# IMMUNOGLOBULINS- ANTIGEN-ANTIBODY REACTIONS AND SELECTED TESTS

## AFFINITY AND AVIDITY

### Affinity

Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody.
Affinity is the equilibrium constant that describes the antigen-antibody reaction. Most antibodies have a high affinity for their antigens.

### Avidity

Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities. To repeat, affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.

## SPECIFICITY AND CROSS REACTIVITY

### Specificity

Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen. In general, there is a high degree of specificity in antigen-antibody reactions. Antibodies can distinguish differences in:

- The primary structure of an antigen
- Isomeric forms of an antigen
- Secondary and tertiary structure of an antigen

### Cross reactivity

Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen. Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).

## TESTS FOR ANTIGEN-ANTIBODY REACTIONS

### Factors affecting measurement of antigen-antibody reactions

The only way that one knows that an antigen-antibody reaction has occurred is to have some means of directly or indirectly detecting the complexes formed between the antigen and antibody. The ease with which one can detect antigen-antibody reactions will depend on a number of factors.

### Affinity

The higher the affinity of the antibody for the antigen, the more stable will be the interaction. Thus, the ease with which one can detect the interaction is enhanced.

### Avidity

Reactions between multivalent antigens and multivalent antibodies are more stable and thus easier to detect.

### Antigen to antibody ratio

The ratio between the antigen and antibody influences the detection of antigen-antibody complexes because the size of the complexes formed is related to the concentration of the antigen and antibody.

### Physical form of the antigen

The physical form of the antigen influences how one detects its reaction with an antibody. If the antigen is a particulate, one generally looks for agglutination of the antigen by the antibody. If the antigen is soluble one generally looks for the precipitation of the antigen after the production of large insoluble antigen-antibody complexes.

## Agglutination Tests

### Agglutination/Hemagglutination

When the antigen is particulate, the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen. The general term agglutinin is used to describe antibodies that agglutinate particulate antigens. When the antigen is an erythrocyte the term hemagglutination is used. All antibodies can theoretically agglutinate particulate antigens but IgM, due to its high valence, is particularly good agglutinin and one sometimes infers that an antibody may be of the IgM class if it is a good agglutinating antibody.

### Qualitative agglutination test

Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen. For example, a patient's red blood cells can be mixed with antibody to a blood group antigen to determine a person's blood type. In a second example, a patient's serum is mixed with red blood cells of a known blood type to assay for the presence of antibodies to that blood type in the patient's serum.

### Quantitative agglutination test

Agglutination tests can also be used to measure the level of antibodies to particulate antigens. In this test, serial dilutions are made of a sample to be tested for antibody and then a fixed number of red blood cells or bacteria or other such particulate antigen is added. Then the maximum dilution that gives agglutination is determined. The maximum dilution that gives visible agglutination is called the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination.

**Prozone effect -** Occasionally, it is observed that when the concentration of antibody is high (i.e. lower dilutions), there is no agglutination and then, as the sample is diluted, agglutination occurs. The lack of agglutination at high concentrations of antibodies is called the prozone effect. Lack of agglutination in the prozone is due to antibody excess resulting in very small complexes that do not clump to form visible agglutination.

### Applications of agglutination tests

i. Determination of blood types or antibodies to blood group antigens.
ii. To assess bacterial infections
*e.g.* A rise in titer of an antibody to a particular bacterium indicates an infection with that bacterial type.
N.B. a fourfold rise in titer is generally taken as a significant rise in antibody titer.

### Passive hemagglutination

The agglutination test only works with particulate antigens. However, it is possible to coat erythrocytes with a soluble antigen (e.g. viral antigen, a polysaccharide or a hapten) and use the coated red blood cells in an agglutination test for antibody to the soluble antigen. This is called passive hemagglutination. The test is performed just like the agglutination test. Applications include detection of antibodies to soluble antigens and detection of antibodies to viral antigens.

