BLOOD GROUPING REAGENTS

Monoclonal Antibodies to ABO Rh(o)D
(IgG/IgM, IgG + IgM)

ISO 9001:2008
ISO 13485:2013

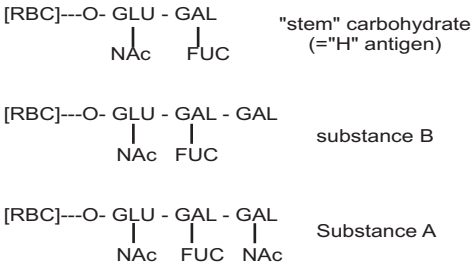
CLINICAL SIGNIFICANCE

Human blood transfusions requires compatibility for the two major blood group antigen systems, namely ABO and Rh. There are about100 known blood group determinants so far identified that comprise 15 genetically distinct blood group systems. ABO system is defined by two red blood cells (RBC) antigens, A and B, whose presence or absence is determined by three alleles (A, B, O) at one genetic locus. The presence or absence of Rh antigens on red blood cells is determined by two alleles at another locus, Rh. Rh INCOMPATIBILITY between mother and infant may result in *Erythroblastosis Fetalis*, which can be prevented by passive immunization of the mother with anti-Rh antibodies.

The success of blood transfusions depends on ensuring the compatibility of the blood types between donor and recipient. If the recipient has antibodies to the infused red cells, these red cells will be rapidly destroyed, resulting in a potentially lethal **transfusion reaction**. Type A blood given to a type B recipient, for instance, can result in such a reaction, since the recipient's serum contains anti-A antibodies.

PRINCIPLE

The blood group substances A and B represent are two modified forms of a "stem" carbohydrate present on red blood cells and other tissues. Their structures are shown below:



Where GLU = Glucose or Glucosamine, GAL = Galactose or galactosamine, FUC is fucose, NAc represents an N Acetyl group

The presence of ABO antigens and antibodies (*isoagglutinins)* in the four blood types is summarized below:

BLOOD TYPE	RBC ANTIGENS	SERUM ANTIBODIES	FREQUENCY
A	A	anti-B	40%
B	B	anti-A	10%
AB	A *and* B	none	5%
O	none	anti-A*and* anti-B	45%

The presence of A and B carbohydrates in our tissues is determined by three alleles at a single genetic locus. One allele encodes an enzyme which produces the A substance, another the B substance; and when both of these alleles are present in a heterozygote both carbohydrates are made. The third allele, O, behaves essentially as a "null" allele, producing neither A nor B substance. Thus, while the ABO system yields only four blood types (phenotypes), there are six possible genotypes:

Genotype	**Blood Type** (Phenotype)
A/A	A
A/O	A
B/B	B
B/O	B
A/B	AB
O/O	O

Only a single genotype can produce the phenotype AB, namely the heterozygous state A/B. Likewise, type O individuals must be homozygous O/O. However, type A or type B individuals can be either homozygous or heterozygous, the O allele being effectively recessive since it does not contribute either of the two antigens. The inheritance of the ABO blood groups follows simple Mendelian rules. For instance, a homozygous type A mother and a type AB father (below, left) can yield only two kinds of offspring, type A (genotype A/A) or type AB (genotype A/B). A heterozygous type A and a heterozygous type B, on the other hand (below, right), can yield four genotypes and four corresponding phenotypes.

A/A x A/B		**Parents**	**A/O x B/O**				**Parents**
A/A	x A/B	Offspring	A/B	A/O	B/O	O/O	Offspring
0.50	0.50	Frequency	0.25	0.25	0.25	0.25	Frequency

Rh BLOOD GROUPS

While many blood group systems are known other than the ABO system, the Rh system is of special importance,. This was originally defined by a rabbit antibody directed against the red blood cells of Rhesus monkeys, an antibody which turned out to be capable of distinguishing between the red blood cells of different human individuals. In simple terms, this system is defined by the presence or absence of a single red blood cell antigen, representing the two blood types Rh(+) and Rh(-). These are determined by two alleles at a single locus, which segregate independently of the ABO blood group locus. Thus an Rh(+) individual may be homozygous (+/+) or heterozygous (+/-), while an Rh(-) individual must be homozygous (-/-).

