



Serotyping Techniques

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ABO Blood Groups

<u>Blood Group</u>	<u>RBC Antigens</u>	<u>Serum Antibodies</u>	<u>Percentage</u>
O	none	Anti A & B	45%
A	A	Anti B	42%
B	B	Anti A	8-10%
AB	AB	none	2-4%

Genotype = inherited genetic profile.

Phenotype = expression of genetic inheritance.

<u>Blood group</u>	<u>Antigens</u>	<u>Anti A</u>	<u>Anti A1</u>
A ₁	A, A ₁	+	+
A ₂	A	+	-

Serum from Blood Group B donors contains 2 isohemagglutinins, Anti A and Anti A₁.

Anti A₁ is detectable in the serum of 1%-2% of people who are typed A₂ and about 26% of people typed A₂ B.

Commercial Blood typing reagents do not discriminate between A₁ and A₂.

Blood type A₂ makes up about 20% of persons who are blood type A and is determined by using lectin from *Dolichos biforus*.

<u>Genotype</u>	<u>Phenotype</u>
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A1, A1	A1
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A1, A2	A1
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A1, O	A1
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A2, A2	A2
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A2, O	A2
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B, B	B
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B, O	B
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A1, B	A1 B
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A2, B	A2 B
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OO	O
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Other Subgroups of Blood Group A

Blood group A3 was first recognized in 1936 by Friedenreich as a weak agglutination of cells among vast numbers of un-agglutinated RBC's when exposed to Anti A and blood type O serum.

AX, Am, Aint, Aend, Afiin, Ael, Abantu are all variants of the A gene found in different populations or family lines that exhibit various agglutination patterns.

Generally considered not to be of significance clinically.

Blood Group B

Variants of blood group B are much rarer than type A, probably due to the greater prevalence of blood type A.

Serum Isohemagglutinins

There are 2 types:

Natural – are usually IgM and are directed against A & B blood antigens.

Acquired – usually IgG and are directed against lesser surface antigens.

Reaction of an IgM isohemagglutinin and incompatible RBC's results in complement activation/fixation and hemolysis.

Begin to be formed about 6 months of age.

Isohemagglutinin Titers

Natural isohemagglutinins are primarily IgM, but some IgG are also found.

Titers determine the strength of a particular substance and are recorded as the last dilution where a reaction occurred,(1: 256).

Appears to have a correlation with the reactivity potential of individuals in that the higher the titer, the greater the level of response to an offending allergen.

Slide Testing

A simple procedure that requires specific A or B antiserum.

A 35% to 45% saline suspension of RBC's, glass slides, and applicator sticks for mixing.

Add a drop of antiserum to 2 drops of suspended cells and check for agglutination.

Advantages are that it is quick, easy and can be done on a finger stick sample.

Another advantage is that you can see weak agglutination easier than with tube testing, which may denote a subgroup.

Disadvantages are that the testing must be done at the time of obtaining the sample, whereas tube testing allows for storage and testing at a later date.

Tube Testing

Requires a 2% to 5% saline suspension of RBC's, a serofuge and antiserum.

Add 1 drop of antiserum to appropriate tube and 2 drops of saline suspended RBC's, mix, spin for 30 to 45 seconds and read for agglutination.

Advantages are that you can perform a lot of tests at one time and that they are easier to read than slide or card testing.

Disadvantage is that weak reacting blood groups can be missed more easily.

Blood Group Changes with Aging

Blood group antigens have been found to be present in fetal tissue.

At birth, blood group antigens generally exhibit a weaker expression. At about 3 years of age, blood group antigens reach the full strength of their expression.

The number of antigen sites on the cell surface ranges from 120,000 up to > 1 million depending upon the blood type and typing serum used.

As a person ages, there is some loss of cell surface antigenicity which may make it difficult to determine blood type for transfusion in older people.

Aging RBC's (those approaching 120 days) do not seem to lose their cell surface antigen content.

Rh(D) Blood Groupings

Rh+ (positive) and Rh- (negative) refer to the D blood group antigen.

Most important following ABO groupings in blood transfusion reactions.

Rh(D) + = 85%

Rh(D) - = 15%

Certain areas of the world will show slightly higher percentages of each antigen.

Wierner System

Rh(D) is determined by a single gene and each gene gives rise to several different factors which are measured by the antibodies produced in response to them.

DCE system: Fischer-Race Theory

There are 3 sets of 2 allelic genes, with one gene of each of the 3 pairs linked together at a single locus.

D -; Cc; Ee

D must be present in order for the person to be Rh+, while its absence denotes Rh-.

Du variant

Du is a variant of D and must undergo special testing procedures to uncover.

Considered Rh- for transfusion purposes and is < 1% of the population.

Anti Human Globulin – Coombs Test

Normal RBC's repel one another due to their negative surface charge (Zeta potential).

Smaller surface antigens that react with an IgG antibody may not be able to overcome the repelling forces.

Zeta potential overcome by adding 30% albumin or LISS.

Anti Human Globulin acts as a bridge to link IgG/antigen complexes to produce agglutination for detection.

Both direct and indirect antiglobulin tests performed.

Role in Erythroblastosis Fetalis

Most often caused by Rh(D) incompatibility where the mother is Rh- and the fetus is Rh+.

