LORNE LABORATORIES LTD.
GREAT BRITAIN

MONOCLONAL BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE

Monoclonal Anti-C^w: For Tube, Bio-Rad-ID, Ortho BioVue and Slide Techniques.

SUMMARY

Levine and Stetson discovered the Rh blood group system in 1940. Apart from D the other major Rh antigens are C, E, c and e. The D antigen is highly immunogenic and the C and e antigens are less immunogenic than E and c. The C^w antigen is one of the more rare antigens, but Anti-C^w is a fairly commonly encountered antibody. All the Rh antibodies are clinically significant since they may cause both Transfusion Reactions and Haemolytic Disease of the Newborn.

CDE Term	Caucasian %	CDE Term	Caucasian %
D	85%	**c**	80%
C	70%	**e**	98%
E	30%	**C^w**	1%

Table 1: Frequency of each antigen.

PRINCIPLE

This reagent will cause agglutination (clumping) of red cells that carry the Cw antigen, after centrifugation. No agglutination generally indicates the absence of the Cw antigen (see **Limitations**).

REAGENTS

Lorne Monoclonal Anti-Cw blood grouping reagent is a reagent containing a human monoclonal IgM antibody, diluted in a phosphate buffer containing sodium chloride (0.6 g%), bovine albumin (6 g%) and a preservative. The reagent is supplied at optimal dilution for use with all recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

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 37°C and –25°C 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 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 reagents 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 heterozygous) 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. Weak Cw antigens may be poorly detected by the slide technique. It is recommended that weak Cw antigens are tested using the tube technique.
3. It is important that when typing the cells of patients known to have auto-antibodies or protein abnormalities to use a reagent control. Lorne Negative Control for Monoclonal Anti-D Reagents (Cat. # 650010) is recommended.
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 reagent is in use.
6. The user must the determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Applicator sticks.
- Bio-Rad ID-Cards (NaCl, Enzyme tests and Cold Agglutinins).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or ID-Diluent 2.
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.
- Glass microscope slides.
- Card tiles.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5)
- Positive (ideally heterozygous) and negative control red cells.
- Test tube centrifuge.
- 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 reagent and 1 volume of red cell suspension.
3. 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.

B. 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 on Neutral cassettes 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.

C. 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 on NaCl, Enzyme tests and Cold Agglutinins ID-Card(s) as needed.
3. Place in appropriate microtube: 50µl of red cell suspension and 25µl of Lorne reagent.
4. Centrifuge the ID-Card(s) in a Bio-Rad ID centrifuge.
5. Read macroscopically for agglutination.

D. Microplate Technique, using "U" wells

0. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
1. Place in the appropriate well: 1 volume Lorne reagent and 1 volume of red cell suspension.
2. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
3. Incubate at room temperature for 15 minutes (time dependant on user).
4. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
5. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker
6. Read macroscopically or with a validated automatic reader.
7. Any weak reactions should be repeated by the tube technique.

D. Slide Technique

1. Prepare a 35-45% suspension of red cells in serum, plasma or PBS or Isotonic saline. If this is not possible, whole anti-coagulated blood may also be used as the sample.
2. Place on a labelled glass slide or card tile: 1 volume of Lorne reagent and 1 volume of red cell suspension.
3. Using a clean applicator stick, mix the reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for a 1 minute period.
5. Read macroscopically after 1 minute over a diffuse light, 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 red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the Cw 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 Cw antigen on the red cells.

STABILITY OF THE REACTIONS

1. Tube tests must be read immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes leading to false negative, or weak positive reactions.
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 reagent.
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. Many monoclonal human IgM Rhesus antibodies have been shown to posses Anti-I/i cold agglutinin activity, particularly with Cord cells or enzyme treated cells. This may become apparent if tests are incubated below the recommended temperature.
2. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative reactions and so caution should always be exercised when assigning genotypes on the basis of test results.
3. 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 Anti-C^w reagent 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 Quality Control of the reagents was performed using red cells that had been washed twice with PBS or Isotonic saline prior to use.
5. 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 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[6].

BIBLIOGRAPHY

1. Widman FK. Technical Manual, 9th Edition. American Association of Blood Banks, Arlington, VA, 1985; Chapter 8
2. Race RR, Sanger R. Blood Groups in Man, 6th Edition. Blackwell Scientific, Oxford 1975; Chapter 2
3. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition. Blackwell Scientific, Oxford 1987; Chapter 7
4. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6
5. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
6. 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

Lorne Monoclonal Anti-C^w	2 ml	750002
	1000 ml	750000*

***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		

Lorne Laboratories Limited
Unit 1 Cutbush Park Industrial Estate
Danehill
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