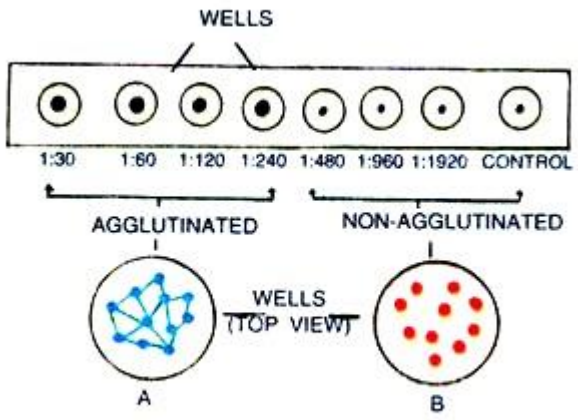



**Fig. 22.24:** Direct agglutination test.

**Coomb's Test (Antiglobulin Test)**

**Direct Coomb's Test**

When antibodies bind to erythrocytes, they do not always result in agglutination. This can result from the antigen/antibody ratio being in antigen excess or antibody excess or in some cases electrical charges on the red blood cells preventing the effective cross linking of the cells. These antibodies that bind to but do not cause agglutination of red blood cells are sometimes referred to as incomplete antibodies. In no way is this meant to indicate that the antibodies are different in their structure, although this was once thought to be the case. Rather, it is a functional definition only. In order to detect the presence of non-agglutinating antibodies on red blood cells, one simply adds a second antibody directed against the immunoglobulin (antibody) coating the red cells. This anti-immunoglobulin can now cross link the red blood cells and result in agglutination. This test is illustrated in Figure 10 and is known as the Direct Coomb's test.

**Indirect Coomb's Test**

If it is necessary to know whether a serum sample has antibodies directed against a particular red blood cell and you want to be sure that you also detect potential non- agglutinating antibodies in the sample, an Indirect Coomb's test is performed. This test is done by incubating the red blood cells with the serum sample, washing out any unbound antibodies and then adding a second anti-immunoglobulin reagent to cross link the cells.

## Applications

These include detection of anti-rhesus factor (Rh) antibodies. Antibodies to the Rh factor generally do not agglutinate red blood cells. Thus, red cells from $Rh^+$ children born to $Rh^-$ mothers, who have anti-Rh antibodies, may be coated with these antibodies. To check for this, a direct Coombs test is performed. To see if the mother has anti-Rh antibodies in her serum an Indirect Coombs test is performed.

**Hemagglutination Inhibition**

The agglutination test can be modified to be used for the measurement of soluble antigens. This test is called hemagglutination inhibition. It is called hemagglutination inhibition because one measures the ability of soluble antigen to inhibit the agglutination of antigen-coated red blood cells by antibodies. In this test, a fixed amount of antibodies to the antigen in question is mixed with a fixed amount of red blood cells coated with the antigen (see passive hemagglutination above). Also included in the mixture are different amounts of the sample to be analyzed for the presence of the antigen. If the sample contains the antigen, the soluble antigen will compete with the antigen coated on the red blood cells for binding to the antibodies, thereby inhibiting the agglutination of the red blood cells.

By serially diluting the sample, you can quantitate the amount of antigen in your unknown sample by its titer. This test is generally used to quantitate soluble antigens and is subject to the same practical considerations as the agglutination test.

**Precipitation tests**

**Radial Immunodiffusion (Mancini)**

In radial immunodiffusion antibody is incorporated into the agar gel as it is poured and different dilutions of the antigen are placed in holes punched into the agar. As the antigen diffuses into the gel, it reacts with the antibody and when the equivalence point is reached a ring of precipitation is formed.

The diameter of the ring is proportional to the log of the concentration of antigen since the amount of antibody is constant. Thus, by running different concentrations of a standard antigen one can generate a standard cure from which one can quantitate the amount of an antigen in an unknown sample. Thus, this is a quantitative test. If more than one ring appears in the test, more than one antigen/antibody reaction has occurred. This could be due to a mixture of antigens or antibodies. This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

**Immunoelectrophoresis**

In immunoelectrophoresis, a complex mixture of antigens is placed in a well punched out of an agar gel and the antigens are electrophoresed so that the antigen are separated according to their charge. After electrophoresis, a trough is cut in the gel and antibodies are added. As the antibodies diffuse into the agar, precipitin lines are produced in the equivalence zone when an antigen/antibody reaction occurs.

This tests is used for the qualitative analysis of complex mixtures of antigens, although a crude measure of quantity (thickness of the line) can be obtained. This test is commonly used for the analysis of

components in a patient' serum. Serum is placed in the well and antibody to whole serum in the trough. By comparisons to normal serum, one can determine whether there are deficiencies on one or more serum components or whether there is an overabundance of some serum component (thickness of the line). This test can also be used to evaluate purity of isolated serum proteins.

**Countercurrent electrophoresis**
In this test the antigen and antibody are placed in wells punched out of an agar gel and the antigen and antibody are electrophoresed into each other where they form a precipitation line. This test only works if conditions can be found where the antigen and antibody have opposite charges. This test is primarily qualitative, although from the thickness of the band you can get some measure of quantity. Its major advantage is its speed.

