LORNE LABORATORIES LTD.
GREAT BRITAIN

HUMAN BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE

Polyclonal Anti-C^w: For Tube, DiaMed-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 test red cells that carry the Cw antigen, after centrifugation. No agglutination generally indicates the absence of the Cw antigen (see **Limitations**).

REAGENTS

Lorne Human Anti-Cw blood grouping reagent is prepared from human serum diluted in a sodium chloride (0.9%) solution containing macromolecular potentiators (1.6%) and bovine albumin (14–20%). 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

Do not freeze. 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.

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 48 hours. 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 before being tested. Blood showing 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 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. When typing red cells from a patient suspected of having auto- antibodies or protein abnormalities a reagent control is needed due to high protein levels in the Anti-C^w reagent. Lorne Rh-Hr Control Serum (Cat. # 200010) is recommended.
3. Weak Cw antigens may be poorly detected by the gel card and slide technique. It is recommended that weak Rhesus antigens are tested using the tube technique.
4. Agglutination of test red cells with Lorne Rh-Hr Control Serum invalidates the Rh typing results.
5. The Rh system antibodies are enhanced when tested against enzyme-treated red blood cells
6. In the **Recommended Techniques** one volume is approximately 40µl when using the vial dropper provided.
7. 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.
8. The user must the determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Applicator sticks.
- DiaMed ID-Cards (Neutral).
- DiaMed ID-Centrifuge.
- DiaMed ID-Diluent: e.g. ID-CellStab.
- DiaMed ID-Incubator equilibrated at 37ºC ± 2ºC.
- Glass microscope slides.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Heated Rh-view box.
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho BioVue System Heat Block equilibrated to 37ºC ± 2ºC.
- Ortho 0.8% Red Cell Diluent.
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22ºC ± 1ºC
- Positive (ideally heterozygous) and negative control red cells.
- Reagent control i.e. Lorne Rh-Hr Control Serum (Cat. # 200010).
- Test tube centrifuge.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrate to 37ºC ± 2ºC.

RECOMMENDED TECHNIQUES

A. Tube Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Lorne reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination
6. Test Lorne Rh-Hr Control Serum in parallel with the reagent.

B. DiaMed-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in an ID-Diluent.
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 reagent.
4. Incubate the ID-Card(s) for 15 minutes at 37ºC ± 2ºC.
5. Centrifuge the ID-Card(s) for 10 minutes at 90 rcf or for a suitable alternative time and force.
6. Read macroscopically for agglutination.
7. Any weak reactions should be repeated by the tube technique.

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: 50µl of test red cell suspension and 40µl of Lorne reagent.
4. Incubate the cassette(s) for 15 minutes at 37ºC.
5. Centrifuge cassette(s) for 5 minutes in an Ortho BioVue System Centrifuge.
6. Read macroscopically for agglutination.

D. Slide Technique

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS.
2. Place on a labelled microscope slide pre-warmed to 40-50ºC: 1 volume of Lorne and 1 volume of test 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 30 seconds, with occasional further mixing during the 2-minute period, maintaining the slide at 40-50ºC.
5. Read macroscopically after 2 minutes over a diffuse light, do not mistake fibrin strands as agglutination
6. Test Lorne Rh-Hr Control Serum in parallel with the reagent.
7. 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 appropriate Rh 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 Rh 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. 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 two minutes 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. Some red cells express variant Rh antigens and may give weaker reactions than seen with randomly selected positive control cells. Anti-C may give weaker reactions with C antigen of R_2R_Z individuals. Similarly, Anti-e may give slightly weaker reactions in absence of C antigen, e.g. R_2r, r"r and rr.
2. Antibodies directed at low frequency antigens may occur as unsuspected contaminants in blood grouping antisera. In addition, certain antigens (eg. Bg, Sd^a) can be present in an exalted state on red blood cells. These phenomena may be the source of rare false positive reactions, which may occur with more than one lot of a given specificity.
3. It is not possible to claim the absence of all contaminating antibodies, as red cells carrying antigens of low frequency or exalted antigens are not always available for testing.
4. 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.
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 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. Due to the presence of macromolecular potentiators in this reagent, typing of blood samples that are negative for the C^w antigen may show a slight reddish tinge of the gel in Diamed Inert gel cards. This reddish tinge should not be mistaken for haemolysis or a weak positive reaction. It essential that a red cell negative for the C^w antigen is incorporated in the Diamed gel card test as a negative control and is used as a comparison when reading the gel card results of patient red cells.
4. The presence of contaminating antibodies to antigens with an incidence of 1% or greater within the random population has been excluded either in tests employing the appropriate antigen-negative red cells or in tests employing reagents previously absorbed to remove interfering specificities.
5. Antibodies to Xg^a, Do^a, Yt^a, Co^b, Wr^a, Bg^a and V^w may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cell. This can also be said for Yt^b, M^g and V^w and other low frequency antigens which may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cells
6. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS 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[6].

BIBLIOGRAPHY

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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 Human Anti-C^w	2 ml	216002
	1000 ml	216000

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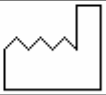
TABLE OF SYMBOLS

LOT	Batch Number	IVD	*in-vitro* Diagnostic
REF	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		