REAGENTS

These reagents are intended for in-vitro diagnostic slide, tube or Automated Microplate System use only. The ready to use reagents are supplied in glass or plastic vials. Do not use any reagent past the expiration date marked on each labels. Blood Grouping Reagents are of antibodies class IgG or IgM, or IgG+IgM Monoclonal Anti-A, Anti-B, Anti-A,B, Anti-D, Manufactured from antibodies derived from the supernatants of in vitro cultures of hybridomas of murine or human origin. These reagents contain sodium azide (<0.1%), sodium arsenite (0.02%) and BSA.

REAGENT PREPARATION

1. The reagents are intended for use as supplied. No prior preparation or dilution of the reagents is required or permitted.
2. All reagents should be brought to room temperature (+15 - +30°C) before use.
3. Effort should be made to minimize contamination during use of the product.
4. Do not transfer reagents back into the original container or between containers once dispensed or put into use.

STORAGE

1. All reagents are stable till the expiration date marked on each labels when stored at 2 to 8°C and should not be frozen to ice.
2. Do not use beyond the expiration date.
3. Do not interchange cap/s or dispenser/s

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the donor is required prior to specimen collection. Whole blood samples must be collected in EDTA anticoagulant in either glass or plastic tubes. Clotted or hemolysed blood samples should not be used when red blood cell testing is being carried out. Specimens from donors with protein abnormalities may give erroneous results. Lipemic, icteric or hemolyzed samples may produce erroneous results in plasma ABO testing (reverse ABO grouping). Anticoagulated samples containing clots may also give erroneous results in ABO cell testing. Specimen should be stored at 4° to 8°C If testing must be postponed for longer than 24 hours from collection. Samples must be returned to room temperature (15° C to 30° C) prior to analysis. Testing should be carried out within five (5) days of collection. Bacterial contamination of the specimen may cause erroneous test results.

QUALITY CONTROL

A series of quality control samples should be run at the beginning and end of each test run. Quality control samples should be tested in the same manner as all other samples. The control samples should be selected to verify positive and negative reactions with every reagent. The positive controls should produce (+) reactions and the negative controls should produce negative (-) reactions with the appropriate reagent. If the expected results are not obtained with an individual control sample, the suspect quality control sample should be inspected for both adequate quantity and compliance with the sample requirements. Failure of controls to perform as expected may indicate contamination or deterioration of one or more of the reagents comprising the system and should be re-assyed with new batch of reagent/s.

LIMITATIONS

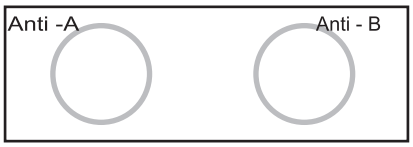
All Blood Grouping reagents are for professional IVD use only. **Avidity of reagents may progressively be deteriorated upon freezing to ice, exposing higher temperatures, cross contamination, bacterial contamination etc. Failure to react may lead to wrong results.**

TEST PROCEDURE

ABO BLOOD GROUPING PROCEDURE

A. Slide Test Procedure

1. On to a clean slide draw two circles of a coin size and mark as " Anti-A" and "Anti- B" using a suitable glass marking wax pencil as given under.



2. Place one drop of blood grouping reagent Anti-A (color coded as blue) in to circle marked "Anti-A"
3. Place one drop of blood grouping reagent Anti-B (color coded as yellow) in to circle marked "Anti-B" .
4. To each of the reagent add one drop of whole blood. Mix well with a separate applicator stick or tooth pick.
5. Rock the slide gently back and forth.
6. Observe for agglutination at the end of 2 to 3 minutes. Peripheral drying should not be interpreted as a positive test result.

Tube Test Procedure

Step-1. Prepare a 2 - 5% RBC suspension of the sample to be tested in isotonic saline.