**Radioimmunoassay (RIA)/Enzyme Linked Immunosorbent Assay (ELISA)**
Radioimmunoassays (RIA) are assays that are based on the measurement of radioactivity associated with immune complexes. In any particular test, the label may be on either the antigen or the antibody. Enzyme Linked Immunosorbent Assays (ELISA) are those that are based on the measurement of an enzymatic reaction associated with immune complexes. In any particular assay, the enzyme may be linked to either the antigen or the antibody.

**Competitive RIA/ELISA for Ag Detection**
The method and principle of RIA and ELISA for the measurement of antigen. By using known amounts of a standard unlabeled antigen, one can generate a standard curve relating radioactivity (cpm) (Enzyme) bound versus amount of antigen. From this standard curve, one can determine the amount of an antigen in an unknown sample.
The key to the assay is the separation of the immune complexes from the remainder of the components. This has been accomplished in many different ways and serves as the basis for the names given to the assay:

**Precipitation with ammonium sulphate**
Ammonium sulphate (33 - 50% final concentration) will precipitate immunoglobulins but not many antigens. Thus, this can be used to separate the immune complexes from free antigen. This has been called the Farr Technique

**Anti-immunoglobulin antibody**
The addition of a second antibody directed against the first antibody can result in the precipitation of the immune complexes and thus the separation of the complexes from free antigen.

**Immobilization of the Antibody**
The antibody can be immobilized onto the surface of a plastic bead or coated onto the surface of a plastic plate and thus the immune complexes can easily be separated from the other components by simply washing the beads or plate. This is the most common method used today and is referred to as Solid phase RIA or ELISA. In the clinical laboratory, competitive RIA and ELISA are commonly used to quantitate serum proteins, hormones, drugs metabolites.

**Non-competitive RIA/ELISA for Ag or Ab**
Non-competitive RIA and ELISAs are also used for the measurement of antigens and antibodies. The bead is coated with the antigen and is used for the detection of antibody in the unknown sample. The amount of labeled second antibody bound is related to the amount of antibody in the unknown sample. This assay is commonly employed for the measurement of antibodies of the IgE class directed against particular allergens by using a known allergen as antigen and anti-IgE antibodies as the labeled reagent. It is called the RAST test (radioallergosorbent test). The bead is coated with antibody and is used to measure an unknown antigen. The amount of labeled second antibody that binds is proportional to the amount of antigen that bound to the first antibody.

**Tests for Cell Associated Antigens**
**Immunofluorescence**
Immunofluorescence is a technique whereby an antibody labeled with a fluorescent molecule (fluorescein or rhodamine or one of many other fluorescent dyes) is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.

**Direct Immunofluorescence**

In direct immunofluorescence, the antibody specific to the antigen is directly tagged with the fluorochrome.

**Indirect Immunofluorescence**

In indirect immunofluorescence, the antibody specific for the antigen is unlabeled and a second anti-immunoglobulin antibody directed toward the first antibody is tagged with the fluorochrome. Indirect fluorescence is more sensitive than direct immunofluorescence since there is amplification of the signal.

**Flow Cytometry**

Flow cytometry is commonly used in the clinical laboratory to identify and enumerate cells bearing a particular antigen. Cells in suspension are labeled with a fluorescent tag by either direct or indirect immunofluorescence. The cells are then analyzed on the flow cytometer.

The principle of flow cytometry. In a flow cytometer, the cells exit a flow cell and are illuminated with a laser beam. The amount of laser light that is scattered off the cells as they passes through the laser can be measured, which gives information concerning the size of the cells. In addition, the laser can excite the fluorochrome on the cells and the fluorescent light emitted by the cells can be measured by one or more detectors.

The type of data that is obtained from the flow cytometer. In a one parameter histogram, increasing amount of fluorescence (*e.g.* green fluorescence) is plotted on the x axis and the number of cells exhibiting that amount of fluorescence is plotted on the y axis. The fraction of cells that are fluorescent can be determined by integrating the area under the curve. In a two parameter histogram, the x axis is one parameter (*e.g.* red fluorescence) and the y axis is the second parameter (*e.g.* green fluorescence). The number of cells is indicated by the contour and the intensity of the color.

***1. Precipitation Reactions:***

The reaction of soluble antigens with IgG or IgM antibodies to form a large interlocking aggregates (lattices) is called precipitation reaction. The precipitates formed by antibodies are known as precipitins.

**The precipitation reactions occur in two stages:**

(i) Rapid interactions within a second between antigen and antibodies and formation of complex.

(ii) Slow rate of reaction completing even within a few minutes or hours and forming lattices from antigen-antibody complexes.

When the antibodies and antigens are in proper ratio, precipitation reactions normally occur. When there is excess amount of either of two, no visible precipitate is formed.

