

PACKAGING

Ref.: 101-0538	Cont.: 5 mL
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Store 2 - 8° C.

CLINICAL SIGNIFICANCE

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.

Major Rh Antigens				
D	C	E	c	e
85	70	30	80	98

Table 1: Frequency of each antigen in Caucasian population.

PRINCIPLE OF THE METHOD

The reagents will cause direct agglutination (clumping) of test red cells that carry the corresponding Rh antigen. No agglutination generally indicates the absence of the corresponding Rh antigen (see **Limitations**)

REAGENTS

Chronolab Monoclonal IgM Anti-Rh blood grouping reagents are low protein reagents containing human monoclonal antibodies diluted with sodium chloride (0.9 g%), bovine albumin (6 g%) and macromolecular potentiators. 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
Anti-C+D+E	388F3 + RUM-1 + MS-258

Table 2: Human IgM Cell Lines/Clones used.

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.
10. For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

NOTES

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 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.
3. Weak Rhesus antigens may be poorly detected by the gel card, microtitre plate and slide technique. It is recommended that weak Rhesus antigens are tested using the tube test technique.
4. In the techniques here detailed one volume is approximately 40 µl when using the vial dropper provided.
5. 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.
6. The user must determine suitability of the reagents for use in other techniques.

STORAGE

Do not freeze. 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.

MATERIAL REQUIRED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Test tube centrifuge.
- Volumetric pipettes.
- Glass microscope slides.
- Applicator sticks.
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22°C ± 1°C.
- Positive (ideally R₁r) and negative (rr) control red cells.
- DiaMed ID-Cards (Neutral).
- DiaMed ID-Centrifuge.
- DiaMed ID-Diluent: e.g. ID-CellStab.
- Plate shaker.
- Automatic plate reader.
- Validated "U" well microplates.
- Microplate centrifuge.

SAMPLE

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed then store specimens at 2-8° C. 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 samples showing evidence of lysis may give unreliable results.

PROCEDURE

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 Chronolab Anti-Rh reagent and 1 volume 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 a negative or questionable result, 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 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 Chronolab Anti-Rh reagent.
4. Centrifuge the ID-Card(s) for 10 minutes at 90 rcf or for a suitable alternative time and force.
5. Read macroscopically for agglutination.

c. Microplate Technique, using "U" wells

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in the appropriate well: 1 volume Chronolab Anti-Rh 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.

d. Slide Technique

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS.
2. Place on a labelled glass slide: 1 volume of Chronolab Anti-Rh 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 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. 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. Chronolab Anti-Rh reagents are not suitable for use with enzyme treated cells or for use in indirect antiglobulin techniques.
2. 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 R₂R_Z individuals. Similarly, Anti-e may give slightly weaker reactions in absence of C antigen, e.g. R₂r, r^rr and rr.
3. 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.
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
5. The user is responsible for the performance of the reagents by any methods other than those mentioned in the **Recommended Techniques**.
6. Any deviations from the **Recommended Techniques** should be validated prior to use.

PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by all the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Chronolab Monoclonal Anti-C+D+E 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 prior to use.
5. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

BIBLIOGRAPHY

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