Anti A, Anti B and Anti AB are monoclonal antibodies of IgM type specific against red blood antigens A and B.

Application: For invitro use in Haemagglutination slide or saline test for human blood grouping.

Reagent: Mouse monoclonal antibody raised against blood group antigens A & B.

Source: Obtained by immunizing Balb/c mouse with red cells of blood groups A and B and fusing the splenocytes of that mouse with myeloma (SP2/0) cells.

Storage: This product will be well-preserved within utility limit till the expiry date, if stored at temperature between +2°C and +8°C.

Caution: Do not freeze.

PROCEDURE:

Principle: Red blood cell antigen A or B, when mixed with their respective antibodies, agglutinate.

Phenotyping (grouping) of them is done by reacting the blood sample with Anti A or Anti B. Presence of haemagglutination determines the group of the tested blood.

Specimen: Use properly stored anticoagulated blood or 10% RBC-saline suspension. To prepare 10% RBC Saline suspension, add approximately 5 volumes of isotonic saline to the whole blood and centrifuge for 2 minutes. Remove the supernatant and wash the sedimented red cells three more times with normal saline as above. After final wash, take 100 microlitres of sedimented red cells, dilute to one ml with saline and mix thoroughly before use.

PHENOTYPING:

MACROSCOPIC SLIDE TEST

1. Label two glass slides with name or number of the patient and make two circles on each slide. Label the circles as A, B, AB and S.
2. Add one drop of Anti A antibody in Circle A, Anti B antibody in circle B, Anti AB antibody in circle AB and a drop of saline in the fourth circle.
3. Add one drop of patient’s whole blood or red cell saline suspension to each circle.
4. Mix the red cells and the antibody immediately with an applicator stick and spread it over an area of about one square inch within the circle.
5. Gently tilt the slides forward and backward at room temperature for a maximum of two minutes.
6. Read the slides for haemagglutination.

MICROSCOPIC TUBE TEST (FOR ENCHANCED SENSITIVITY)

1. Use 8x50 mm small glass test tubes. For each specimen, take 4 tubes and label them with the name or number of patient. Mark the tubes as A, B, AB and saline.
2. Add one deep of Anti A, Anti B, Anti AB antibody and saline to the respective tubes.
3. Add one deep of 2-3% RBC-saline suspension to each tube.
4. Shake each tube thoroughly and centrifuge for 1 minute at 1000 rpm (125g) or 3400 RPM (1000 g) for 20 secs or incubate at Room Temperature for 1 hour.
5. Gently dislodge the sedimented cells and read for haemagglutination, either macroscopically or microscopically.

Interpretation

Agglutination of red blood cells within two minutes indicates the corresponding antigens in the patient’s red blood cells. Absence of agglutination indicates the absence of such antigens on the red blood cells.

Agglutination results are as interpreted follows for phenotyping.

<table>
<thead>
<tr>
<th>Sample red cell reacted with</th>
<th>Anti A</th>
<th>Anti B</th>
<th>Anti AB</th>
<th>Saline</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>+   -   -    -</td>
<td>'A' Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-   +   -    -</td>
<td>'B' Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+   +   -    -</td>
<td>'AB' Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-   -   -    -</td>
<td>'O' Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+   +   +    +</td>
<td>Weaker variants of 'A' or 'B' Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(*) suggestiv of antibody in the blood giving a non specific reaction. The entire test is to be repeated using 10% saline suspension of red cells.</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Even though Anti A and Anti B antiseras are sufficient for ABO phenotyping 'O' blood it is advisable to use Anti AB sera to rule out any doubt arising due to weaker variants of subgroup A and B.

Run positive and negative test controls for each batch of blood grouping sera every time before proceeding with the actual test samples.

Precautions

1. The blood drop on the slide should not be allowed to dry, partial drying of the blood could be misinterpreted as agglutination.
2. Centrifugation should be perfect. Over-centrifugation or under-centrifugation may result in false positive or false negative interpretation.
3. Dislodgement of sedimented red cells in tube test should be done as gently as possible, rough dislodgement may disrupt small or weak agglutinates and hence may lead to false negative interpretation.
4. The entire procedure should be carried out at room temperature. Warm or cold antibodies in the tested blood can cause agglutination and may lead to wrong interpretation.
5. Haemolysed blood samples should not be used.
6. Improper antigen antibody concentrations may cause false or delayed agglutination.

References

Vox sanguinis, 1989, 56, 122.