One can produce the optimal ratio of these two by putting antigens and antibody adjacent to each other and waiting for their diffusion together. In precipitation test, a precipitation ring appears which display the creation of optimal ratio. This zone is known as the zone of equivalence.

***2. Immunodiffusion Test (IDT):***

Immunodiffusion tests are performed in a gelled agar medium. One of the IDTs is Ouchterlony test. In Ouchterlony test wells are cut, into which a purified antiserum (a serum containing antibodies) is added, and to each surrounding well, soluble form of test antigens are added.

Thereafter, a line of visible precipitate is formed between the wells where after diffusion optimal ratio of antigen-antibody is formed. Through the Ouchterlony test, the presence of antibodies in the serum against more than one antigen at a time can be demonstrated. Through this test, identical, partially identical and different types of antigens can also be found out.

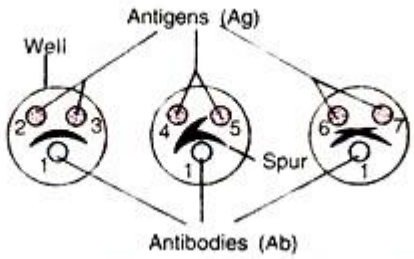



**Fig. 22.22:** The Ouchterlony immunodiffusion test. 1, antiserum filled in wells, 2-7, different types of antigens; A, line of identity (diffusion of antigens and antibodies does not occur, therefore, they react and precipitate to form the dark line); B, lines of partial identity (antigens are not identical but they share many antigenic determinant sites and related to each other); C, line of non-identity (antiserum contains antibodies against antigens in wells). The antigens are not related because they diffuse across each others' zone of precipitation.

***3. Counter Current Immunoelectroptioresis Test (Counter Innmunoelectroptioresis (cie)):***

CIE not only depends entirely on diffusion of antigen and antibody in a gel, but also uses electrophoresis for their rapid movement. By using this method protein can be separated within an hour. CIE is useful for the diagnosis of bacterial meningitis and the other diseases.

The principle of CIE is based on the movement of antigens and antibodies to opposite poles after applying electric current in buffers of correct electric strength and pH, because some of the antigens and antibodies have the opposite charges. If a reaction occurs, a precipitation line appears within an hour.

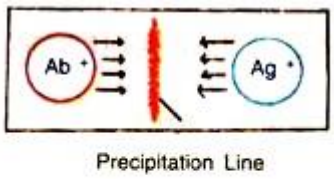



**Fig. 22.23:** Counter immunoelectro-phoresis (CIE). Ag, antigen; Ab, antibodies.

***4. Complement Fixation Reactions:***

A group of 20 or more serum protein is collectively known as complement. During reaction, the complement binds to antigen-antibody complex and is used up or fixed. This process of complement fixation may be used to measure even very small amount of antibody that does not produce a visible reaction such as precipitation or agglutination. Therefore, it is necessary to use indicator system.

This method is used in diagnosis of diseases such as leptospirosis, mycoplasmal pneumonia, Q fever, polio, rubella, histoplasmosis, coccidiodomycosis and streptococcal infections. The test requires patient's serum, test antigen, complement from guinea pig and antibodies of sheep RBCs to determine whether sheep RBCs may be lysed by guinea pig complement.

**The test is accomplished in the following two stages:**

**Stage 1:**

The patient's serum is heated at 56°C for 30 minutes so that the complement should be inactivated. The heated serum is diluted and then added to known amount of specific antigen and complement. The test antigen may correspond to the diseases.

For example, if a patient is suffering from a disease caused by streptococci the test antigen would be the streptococcal antigen. If the patient's serum contains antibodies against streptococci, the test antigen will form complement sequence. This mixture is again incubated for about 30 minutes. At this point, no antigen-antibody reaction occurs.

**Stage 2:**

In stage 2, the complement fixed by antigen-antibody reaction is detected by an indication system. This system consists of sheep RBCs containing specific antibodies attached to their surfaces.

When these are added to complement, haemolysis of RBCs occurs that impart changes in colour of the mixture. This shows that the complements have not been fixed during the first stage; therefore, these become available to cause haemolysis. This indicates that the patient has no streptococcal pneumonia. However, if the guinea pig complements are destroyed, they will not be able to cause the lysis of RBCs. On the other hand, if the complements are fixed (by antigen- antibody reaction) during the first stage, these will not be available to cause haemolysis during the second stage. This indicates that the patient has the infection of streptococci.

