



**BLOOD GROUPING REAGENTS  
DIRECTIONS FOR USE**

**Anti-C, Anti-E, Anti-c and Anti-e Monoclonal: For Tube, Bio-Rad-ID, Ortho BioVue, Microplate 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; the C and e antigens are less immunogenic than E and c. The corresponding antibodies are all clinically significant since they may cause both Transfusion Reactions and Haemolytic Disease of the Newborn.

MODIFIED WIENER HAPLOTYPE	PREVALENCE (%)		
	WHITE	BLACK	ASIAN
R <sub>1</sub>	42	17	70
R <sub>2</sub>	14	11	21
R <sub>0</sub>	4	44	3
R <sub>Z</sub>	<0.01	<0.001	1
r	37	26	3
r'	2	2	2
r''	1	<0.01	<0.01
r <sup>Y</sup>	<0.01	<0.01	<0.01

**INTENDED PURPOSE**

The Rh reagents are blood grouping reagents intended to be used to qualitatively determine the presence or absence of Rh antigens on the red cells of blood donors or patients requiring a blood transfusion when tested in accordance with the recommended techniques stated in this IFU.

**PRINCIPLE**

The reagents contain antibodies to the appropriate Rhesus antigen on human red cells and will cause direct agglutination (clumping) of red cells that carry the corresponding Rh antigen. No agglutination (no clumping) generally indicates the absence of the corresponding Rh antigen (see **Limitations**).

**REAGENTS**

Lorne Monoclonal IgM Anti-Rh blood grouping reagents (REF: 690005, 691005, 692005, 693005, 700010) are low protein reagents containing human monoclonal antibodies directed against the appropriate Rh antigens (D, C, c, E, e) diluted with sodium chloride, bovine albumin and macromolecular potentiators (see **Limitations**). The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. Each reagent is supplied at optimal dilution for use with all recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date (see Vial Label).

Reagent	Cell Line / Clone	Antibody Type
Anti-C Monoclonal	MS-24	Hybridoma cell line secreting Human IgM
Anti-E Monoclonal	MS-258	Hybridoma cell line secreting Human IgM
Anti-c Monoclonal	MS-33	Hybridoma cell line secreting Human IgM
Anti-e Monoclonal	MS16	Hybridoma cell line secreting Human IgM
	MS-63	Hybridoma cell line secreting Human IgM

**STORAGE**

Reagent vials should be stored at 2 - 8°C 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. According to the reagents' in-use stability studies, once vials are 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.

**SAMPLE COLLECTION AND PREPARATION**

Blood samples can be collected into EDTA 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 between 2°C and 8°C. EDTA or clotted blood may be stored up to 1 day after collection at ambient temperature and up to 21 days at refrigerated temperature (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 blood samples with PBS or Isotonic saline before being tested.

**PRECAUTIONS**

- The reagents are intended for *in vitro* diagnostic use only.
- If a reagent vial is cracked or leaking, discard the contents immediately.
- Do not use the reagents past the expiration date (see **Vial Label**).
- Do not use the reagents if a precipitate is present.
- Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
- The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden, but is not supplied sterile. 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.
- The reagents contain < 0.1% sodium azide (NaN<sub>3</sub>). 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.

- 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.
- 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**

- A positive control (ideally heterozygous) and a negative control shall be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
- When typing red cells from patients known or suspected to have auto-antibodies, protein abnormalities or a positive Direct Antiglobulin Test (DAT), it is important that a reagent negative control is tested in parallel. For the reagent negative control, only Lorne Monoclonal Rh Control, catalogue number 640010, must be used.
- Weak Rh antigens may be poorly detected by the gel card, microtitre plate and slide technique. It is recommended that weak Rh antigens are tested using the tube test technique.
- Before use, let the reagent warm up to room temperature. As soon as the reagent has been used, put the reagent back in storage at 2-8°C.
- In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
- The use of reagents and interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use. The user must determine suitability of the reagents for use in other techniques.

**REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED**

**Tube Technique**

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Centrifuge capable of spinning at 1000 g for 20 seconds.
- PBS solution (pH 6.8-7.2) or Isotonic saline solution (pH 6.5-7.5).
- Positive and negative control red cells: Monoclonal Anti-C: R<sub>1</sub>r (positive control) and rr (negative control). Monoclonal Anti-E: R<sub>2</sub>r (positive control) and rr (negative control). Monoclonal Anti-c: R<sub>1</sub>r (positive control) and R<sub>1</sub>R<sub>1</sub> (negative control). Monoclonal Anti-e: R<sub>2</sub>r (positive control) and R<sub>2</sub>R<sub>2</sub> (negative control).

**Bio-Rad-ID Micro Typing Technique**

- Bio-Rad ID-Cards (NaCl, Enzyme tests and Cold Agglutinins).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or ID-Diluent 2.

**Ortho BioVue Typing Technique**

- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.

**Microtitre plate Technique**

- Validated "U" well microtitre plates.
- Microtitre plate centrifuge.
- Microtitre plate shaker.

**Slide Technique**

- Glass microscope slides or white card tiles.
- Applicator sticks.
- Timer or stopwatch

**All Techniques**

- Volumetric pipettes.

