Tulip Group

TECHNOTES

Antibody Screening

Antibody Screening in Pre-transfusion Testing and Antenatal Screening

For Private Circulation Only

www.tulipgroup.com

Tulip Block, Dr. Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Bambolim, Goa - 403 202, India.
Tel.: + 91 832 2458546-51. Fax: +91 832 2458544. e-mail: sales@tulipgroup.com

Setting Trends

Antibody Screening in Pre-transfusion Testing and Antenatal Screening

Q. What are naturally occurring or expected antibodies?
Q. What are atypical or unexpected antibodies?
Q. How atypical antibodies are formed and which are clinically significant antibodies?
Q. What are autoantibodies?
Q. What is pre transfusion testing?
Q. What is Antibody Screening?
Q. How Antibody Screening is done?
Q. What is Antibody Identification?
Q. What is the difference between Crossmatch and Antibody Screening?
Q. What is Type and Screen Policy?
Q. What is the importance of Antibody Screening in antenatal cases?

Naturally occurring or expected antibodies.

If an ABO antigen is missing from an individual's red cell membrane, then it is **EXPECTED** that the individual will produce an antibody to that antigen. These have also been called **"naturally occurring"** antibodies. Example: anti-A, anti-B, anti-AB. Healthy adults always have anti-A and/or anti- B antibodies in their serum if they lack the corresponding antigen on their red cells.

Atypical or Unexpected antibodies.

In all blood group systems other than ABO, if the antigen is missing from the red cell, the individual is **NOT** expected to produce an antibody against it, normally. When these antibodies are produced they are termed **UNEXPECTED or ATYPICAL or ALLOANTIBODIES**. The production of these antibodies is a result of an event, like blood transfusion or Pregnancy. These antibodies are normally of IgG class. Example: anti-D, anti-C, anti Fy^a.

Formation of Atypical or Alloantibodies.

Alloantibodies are the antibodies produced against foreign antigen. These antibodies can be made in response to a transfusion of red cells or exposure to fetal red cells during pregnancy or delivery. These antibodies are directed to a non-self antigen and thus called as alloantibodies. There are now about 270 authenticated blood group antigens. Many of these blood group antigens fall into one of 26 blood group systems.

Apart from these 26 Blood group systems there are few more antigens which are not assigned to any blood group system. Antibodies to all these antigens are not capable of causing Hemolytic Transfusion Reaction or HDFN. There are some antibodies which are clinically significant and capable of causing Hemolytic Transfusion Reaction and HDFN, such few antibodies are listed below.

Name of Blood Group System	Clinically Significant Antibodies
ABO	A, B & AB
Rh	D, C, E, c, e
Kell	K
Kidd	Jk^a, Jk^b
Duffy	Fy^a, Fy^b
MNS	S, s (rarely M, N)
Lewis	Le^a (rarely)

Autoantibodies.

Autoantibodies are antibodies, usually formed by a disease process or medication. These antibodies are produced against person's own red cells.

Pre-transfusion testing.

The objective of pre-transfusion testing is to ensure that enough red cells and components will survive when transfused, or in other words, to transfuse a blood component to a patient that will provide maximum benefit while causing less harm.

In most blood banks, pre-transfusion testing involves (i) determining the ABO and Rh types of patient and donor blood, (ii) screening patient and donor sera for RBC alloantibodies, and (iii) performing a major crossmatch. Pre-transfusion testing can assure ABO compatibility between donor and patient blood as well as detect most clinically significant RBC alloantibodies that can react with donor's red cell antigens.

Antibody screening.

Apart from clerical checking, grouping and typing of donor and patient blood, the serum or plasma of the patient must be tested against a panel of group O reagent red cells. Such reagent red cells are selected because they carry the blood group antigens necessary for detecting the most important ***"clinically significant"*** RBC alloantibodies. This procedure is known as antibody screening and these reagent red cells are known as screening cell panels. The antibody screening determines whether an antibody to a red cell antigen has been produced. Antibody screening is performed to detect antibodies in:

- Patients requiring blood transfusion
- Women who are pregnant
- Patients with suspected transfusion reactions
- Blood and plasma donor

Antibody screening cells are reagent red cells that provide a combination of antigens other than A and B antigens. These cells are tested with patient's serum/plasma to determine whether an unexpected antibody exists. Below is an example of an antigram for a three cell screening panel. An antigram lists the antigens present in the red cell suspensions. A reaction to either of the screening cells demonstrates the presence of an atypical antibody. Three cell panel is always preferred over two cell panel because it provides an *rr* (Rh Negative) cell and homozygous cells for the Duffy and Kidd blood groups. Most common, clinically significant antibodies reacts with three cell panel and initial conclusions regarding the type of antibody can often be made when screen is complete.

matrix™ GEL•SYSTEM ERYGEN AS Antigram.