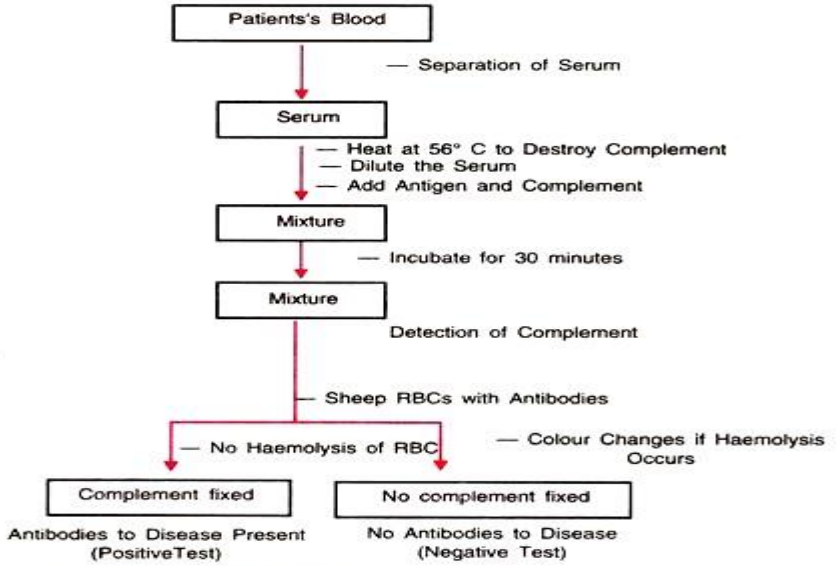



**Fig. 22.25:** Procedure for complement fixation test.

***5. Neutralization Reactions:***

The neutralization reactions are the reactions of antigen- antibody that involve the elimination of harmful effects of bacterial exotoxins or a virus by specific antibodies. These neutralizing substances i.e. antibodies are known as antitoxins. This specific antibody is produced by a host cell in response to a bacterial exotoxin or corresponding toxoid (inactivated toxin). The antitoxin reacts with exotoxin and neutralizes it. These antitoxins can be artificially induced in animals such as horses. Thus, the antitoxin of animal sources in turn can be injected into human which provides a passive immunity against a toxin present in human body produced by the pathogens causing diphtheria, tetanus, etc.

**Diagnosis of Viral Infections:**

Neutralization test is very useful in diagnosis of viral infections in humans. After introduction of a virus, antibodies are produced in response and bind to receptor sites present on the viral surfaces. After binding of antibodies, viral particles fail to reach to the cells. Thereafter, the virus is destroyed. Artificially, the virus is capable of destroying their cell-damaging effect in cell culture or embryonic eggs can be used to determine the presence of antibodies against them. However, when serum contains antibodies against a particular virus, the antibodies will not allow the virus to infect the cell in cell culture; consequently the cells will not be damaged.

***6. Radioimmunoassay (RIA):***

It is such a technique which is highly sensitive and can measure even the less concentration (i.e. 0.001 µg/ml) of antigen or antibody. In 1960, for the first time this technique was developed by S.A. Berson and R. Yalow when they were engaged in determining the concentration of insulin and anti-insulin

complexes in diabetics. Thereafter, Berson died, and significance of this technique was realised. In 1977, Yalow was awarded a Nobel Prize. There are two methods of measuring RIA: the liquid phase and the solid phase RIAs.

**1. Liquid Phase RIA:**

The liquid phase RIA is based on competitive binding of radiolabeled antigen and un-labelled antigen, to a high affinity antibody. The antigen labelled with $^{125}I$ is mixed with such a concentration of antibody that can just saturate the antibody. Therefore, the increasing amount of antigen (un-labelled) of unknown concentration is added. The two types of antigens now compete for available sites of the antibody. The antibody does not differentiate the labelled antigen from the un-labelled one. Upon gradually increasing concentration of un-labelled antigen, the labelled antigen could be displaced from the binding sites available on antibody. The labelled antigens are made free in the solution. The amount of labelled antigen in solution is measured, and the concentration of un-labelled antigen can be determined.

**2. Solid Phase RIA:**

In solid phase RIA, either antigen or antibody is immobilized on a solid phase matrix. It is simple and easy in handling as compared to liquid phase RIA.

***7. Enzyme-Linked Immunosorbent Assay (ELISA):***

The principle of ELISA is similar to RIA, but differs slightly. In RIA radiolabelled antigen is used, whereas in ELISA enzyme is used that reacts with a colourless substrate and develops a coloured reaction product. There is a large number of enzymes such as alkaline phosphatase, horse radish peroxidase, and p-nitro-phenyl phosphatase which are employed in ELISA. As compared to RIA, this assay is both cheaper and safer. On the basis of known concentration of antigen or antibody a standard curve is prepared from which the unknown concentration of sample is measured. A microliter plate with numerous shallow wells is used in this method. It is very useful in testing for AIDS antibodies. However, now-a days a number of ELISA kits have been developed and are in current use.