**RECOMMENDED TECHNIQUES**

**A. Tube Technique**

- Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
- Place in a labelled test tube: 1 volume Lorne Anti-Rh reagent and 1 volume red cell suspension.
- Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
- Gently resuspend red cell button and read macroscopically for agglutination
- Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
- Following incubation, repeat steps 3 and 4.

**B. Bio-Rad ID Technique (NaCl, Enzyme and Cold agglutinins cards)**

- Prepare a 0.8% suspension of red cells in ID-CellStab or ID-Diluent 2.
- Remove aluminium foil from as many microtubes on the NaCl/Enzyme/Cold agglutinins ID-Card as needed.
- Place in appropriate microtube: 50µl of red cell suspension and 25µl of Lorne Anti-Rh reagent.
- Centrifuge the ID-Card(s) in a Bio-Rad ID centrifuge.
- Read macroscopically for agglutination.

**C. Ortho BioVue Technique (Neutral cassettes)**

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 the Neutral cassette as needed.
3. Place in appropriate reaction chamber: 50µl of red cell suspension and 40µl of Lorne Anti-Rh reagent.
4. Centrifuge cassette(s) for 5 minutes 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 Lorne Anti-Rh reagent and 1 volume 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. 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 Anti-Rh reagent and 1 volume of 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 1-minute, 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.

**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 appropriate Rh 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 appropriate Rh antigen on the red cells.
3. **Control:** The reaction can be interpreted if the results obtained with the control samples are valid. Test results of cells that are agglutinated using Monoclonal Rh Control (ref.640010) 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 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.
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

**LIMITATIONS**

1. Lorne Anti-Rh reagents are not suitable for use with enzyme treated cells or for use in indirect antiglobulin techniques.
2. Many Monoclonal human IgM anti-Rh antibodies have been shown to possess 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.
3. 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<sub>2</sub>Rz individuals. Similarly, Anti-e may give slightly weaker reactions in absence of C antigen, e.g. R<sub>2</sub>r, r<sup>+</sup>r and rr.
4. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative reactions. For these reasons, caution should always be exercised when assigning genotypes on the basis of test results.
5. In case of ambiguous results, it is recommended to wash red blood cells at least 2 times.
6. 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. Prior to release, each lot of Rh reagent was tested using the recommended test methods listed in this IFU. The tests complied with the test requirements as stated in the current version/issue of the "Guidelines for the Blood Transfusion Services in the United Kingdom" and the "Common Specifications.
2. Specificity of source Monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
3. The Quality Control of the reagents was performed using red cells with phenotypes that were verified by a UK blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.
4. The Quality Control of the reagents was performed using red cells with phenotypes that were verified by a UK blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.
5. The performance of the reagents was confirmed against well-established CE marked comparator IVD reagent(s) in a comparison study where the reagents were tested in parallel with all recommended methods. The overall statistical analysis results of the study are shown below:

	Method	N	Sensitivity	Specificity	PPV	NPV
Anti-C	Test Tube	475	99.6%	100.0%	100.0%	99.5%
	Ortho	475	100.0%	99.1%	99.2%	100.0%
	Bio-Rad	475	100.0%	98.6%	98.8%	100.0%
	Microplate	475	100.0%	99.5%	99.6%	100.0%
	Slide	438	99.6%	100.0%	100.0%	99.5%
Anti-E	Test Tube	479	100.0%	99.7%	99.2%	100.0%
	Ortho	479	100.0%	100.0%	100.0%	100.0%
	Bio-Rad	479	100.0%	99.7%	99.2%	100.0%
	Microplate	479	97.5%	100.0%	100.0%	99.2%
	Slide	447	99.1%	100.0%	100.0%	99.7%
Anti-c	Test Tube	475	100.0%	95.1%	99.0%	100.0%
	Ortho	475	100.0%	96.3%	99.2%	100.0%
	Bio-Rad	475	100.0%	95.1%	99.0%	100.0%
	Microplate	475	100.0%	100.0%	100.0%	100.0%
	Slide	444	100.0%	97.4%	99.5%	100.0%
Anti-e	Test Tube	474	100.0%	94.7%	99.8%	100.0%
	Ortho	474	100.0%	94.7%	99.8%	100.0%
	Bio-Rad	474	100.0%	100.0%	100.0%	100.0%
	Microplate	474	99.8%	94.7%	99.8%	94.7%
	Slide	443	99.5%	94.7%	99.8%	90.0%

**DISCLAIMER**

1. The end 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<sup>5</sup>.

**BIBLIOGRAPHY**

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2. AABB Technical Manual, 16<sup>th</sup> edition, AABB 2008.
3. 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
4. Guidelines for the Blood Transfusion Service in the United Kingdom, 6<sup>th</sup> Edition 2002. The Stationary Office.
5. 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	Tests per vial
Anti-C Monoclonal	5 ml	690005	100
Anti-E Monoclonal	5 ml	691005	100
Anti-c Monoclonal	5 ml	692005	100
Anti-e Monoclonal	5 ml	693005	100

**TABLE OF SYMBOLS**

Symbol	Definition	Symbol	Definition
	Manufacturer	<b>REF</b>	Catalogue number
	Temperature limitation		Use by YYYY-MM-DD
<b>IVD</b>	In vitro diagnostic medical device		Consult instructions for use.
<b>EC REP</b>	Authorised Representative	<b>LOT</b>	Lot number
	CE symbol		



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