Label 3 test tubes (12 x 75 mm) as ... And with sample ID	A	B	Ctrl
Add Anti A (Blue color coded) drop	1	-	-
Add Anti B (Yelow color coded) drop	-	1	-
Add 2-5 % RBC suspension Step-1 drop	1	1	1

Mix gently for 30 seconds and let the tube un-disturbed for about 10 mins at RT. Gently dislodge the cells and observe agglutination against Cntrl

.2. Rh TYPING

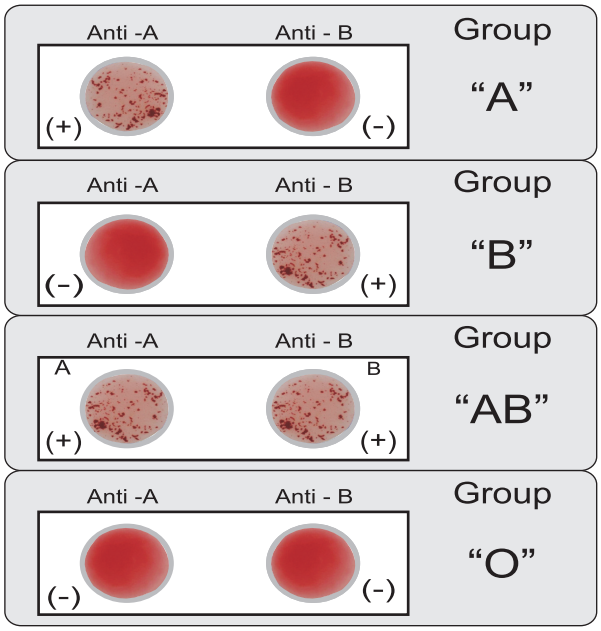
A. Slide test

1. Use whole blood or prepare 40% suspension of red blood cells in individuals own plasma or normal saline.
2. Place one drop each of Anti-D and whole blood or above cell suspension on a glass slide using a Pasteur pipette.
3. Mix well using an applicator stick or with sterile tooth pick.
4. Rock the slide gently back and forth.
5. Observe for agglutination macroscopically or using a hand lens within 2 to 3 minutes. Peripheral drying should not be interpreted as a positive test result.

B. Tube test

1. Prepare a 5% suspension of red blood cells in individuals own plasma or in normal saline.
2. To two small test tubes (12 x 75 mm) labeled as test (T) and control (C),add one drop of above cell suspension to both tubes using a Pasteur pipette.
3. Add one drop of Anti-D to tube (T) and one drop of 22% Bovine Albumin to tube (C).
4. Mix well and centrifuge both the tubes at 1000 rpm for one minute. Alternatively the tubes can be incubated at 37°C for 30 minutes.
5. Gently dislodge the cell button and examine macroscopically for agglutination.

INTERPRETATION OF RESULTS



1. Agglutination of red blood cells in presence of Anti-A only indicates the presence of A antigen on red blood cells (Group A).
2. Agglutination of red blood cells in presence of Anti-B only indicates the presence of B antigen on red blood cells (Group B).
3. Agglutination of red blood cells in presence of Anti-A as well as Anti-B indicates the presence of both A and B antigens on red blood cells(Group AB).
4. Absence of agglutination of red blood cells in presence of Anti-A and Anti-B indicates absence of both A & B antigens(Group O).
5. Agglutination of the red blood cells in presence of Anti-D antibody with both slide test and tube test indicates the presence of D (Rho) antigen on red blood cells and hence Rh D Positive.
6. Absence of agglutination of the red blood cells in presence of Anti-D antibody with both slide test and tube test is a negative test. It generally indicates that D (Rho) antigen is not demonstrable. Confirm through indirect Coomb's test using Anti-D(Rho) Monoclonal (IgM/IgG / IgM+IgG) reagent.
7. No interpretation should be made if the agglutination appears in negative control with either slide test or tube test.

LIMITED EXPRESSED WARRANTY DISCLAIMER.

The reagent meets the requirements of the Common Technical Specifications for products defined in Annex II, List A of Directive 98/79/EC on in vitro Diagnostic Medical Devices. When used in accordance with the recommended Instructions for Use the reagent has been tested and found to specifically agglutinate human red cells if the corresponding antigen is present. The reactivity of each lot has been verified with a panel of red cells tested in accordance with the recommended Instructions for Use.

Every effort is made to supply specified material as per the samples or specifications, however, due to continuous development/s the company reserve the right to improve / change any specifications / component /s without prior information / notice to the purchaser / actual user.

The manufacturer limits the warranty to this test kit, as much as that the test kit will function as an in vitro diagnostic assay within the Nature of Sample, Procedure limitations and specifications as described in the product procedure manual, when used strictly in accordance with the instructions contained. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever / whomsoever caused by the product in the use or in the application there of.

Bibliography

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