**1. Indirect ELISA:**

It is used to measure antibody. Known antigen is coated on the plastic lining of the wells of microtiter plate which is made up of polystyrene latex. To test for the presence of antibodies against this antigen in the patient, his blood serum is added to the wells. If the patient's serum contains antibody specific to antigen, the antibody will bind to the absorbed antigen otherwise not. After incubation the wells are washed and the enzyme, labelled with antihuman gamma globulin (anti-Hgg), is added to the wells. Anti-Hgg can react with antigen antibody complex. The mixture of wells is washed to remove the excess of unbound labelled anti- Hgg. Finally the correct substrate for the enzyme is added which is hydrolysed by the enzyme and develops a colour. Varying concentrations of antibody in serum shows changes in the intensity of colour. This method is very useful in detection of antibodies to HIV, Salmonella, Yersinia, Brucella, Treponema and streptococci.

**2. Double Antibody Sandwich ELISA:**

This method detects antigen. In this case antibody (antiserum) is immobilised on the surface of wells of microtiter plate. A test antigen is added to each well and allowed to react with the bound antibody. It is incubated during this period. If antigen combines specifically with antibody absorbed to wells, the antigen will be retained even after washing and unbound antigen would be made free. Thereafter, a second

enzyme-linked antibody (e.g. alkaline phosphatase tagged to antibody) is added to react with bound antigen. It is again incubated for a few seconds, the enzyme labelled antibody reacts with the antigen-antibody complex already formed in the wells and results in the development of a “sandwich’. The mixture in wells is washed again to remove the excess of labelled enzyme. A chromogenic substrate e.g. nitro-phenyl phosphate is added which reacts with enzyme and develop yellow colour. The reaction can be stopped simply by changing the pH or denaturing the enzyme. The change in colour is measured visually or spectrophotometrically. Change in colour shows the presence of desired antigen in the sample. This technique is useful in detection of toxins of Vibrio cholerae, E. coli, Staphylococcus enterotoxin-A and antigens of rotavirus.

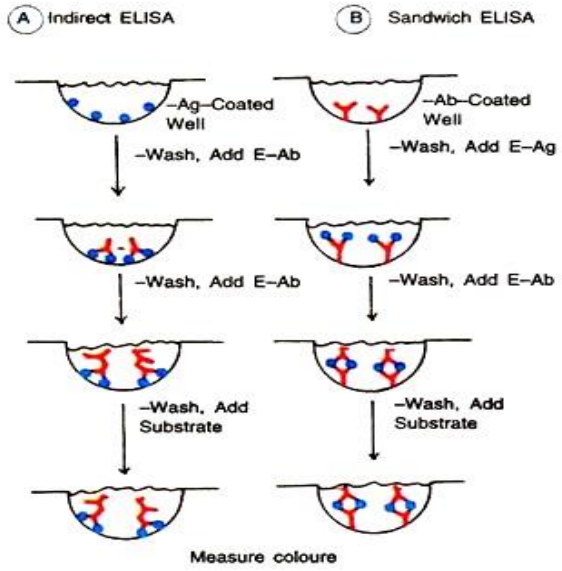



**Fig. 22.26:** Enzyme-linked immunosorbent assay (ELISA) technique for detection of antibody (A) and antigen (B).

### *9. Fluorescent Antibody (FA) Technique:*

The FA technique is used to detect the microorganisms present in clinical specimens, and specific antibodies present in serum. If the antibodies bind to cell or tissues, it can be observed by tagging the antibody with a fluorescent dye such as fluorescein isothiocyanate and rhodamine. Both the dyes can conjugate the FC region of antibody without affecting the specificity and make the antibody fluorescent when exposed to UV light. Fluorescein absorbs blue light (490 nm) and emits yellow green fluorescence (517 nm). Similarly, rhodamine absorbs the yellow green light (515 nm) and emits deep red fluorescence (546 nm). The FA technique is very useful in testing for rabies within a few hours with 100% accuracy. There are two methods of FA test, direct FA test and indirect FA test. Direct FA test is used to identify the microorganisms present in clinical specimen. The specimen containing antigen is fixed onto a slide and, thereafter, fluorescein-labelled antibodies are added on the specimen. It is incubated for a few minutes. The slide is washed to remove unbound antibody and observed under the UV microscope for yellow-green fluorescence. The indirect FA test is useful for the detection of specific antibodies in serum formed by a microorganism.