Developed during 1st pregnancy but affects 2nd childbirth.

Rarely other antigens are implicated such as ABO.

Anti Rh(D) administered post partum to neutralize antibody formation.

Rh(D) Testing Reagent

A blend of monoclonal IgM and IgG in buffered saline and bovine albumin.

Slide Testing

Pre-warmed slides needed (40-50 degrees C).

Rocking light box.

Add anti Rh(D) to 2 drops of a 35% to 45% saline suspension of RBC's.

Mix and rock for not longer than 2 minutes.

Check for agglutination

Tube Testing

Add 2 drops of a 3% to 5% washed saline suspension of RBC's to 1 drop of anti- Rh(D) in a test tube.

Mix gently and centrifuge at 1500-2000 rpm for 30 to 45 seconds.

Gently re-suspend the button and look for agglutination.

All Rh(D) negatives should be tested for the Du variant.

The Du test involves incubation at 37° C and the addition of AHG.

MN Blood Group System

Significance

Part of the MNSs system as they are linked on the same chromosome.

MM, MN, NN Genotypes.

MM = 28% of whites & 26% of blacks.

MN = 50% of whites & 44% of blacks.

NN = 22% of whites & 30% of blacks.

Rare cause of transfusion reactions.

M,N Testing Reagent

Monoclonal IgG antibodies to M and N antigens in buffered saline and bovine albumin.

Testing Procedure

Tube testing must be performed due to incubation period.

1 drop of anti M or N is added to a 3% to 5% solution of washed RBC's.

Mix and incubate at room temperature (23° C +/- 3° C) for 15 minutes.

Centrifuge @ 1000 rpm for 1 minute or 3400 rpm for 15 seconds.

Re-suspend the button of cells gently and examine for agglutination.

The N antibody + antigen complex is usually more difficult to interpret than the M complex.

False positives and negatives are more common as feeble agglutination may occur with the N antigen. Additionally, once the “button” has been re-suspended, re-centrifuging it and re-reading will often not produce the same result.

Lewis Blood Group System (LeA & LeB)

Significance

Lewis antigens are soluble glycosphingolipids that are adsorbed to RBC membranes from plasma. They are influenced by secretor status and possibly modified by ABO genes.

While genetically inherited, LeA & LeB are not genetically predetermined; rather, they are passively acquired.

The genotype [LeA+, LeB-] is generally associated with a non-secretor status while the presence of [LeA-, LeB+] is associated with a positive secretor status.

Exceptions to this “rule” were discovered by researchers when a [LeA-, LeB-] secretor was discovered.

In 1951 Grubb proposed the presence of a secretor gene Se whose presence was needed to convert LeA to LeB.

Lewis antigens in infants differ from that of adults and the Lewis blood group distribution does not begin to express itself until age 6.

Anti Lewis A & B antibodies can produce hemolytic transfusion reactions if present in high enough titer.

Clinically, the greater majority of chronic degenerative disease patients are non-secretors (about 40% to 45%).

Lewis Blood Group System

<u>Incidence</u>	<u>Whites</u>	<u>Blacks</u>
LeA+, LeB- =	22%	23%
LeA-, LeB+ =	72%	55%
LeA-, LeB- =	6%	22%
LeA+, LeB+ =	rare	rare

Lewis Blood Group Reagent

Monoclonal IgG antibody in phosphate buffer and bovine albumin. LeA & LeB are produced from separate cell lines.

Testing Procedure

Tube testing must be done as incubation @ 18° C to 23° C (64° F to 73° F) is part of the testing procedure.

**In a test tube add 1 drop of anti LeA or LeB to a 2% to 5% saline solution of RBC's.
Mix and incubate at 18° C to 23° C for 10 to 15 minutes.**

Do not perform tests above 23° C.

Centrifuge @ 900-1000 rpm for 15 to 30 seconds, but not longer.

Re-suspend gently and look for agglutination.

Limitations

False positive or false negative results can occur from bacterial contamination.

RBC's must be washed in physiological saline as serum and plasma may neutralize Lewis antibodies.

There is a great deal of variance between individuals with the same genotype, so specimens will vary in strength of reaction.

Certain blood types (O & B) reactivity will be greater than others such as A₁ or A₁B types.

RBC Lewis antigens will deteriorate with age of the specimen and will diminish in number in elderly patients.

False negative tests for LeA⁺ patients will be seen if incubated above 23° C.

Lewis blood group antigens can not be accurately determined in children less than 6 years old.

Agglutination Inhibition Test

Saliva to be tested is mixed with commercial antiserum (anti-A, anti-B or anti-H) and allowed to incubate briefly. After incubation, known blood group A or B cells are added and agglutination is checked for.

If the person is a secretor, then there will be no agglutination of the RBC's because all of the antibodies will have been neutralized by the blood group antigens in the saliva.

If a person is a non-secretor, then agglutination will occur because no inhibition has taken place.

NO Agglutination = Secretor

Agglutination = Non-Secretor

Advantages are that persons typed [Lewis A-, Lewis B-] can be screened for the presence of the secretor gene Se.

Disadvantages are that the Agglutination Inhibition test is very technique dependent and therefore subject to some error.

For Lewis A- and Lewis B- persons, we have found that their clinical presentations and responses to the dietary changes approximate those who are non-secretors.

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.