3. Issitt PD. Applied Blood Group Serology, 3rd Edition, Montgomery Scientific, Miami, 1985, Chapter 10.
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5. Tippett 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
7. 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
8. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
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AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number
Anti-D Clone 1 Monoclonal	10 ml	730010
	1000 ml	730000*
	5000 ml	730000X5*
Anti-D Clone 2 Monoclonall	10 ml	710010
	1000 ml	710000*
	5000 ml	710000X5*

*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
REF	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		

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