**This method follows the following steps:**

(a) Fix a known antigen onto a slide,
(b) Add a test serum (microorganism-specific antibody reacts with antigen and forms a bound complex,
(c) Add fluorescein-labelled anti-Hgg to the slide,
(d) Incubate and wash the slide,
(e) Examine the slide under fluorescence microscope.

The development of fluorescence confirms the presence of antibody specific to antigen fixed on slide.

**IMMUNOLOGY - COMPLEMENT**

## COMPLEMENT FUNCTIONS

Historically, the term complement (C) was used to refer to a heat-labile serum component that was able to lyse bacteria (activity is destroyed (inactivated) by heating serum at 56 degrees C for 30 minutes). However, complement is now known to contribute to host defenses in other ways as well. Complement can opsonize bacteria for enhanced phagocytosis; it can recruit and activate various cells including polymorphonuclear cells (PMNs) and macrophages; it can participate in regulation of antibody responses and it can aid in the clearance of immune complexes and apoptotic cells. Complement can also have detrimental effects for the host; it contributes to inflammation and tissue damage and it can trigger anaphylaxis.

Complement comprises over 20 different serum proteins (see Table 1) that are produced by a variety of cells including, hepatocytes, macrophages and gut epithelial cells. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that, when activated, cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria.

**Table 1. Proteins of the Complement system**

| Classical Pathway | Lectin Pathway | Alternative Pathway | Lytic Pathway |
|---|---|---|---|
| Activation Proteins:<br>C1q<u>rs</u>, C<u>2</u>, C3, C4 | Mannan binding protein (MBP), mannan-asociated serine protease (MASP, MASP2) | C3, Factors <u>B</u> & D*, Properdin (P) | C5, C6, C7, C8, C9 |
| Control Proteins:<br>C1-INH, C4-BP | | Factors I* & H, decay accelerating factor (DAF), Complement receptor 1(CR1), *etc*. | Protein S |

Components <u>underlined</u> acquire enzymatic activity when activated.

Components marked with an asterisk have enzymatic activity in their native form.

## Classical Pathway (Figure 2)

### C1 activation

C1, a multi-subunit protein containing three different proteins (C1q, C1r and C1s), binds to the Fc region of IgG and IgM antibody molecules that have interacted with antigen. C1 binding does not occur to antibodies that have not complexed with antigen and binding requires calcium and magnesium ions. (*N.B.* In some cases C1 can bind to aggregated immunoglobulin [e.g. aggregated IgG] or to certain pathogen surfaces in the absence of antibody). The binding of C1 to antibody is via C1q and C1q must cross link at least two antibody molecules before it is firmly fixed. The binding of C1q results in the activation of C1r which in turn activates C1s. The result is the formation of an activated "C1qrs", which is an enzyme that cleaves C4 into two fragments C4a and C4b.

### C4 and C2 activation (generation of C3 convertase)

The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated "C1qrs" also cleaves C2 into C2a and C2b. C2a binds to the membrane in association with C4b, and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b.

## C3 activation (generation of C5 convertase)

C3b binds to the membrane in association with C4b and C2a, and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the classical pathway.

Several of the products of the classical pathway have potent biological activities that contribute to host defenses. Some of these products may also have detrimental effects if produced in an unregulated manner. Table 2 summarizes the biological activities of classical pathway components.

**Table 2. Biological Activity of classical pathway products**

| Component | Biological Activity |
|---|---|
| C2b | **Prokinin**; cleaved by plasmin to yield kinin, which results in edema |
| C3a | **Anaphylotoxin**; can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells, which may lead to **anaphylaxis** |
| C3b | **Opsonin**; promotes phagocytosis by binding to complement receptors<br><br>Activation of phagocytic cells |
| C4a | **Anaphylotoxin** (weaker than C3a) |
| C4b | **Opsonin**; promotes phagocytosis by binding to complement receptors |

If the classical pathway were not regulated there would be continued production of C2b, C3a, and C4a. Thus, there must be some way to regulate the activity of the classical pathway. Table 3 summarizes the ways in which the classical pathway is regulated.

**Table 3. Regulation of the Classical Pathway**

| Component | Regulation |
|---|---|
| All | **C1-INH**; dissociates C1r and C1s from C1q |
| C3a | **C3a inactivator (C3a-INA**;**Carboxypeptidase B)**; inactivates C3a |
| C3b | **Factors H and I;** Factor H facilitates the degradation of C3b by Factor I |
| C4a | **C3-INA** |
| C4b | **C4 binding protein(C4-BP) and Factor I**; C4-BP facilitates degradation of C4b by Factor I; C4-BP also prevents association of C2a with C4b thus blocking the formation of C3 convertase |

The importance of C1-INH in regulating the classical pathway is demonstrated by the result of a deficiency in this inhibitor. C1-INH deficiencies are associated with the development of hereditary angioedema.