ANTIGEN CHART

		Rheseus						Kell		Duffy		Kidd		Lewis		MNS				P	Test Results		
Cell No.	**Rh Phenotype**	**D**	**C**	**E**	**c**	**e**	**C^w**	**K**	**k**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**Le^a**	**Le^b**	**M**	**N**	**S**	**s**	**P_1**	**AHG**	**ENZ**	**SAL/ 4°C**
I	R_1R_1	+	0	+	+	0	0	0	+	+	0	+	+	0	+	0	+	+	+	+			
II	R_1R_2	+	+	+	+	+	0	+	+	+	+	+	+	0	0	+	+	0	+	0			
III	"	0	0	0	+	+	0	0	+	0	+	+	0	0	0	+	+	NT	+	+			
Patient																							

+ = Positive
0 = Negative
NT = Not tested
W = Weak
S = Strong

Cat. No.	102700032
LOT	823310
Expiry Date	11/11/13

Antibody Screening procedure.

Screening cell panel consists of red cells of three donors' labeled as *Cell I*, *Cell II* and *Cell III.* Patient's serum is tested with these reagent red cells in an IAT phase. Commercial red cell panels are supplied in appropriate cell suspension which makes them ready to use. In Matrix Gel System total procedure for antibody screening is of 30 minutes. 50µl of reagent red cells to be pipetted in to the labeled microtubes of Matrix™ AHG (Coombs) test card followed by 25µl patient's serum or plasma. The card is then incubated for 15 minutes at 37° C followed by centrifugation. Results are then read and interpreted on the antigram provided with the cell panels.

An autocontrol tests the patient's serum with his or her own red cells. Testing an autocontrol routinely with the screen is optional; most blood bankers prefer to perform a DAT only if the screen is positive. The autocontrol and DAT provide useful information in determining whether patient's antibody is directed against his or her red cells or transfused cells.

Antibody identification.

Antibody identification is performed after the positive results of antibody screening. In antibody identification serum or plasma is tested against a panel of reagent red cells. A panel like the screening cells, consists of group O reagent red cells that have been typed for most common antigens specificities. Commercial cell panels are available with variety of antigen configurations, which may include 10, 11, 15, 16 or 20 cells that can be thought of as extended antibody screens. The use of autocontrol with the panel is recommended, especially if it is not routinely tested with the screening cell panel.

Once testing is done results are recorded on antigram provided with the kit for each phase of testing. The phase or reaction temperature at which agglutination appears is an indication that the antibody is IgG or IgM. IgM antibodies typically react at room temperature or on immediate spin. IgM antibodies such as anti-Le^a, -Le^b, -M, -N, -I and P1 should be suspected if immediate spin reactions are detected. IgG antibodies react at the antiglobulin phase. Reactions at different phases indicate more than one antibody and a combination of IgG and IgM antibodies. After recording the results on an antigram provided with the cell panel the antibody is identified by following steps.

- **Ruling Out:** Cells that give negative reaction with all tested phases can be used to rule out the antibodies. If the antigen antibody reaction did not occur, this suggests that the antibody did not react with the antigen present on the panel cell, and respective antigens can be eliminated as a possible antibody. Antigens that are heterozygous should not be cross out because antibody might have been too weak to react. This process is continued for each negative cell.
- **Matching the pattern:** The next step in panel interpretation is to look at the reactions that are positive and match the pattern. When a single antibody is present, the pattern of reactions observed matches with the other cells.
- **Rule of three:** In rule of three, at least three antigen positive red cells that react and three antigen negative red cells that do not react should be observed.
- **Phenotyping the patient:** Individuals do not make alloantibodies to antigens they possess. Another way to confirm antibody identification is to phenotype the patient's red cells to ensure that they are negative for the antigen corresponding to the identified antibody.

Crossmatch Vs Antibody Screening

Antibody Screening is the most reliable and sensitive method of detecting alloantibodies. Crossmatch is often less reliable when compared with Antibody Screening, because some antibodies manifest dosage effect. To explain the dosage effect we take an example of Kidd blood group system having two major antigens Jk^a and Jk^b. Please refer to below table:

Cell	Jk^a	Jk^b	Remarks
Cell I	+	0	This cell is having Homozygous expression of Jk^a
Cell II	0	+	This cell is having Homozygous expression of Jk^b
Cell III	+	+	This cell is having Heterozygous expression of Jk^a and Jk^b

In above example, *cell I* carries the Homozygous expression of Jk^a and results in higher expression of the Jk^a antigen than the *cell III* which carries the heterozygous expression of both Jk^a and Jk^b.

