EXPERIMENT NO: 11

DATE:

ABO BLOOD GROUPING

AIM:

To identify the monoclonal antibodies for phenotyping of human red blood cells.

PRINCIPLE:

To study haemagglutination of human blood grouping mouse monoclonal antibody raised against blood group A and B obtained by immunizing a mouse with red cell of blood group A and B and fusing the splenocytes of the mouse with myloma cells. Red blood cell antigen A or B when mixed with their respective antibodies leads agglutination determines the group of tested blood.

CHARACTERISTICS OF ABO ANTIGENS:

ABO antigens are glycolipid in nature, meaning they are oligosaccharides attached directly to lipids on red cell membrane. These antigens stick out from red cell membrane and there are many antigens sites per red blood cell (approximately 800,000)

Besides their presence on red blood cells, soluble antigens can be present in plasma, saliva, and other secretions. These antigens are also expressed on tissues other than red cells. This last fact is important to consider in organ transplantation.

ABO antigens are only moderately well developed at birth. Therefore ABO-HDN not as serve as other kinds of haemolytic disease of the newborn.

CHARACTERISTICS OF ABO ANTIGENS:

1. These are expected naturally occurring antibodies that occur without exposure to red cells containing the antigen. (There is some evidence that
similar antigens found in certain bacteria, like E.coli, stimulate antibody production in individuals who lack the specific A and B antigens.)

2. immunoglobulin M antibodies, predominantly

3. They react in saline and readily agglutinate. Due to the position of the antigen and the IgM antibodies it is not necessary to overcome the zeta potential.

4. Their optimum temperature is less than 30°C, but reactions do not take place at body temperature.

5. Not only are these antibodies expected and naturally occurring, they are also commonly present in higher titer, 1/128 or 1/256.

6. They are absent at birth and start to appear around 3-6 months as result of stimulus by bacterial polysaccharides. (For this reason, newborn blood is only forward typed.)

**ABO INHERITANCE**

**INHERITANCE TERMINOLOGY:**

**GENE:**

Determines specific inherited trait (ex. Blood type)

**LOCUS:**

Unit of inheritance carries genes 23 pairs of chromosomes per person, carrying many genes. One chromosome inherited from mother, one from father.

**ALLELE:**

Alternate choice of genes at a locus (ex A or B; C or c, Lewis a or Lewis b)
**HOMOZYGOUS:**

Alleles are the same for any given trait are different on each chromosome (ex. A/A)

**HETEROZYGOUS:**

Alleles for a given trait are different on each chromosome (ex. A/B or A/O)

**PHENOTYPE:**

Observed inherited trait (ex. Group A or Rh Positive)

**GENOTYPE:**

Actual genetic information for a trait carried on each chromosome (ex. O/O or A/O)

**DOMINANT:**

The expressed characteristic on one chromosome takes precedence over the characteristic determined on the other chromosome (ex. A/O types as A)

**CO-DOMINANT:**

The characteristic determined by the genes on both chromosomes are both expressed – neither is dominant over the other (ex. A/B types as AB)

**RECESSIVE:**

The characteristic determined by the allele will only be expressed if the same allele is on the chromosome also (ex. Can type as only when genotype is O/O)

**ABO PHENOTYPES AND GENOTYPES:**

1. Group A phenotype = A/A or A/O genotype
2. Group B phenotype = B/B or B/O genotype
3. Group O phenotype = O/O genotype
4. Group AB phenotype = A/B genotype

**PRODUCTION OF A, B AND H ANTIGENS**

The production of A, B and H antigens are controlled by the action of transferases. These transferases are enzymes that catalyze (or control) addition of specific sugars to the oligosaccharide chain. The H, A or B genes each produce a different transferases, which adds a different specific sugar to the oligosaccharide chain.

**PROCEDURE:**

1. Label two glass slides with name or number of the patient and make two circles on each slide. Label the circle as A, B and Rh.
2. Add one drop of monoclonal antibody A in circle A, monoclonal antibody O in circle Rh. Add one drop of patients’ whole blood or its each circle.
3. Mix the red cells and the antibody immediately with an applicator stick and spread it over an area of about one sequence in the circle.
4. Gently till the slides forward and backward at room temperature for a maximum of two minutes. Read the slides for hemagglutination.
**RESULT:**

The RBC cells agglutination in the given sample indicates

<table>
<thead>
<tr>
<th>Sample Red cells reacted with</th>
<th>Result</th>
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<tbody>
<tr>
<td>Mediclone A</td>
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<td>Mediclone B</td>
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<tr>
<td>Mediclone O</td>
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<td>Saline</td>
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A Group

B Group

AB Group

O Group

A or B Group