Agglutination assay on glass slides and test tubes. Store at 2-8 °C. For "in vitro" use only.

PROPOSED USE

This method is valid for qualitative determinations of antigens A and/or B in human blood.

MATERIALS

Glass slide: glass slides, automatic pipette and applicator. Test tube: test tubes, automatic pipette and centrifuge.

FUNDAMENT OF THE METHOD

This method is used for determining blood groups of the ABO system. Problem red blood cells are confronted with murine monoclonal antibodies of known anti-A, anti-B, anti-A+B specificity. Agglutination (or not) of the problem red blood cells to each of the reagents is indicative of the presence or absence of the relevant antigen.

CONTENT

<table>
<thead>
<tr>
<th>CONTENT</th>
<th>Cell Line</th>
<th>IgM</th>
<th>Stabilizing buffer solution</th>
<th>Sodium azide 0.95 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A Blue</td>
<td>11H5</td>
<td>IgM</td>
<td></td>
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<tr>
<td>Anti-B Yellow</td>
<td>BRIC250/6F9</td>
<td>IgM</td>
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<tr>
<td>Anti-A+B Colorless</td>
<td>11H5+ BRIC250/6F9+ES-25</td>
<td>IgM</td>
<td>Stabilizing buffer solution</td>
<td>Sodium azide 0.95 g/L</td>
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</tbody>
</table>

PREPARATION

The reagent is ready for use. Do not dilute.

CONSERVATION AND STABILITY

1. The reagents will remain stable until the date of expiry indicated on the label provided the vials have been stored and properly closed and at a temperature of 2-8 °C and care is exercised when using in order to avoid contamination.

2. The reagents are normally clear and transparent. Turbidity may be an indication of microbial contamination.

SAMPLES

Whole, anti-coagulated or coagulated blood in its own serum can be used if the test is performed immediately. Samples collected in EDTA or heparin must be used within 48 hours. Samples collected in ACD, CPD, and CPDA-1 maintain their reactivity during a period of 21 days. Store samples at 2-8 °C.

PROCEDURE

Qualitative Method

Glass slides

1. Use a pipette to transfer 1 drop (~50 µL) of the problem red blood cells with a hematocrit of approximately 35-40%.

2. Add 1 drop (~50 µL) of the reagent.

3. Mix the blood and the reagent thoroughly with the applicator forming a 2 cm diameter circle.

4. Slowly move the slide with circular movements for 2 minutes.

Reading and interpretation

Reading: After two minutes note the presence or absence of agglutination.
Positive reaction: agglutination can be observed.
Negative reaction: agglutination is not observed after two minutes.

Test Tube Method

1. Prepare a suspension of problem red blood cells after rinsing with 3-5% NaCl 9g/L.

2. Add 1 drop of the reagent (~50 µL) to the test tube.

3. Add 1 drop (~50 µL) of the suspension.
4. Centrifuge at 1000 r.p.m. during 1 minute.

**Reading and Interpretation**

Reading: Gently tap the tube in order to detach the sediment from the glass and examine macroscopically for the presence or absence of agglutination.

*Positive Reaction:* Positive red blood cells remain agglutinated following resuspension.

*Negative Reaction:* Resuspension of red blood cells is homogeneous.

**QUALITY CONTROL**

Make sure the reagents have not lost their activity before using, including that of the positive controls (red blood cells with the relevant antigens) and negative controls. 

**PRECAUTIONS**

Although the reagents are not of human origin and consequently free from HIV and Hepatitis B, both the reagents and the samples should be handled with the necessary precautions.

The preparation contains small amounts of sodium azide. Avoid spilling on irritated skin and on mucosa. Do not use if turbid.

**REFERENCE**


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**PRESENTATION**

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<tr>
<th>Code</th>
<th>Sample</th>
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<th>Determinations</th>
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<tbody>
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<td>Anti-A</td>
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<td>100 determ.</td>
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<td>Anti-A</td>
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<td>1700005</td>
<td>Anti-A+B</td>
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<td>100 determ.</td>
</tr>
<tr>
<td>1700006</td>
<td>Anti-A+B</td>
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<td>200 determ.</td>
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