While performing crossmatch, phenotype (antigenic configuration of red cells) of donor's red cells is not known, and there are possibilities that, patient is having anti- Jk^a and donor red cell carries heterozygous expression of Jk^a. This may lead to a compatible crossmatch even in the presence of corresponding antibody. For these reasons, an antiglobulin crossmatch using donor cells is not the most effective way of detecting a serological incompatibility between donor and patient.

A major crossmatch, involves testing of the patient's serum with donor's RBCs, in IAT phase. In crossmatch we detect the presence of antibodies in patient's serum/plasma corresponding to the antigens present on the donor's red cells.

"***A Negative or Compatible crossmatch shows that antibodies corresponding to the antigens of donor's red cells are absent in patient's serum/plasma. But a compatible crossmatch does not signify that there are no atypical antibodies present in patient's serum/plasma."***

Type and Screen Policy.

In some special instances, crossmatching of blood is excluded from pretransfusion testing according to a policy called "type and screen." This policy stipulates that blood does not have to be crossmatched in advance for patients undergoing surgical procedures usually not requiring blood. The patient's blood is, however, completely tested for ABO group, Rh type, and RBC alloantibodies and then kept in storage by the transfusion service in case it is needed for crossmatching. In most countries, typed and screened patient's blood can be crossmatched by IS-XM (immediate spin cross match or saline crossmatch) and made available in minutes, however in India blood banks following Type and Screen policy performs Coombs crossmatch whenever blood unit is required for the patient.

"Type and Screen" can be very useful for the blood banks having high Crossmatch: Transfusion (CT) ratio. CT ratio is the ratio of blood units crossmatched and blood units transfused in a hospital. Higher CT ratio suggested that number of blood units crossmatched is higher than the number of blood units transfused. Before the introduction of this procedure, many units of donor blood were crossmatched and held in reserve for patients who would probably not need it. At times this would cause shortages of the blood supply and unnecessary outdating of donor units. These factors, along with the added expense of crossmatching blood, caused the Type and Screen (T&S) procedure to gain popularity.

*This procedure is used most frequently to screen pre-operative or gynecological patients whose risk of excessive blood loss is **minimal**.* In case of an emergency, where blood is needed for these patients, IS-XM (saline crossmatched), ABO and D compatible blood can be released with 99.9% assurance of safety, as long as the patient has no unexpected antibodies.

Antibody screening in Antenatal cases.

The antigen that most frequently induces immunization is D but any red cell antigen present on fetal cells and absent from the mother can stimulate antibody production. As these antibodies are of IgG class, they are capable of crossing placenta and may cause hemolysis of fetal red blood cells. HDFN is often classified into three categories on the basis of the specificity of the causative IgG antibody. In descending order of potential severity they are:

1. D hemolytic disease caused by anti-D alone or less often in combination with anti-C or anti-E.
2. "Other" hemolytic disease caused by antibodies against other antigens in the Rh system or against antigens in other systems; anti-c and anti-K are most often implicated.
3. ABO HDFN caused by anti-A,B in a group O woman or by isolated anti-A or anti-B.

In order to detect these antibodies the samples of all pregnant women should be taken early in pregnancy ideally at 10-16 weeks gestation for ABO and D typing and for screening for the presence of red cell alloantibodies. When an antibody screen is positive further tests should be carried out to determine the antibody specificity and significance.

All pregnant women whether D positive or D negative, should have a further blood sample taken at 28 weeks gestation for re-checking the ABO and D group and further screening for red cell alloantibodies. D positive women are just as likely as D negative women to form antibodies other than anti-D late in pregnancy.

When red cell antibodies are detected, further testing of maternal blood should be undertaken to determine the specificity, concentration, origin and level of antibody or antibodies and the likelihood of HDFN. Anti-D, anti-c and anti-K are the antibodies most often implicated in causing haemolytic disease severe enough to warrant antenatal intervention.

Bibliography

1. Basics and applied concepts of Immunohematology
2. BCSH Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories.
3. BCSH Guidelines for blood grouping and antibody testing in pregnancy.
4. Guidelines for pre-transfusion testing; Australian and New Zealand Society of Blood Transfusion.
5. Current issues in transfusion medicine; Pretransfusion testing of red cells-Current status
6. Maximum surgical blood ordering schedule in a tertiary trauma center in northern India: A proposal.
7. AABB, Technical Manual, 15th Edition.