Qualitative test for determination of the Cc, Ee antigen on human red blood cells.

**IVD**

Store at 2-8°C

**PRINCIPLE OF THE METHOD**

The tests will cause 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).

**CLINICAL SIGNIFICANCE**

Levine and Stanier described the Rh blood group system in 1940. Apart from the D other major Rh antigens are C, c, E, and e. The D antigen is highly immunogenic; the C and e antigens are less immunogenic than C e. The corresponding antibodies are all clinically significant since they may cause both Transfusion Reactions and Haemolytic Disease of the Newborn.

<table>
<thead>
<tr>
<th>Major Rh Antigens</th>
<th>D</th>
<th>C</th>
<th>E</th>
<th>C+E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>Ant-C</td>
<td>Anti-c</td>
<td>Anti-E</td>
<td>Anti-C+E</td>
</tr>
<tr>
<td>Cell Line / Clone</td>
<td>McAb</td>
<td>McAb</td>
<td>McAb</td>
<td>McAb</td>
</tr>
<tr>
<td>Dilution</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

**Table 1:** Frequency of each antigen in Caucasian population

**REAGENTS**

Spinreact Monoclonal IgM Anti-Rh blood grouping reagents are low protein reagents containing human monoclonal antibodies diluted with sodium chloride (0.9%), bovine albumin (6%) 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.

**MATERIAL**

Do not freeze. Reagent vials should be stored at 2-8ºC on a solid phase

**NOTES**

1. Tube reactions will be delayed to test cells. When making preparations to test cells, they should be washed at least twice with PBS prior to use.
2. The use of reagents and interpretation of results must be carried out by properly trained individuals.
3. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.
4. The user is responsible for the performance of the reagents by any method other than those mentioned in the Recommended Techniques.
5. Any deviations from the Recommended Techniques should be validated prior to use.

**PACKAGING**

- **Monoclonal Anti-c**
  - Ref: 170001
  - 5 ml
- **Monoclonal Anti-E**
  - Ref: 170003
  - 5 ml
- **Anti-c and Anti-e**
  - Ref: 170035
  - 5 ml
- **Anti-E and Anti-e**
  - Ref: 170037
  - 5 ml

**REFERENCES**

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**MONOCLINIAL Anti-c, Anti-e, Anti-c-e Anti-e**

**Tube, Slide, DiaMed-ID and Microplate Tests**

**Blood Grouping**

**QUALITY ASSURANCE**

- **About the recipient:**
  - The recipient's blood sample should be collected and tested within 24 hours of death.
  - If the recipient's blood sample is not available, a fresh sample should be collected from the donor within 24 hours of death.
  - The sample should be stored at 2-8ºC for up to 48 hours before testing.

- **About the donor:**
  - The donor's blood sample should be collected and tested within 24 hours of death.
  - If the donor's blood sample is not available, a fresh sample should be collected from the recipient within 24 hours of death.
  - The sample should be stored at 2-8ºC for up to 48 hours before testing.

**PROCEDURE**

**A. Tube Method**

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume 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. Slide Method**

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 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. Expose to 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.

**C. 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 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.

**D. 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 Anti-Rh reagent and 1 volume test red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-contamination.
4. Incubate at room temperature for 15 minutes (time dependant on user).
5. Centrifuge the microplate for 1 minute at 140 rcf 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.

**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.
4. Some red cells express variant Rh antigens and may give weaker reactions than seen with normal cells. Anti-C may give slightly weaker reactions in absence of C antigen, e.g. Pr(C,E) and Pr(C,E).
5. 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 grouping on the basis of test results.
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; Errors from the reagent used; The user is responsible for the performance of the reagents by any method other than those mentioned in the Recommended Techniques.
7. Any deviations from the Recommended Techniques should be validated prior to use.

**PERFORMANCE CHARACTERISTICS**

1. The reagents have been characterised by all the procedures here mentioned.
2. Prior to release, each lot of reagents was tested at source and found to be negative for HIV-1, HIV-2, and HTLV-1.

**BIBLIOGRAPHY**

5. Tel. +34 972 69 08 00 Fax +34 972 69 00 99. e-mail: spinreact@spinreact.com