QUALITATIVE TEST FOR DETERMINATION OF A AND/OR B ANTIGENS ON HUMAN RED BLOOD CELLS

1. **IVD**

Store at 2-8°C

**PRINCIPLE**

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

**CLINICAL SIGNIFICANCE**

In 1900, Landsteiner discovered the serum of some people would agglutinate the red cells of others. Four common phenotypes are now recognised: A, B, AB and O. Subgroups of A and B have since been identified.

**REAGENTS**

Spinreact Monoclonal IgG ABO blood grouping reagents contain mouse monoclonal antibodies directed against human A and B antigens. Each reagent is supplied at optimal dilution for use with all the recommended techniques. Stock solutions shall be stored at -20°C. Reagent vials should be stored at 2-8°C. Do not freeze. Reagent vials should be stored at 2-8°C.

1. **PACKAGING**

Product Cell LineClone Colour Eye Used

<table>
<thead>
<tr>
<th>Product</th>
<th>Cell LineClone</th>
<th>Colour</th>
<th>Eye Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>9113D10</td>
<td>Blue</td>
<td>Faintly Blue</td>
</tr>
<tr>
<td>Anti-B</td>
<td>9113D10</td>
<td>Yellow</td>
<td>None</td>
</tr>
</tbody>
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**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.

**REAGENTS AND MATERIALS REQUIRED**

1. Glass test tubes (10 x 75 mm or 12 x 75 mm).
2. Test tube centrifuge.
3. Volumetric pipettes.
5. Applicator sticks.
6. Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22°C ± 1°C.
7. Positive (healthy) red cells of known phenotype.
8. Diaphor ID Cards (Neutral).
10. Dialed ID Dinitro: e.g. ID-CelFab.
11. Plate washer.
14. Microplate centrifuge.

**SAMPLE**

Blood samples drawn with or without anticoagulant may be used for antigen testing. If typing is delayed, store samples at 2-8°C. EDTA and citrate samples should be kept for 48 hours. Samples collected into ACD, CPD or CPDA-1 may be kept up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS before being tested. Blood sample showing evidence of lysis may give unreliable results.

**PROCEDURE**

**A. Tube Technique**

1. Prepare a 2-3% suspension of washed red test cells in PBS.
2. Place in a labelled test tube: 1 volume of Anti-ABO reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at room temperature for 30 minutes.
4. Centrifuge all tubes for 10 seconds at 400 g for a suitable alternative time and force.
5. Gently resuspend red cells and read macroscopically for agglutination.
6. Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
7. Following incubation, repeat steps 4 and 5.

**B. 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 Anti-ABO reagent and 1 volume of test red cell suspension.
3. Place in 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 45 minutes exposure to light and do not mistake fibrin strands as agglutination.

**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-AB reagent.
4. Centrifuge the ID-Cards® for 15 minutes at 800 g 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-AB 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 dependent on user).
5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Resuspend the cells 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 limits of test procedure, indicates the presence of the appropriate ABO antigen on the test red cells.
2. **Negative:** No agglutination of the test red cells constitutes a negative result and within the accepted limits of the test procedure, indicates the absence of the appropriate ABO antigen on the test red cells.
3. **Discrepancies:** If the results obtained with reverse group don’t correlate with forward group, further investigation is required.
4. If a reagent vial is cracked or leaking, discard the contents immediately.
5. False positive or false negative results may also occur due to:
   - Contamination of test material.
   - Improper storage, cell concentration, incubation time or temperature.
   - Improper or excessive centrifugation.
   - Deviation from the recommended techniques.
6. The user is responsible for the performance of the reagents by any method other than those here mentioned.

**LIMITATIONS**

1. ABO reagents are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. When using Monoclonal A-B, blood specimens of weak A or B subgroups (e.g. Ax) may give rise to negative or weak reactions when tested using slides, microtitre plates or gel cards. It is advisable to re-test weak subgroups using the tube technique.
3. Spinreact monoclonal Anti-A and monoclonal Anti-B are not validated to detect Ax and A3 or Bx and B3 antigens and we therefore do not claim reactivity of the monoclonal Anti-A or Anti-B to these antigens.
4. Stored blood may give weaker reactions than fresh blood.
5. False positive or false negative results may also occur due to:
   - Contamination of test material.
   - Improper storage, cell concentration, incubation time or temperature.
   - Improper or excessive centrifugation.
   - Deviation from the recommended techniques.
   - The user is responsible for the performance of the reagents by any method other than those here mentioned.

**Any deviations from the techniques here recommended should be validated prior to use.**

**PERFORMANCE CHARACTERISTICS**

1. The reagents have been characterised by all the procedures here mentioned.
2. Monoclonal Anti-A and Anti-B are tested by the techniques here recommended 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 red cells.
4. Spinreact Anti-B does not react with "Acquired-B" red cells.
5. Spinreact Monoclonal ABO reagents do not detect crypt antigens such as T, Tin or Cedd.
6. The potency of the reagents has been tested against the following minimum performance reference standards obtained from National Institute of Biological Standards and Controls (NIBSC):
   - Anti-A reference standard 86/722 And / Or
   - Anti-B reference standard 86/724

7. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.

8. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

**BIBLIOGRAPHY**