## Lectin Pathway

The lectin pathway (figure 3) is very similar to the classical pathway. It is initiated by the binding of mannose-binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides (mannans). Binding of MBL to a pathogen results in the association of two serine proteases, MASP-1 and MASP-2 (MBL-associated serine proteases). MASP-1 and MASP-2 are similar to C1r and C1s, respectively and MBL is similar to C1q. Formation of the MBL/MASP-1/MASP-2 tri-molecular complex results in the activation of the MASPs and subsequent cleavage of C4 into C4a and C4b. The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated MASPs also cleave C2 into C2a and C2b. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the lectin pathway.

The biological activities and the regulatory proteins of the lectin pathway are the same as those of the classical pathway.

## Alternative Pathway

The alternative pathway begins with the activation of C3 and requires Factors B and D and $Mg^{++}$ cation, all present in normal serum.

## Amplification loop of C3b formation

In serum there is low level spontaneous hydrolysis of C3 to produce C3i. Factor B binds to C3i and becomes susceptible to Factor D, which cleaves Factor B into Bb. The C3iBb complex acts as a C3 convertase and cleaves C3 into C3a and C3b. Once C3b is formed, Factor B will bind to it and becomes susceptible to cleavage by Factor D. The resulting C3bBb complex is a C3 convertase that will continue to generate more C3b, thus amplifying C3b production. If this process continues unchecked, the result would be the consumption of all C3 in the serum. Thus, the spontaneous production of C3b is tightly controlled.

## Control of the amplification loop

As spontaneously produced C3b binds to autologous host membranes, it interacts with DAF (decay accelerating factor), which blocks the association of Factor B with C3b thereby preventing the formation of additional C3 convertase. In addition, DAF accelerates the dissociation of Bb from C3b in C3 convertase that has already formed, thereby stopping the production of additional C3b. Some cells possess complement receptor 1 (CR1). Binding of C3b to CR1 facilitates the enzymatic degradation of C3b by Factor I. In addition, binding of C3 convertase (C3bBb) to CR1 also dissociates Bb from the complex. Thus, in cells possessing complement receptors, CR1 also plays a role in controlling the amplification loop. Finally, Factor H can bind to C3b bound to a cell or in the in the fluid phase and facilitate the enzymatic degradation of C3b by Factor I. Thus, the amplification loop is controlled by either blocking the formation of C3 convertase, dissociating C3 convertase, or by enzymatically digesting C3b. The importance of controlling this amplification loop is illustrated in patients with genetic deficiencies of Factor H or I. These patients have a C3 deficiency and increased susceptibility to certain infections.

## Stabilization of C convertase by activator (protector) surfaces

When bound to an appropriate activator of the alternative pathway, C3b will bind Factor B, which is enzymatically cleaved by Factor D to produce C3 convertase (C3bBb). However, C3b is resistant to degradation by Factor I and the C3 convertase is not rapidly degraded, since it is stabilized by the activator surface. The complex is further stabilized by properdin binding to C3bBb. Activators of the alternate pathway are components on the surface of pathogens and include: LPS of Gram-negative bacteria and the cell walls of some bacteria and yeasts. Thus, when C3b binds to an activator surface, the C3 convertase formed will be stable and continue to generate additional C3a and C3b by cleavage of C3.

## Generation of C5 convertase

Some of the C3b generated by the stabilized C3 convertase on the activator surface associates with the C3bBb complex to form a C3bBbC3b complex. This is the C5 convertase of the alternative pathway. The generation of C5 convertase is the end of the alternative pathway. The alternative pathway can be activated by many Gram-negative (most significantly, *Neisseria meningitidis* and *N. gonorrhoea*), some Gram-positive bacteria and certain viruses and parasites, and results in the lysis of these organisms. Thus, the alternative pathway of C activation provides another means of protection against certain pathogens before an antibody response is mounted. A deficiency of C3 results in an increased susceptibility to these organisms. The alternate pathway may be the more primitive pathway and the classical and lectin pathways probably developed from it.

## Membrane Attack (Lytic) Pathway

C5 convertase from the classical (C4b2a3b), lectin (C4b2a3b) or alternative (C3bBb3b) pathway cleaves C5 into C5a and C5b. C5a remains in the fluid phase and the C5b rapidly associates with C6 and C7 and inserts into the membrane. Subsequently C8 binds, followed by several molecules of C9. The C9 molecules form a pore in the membrane through which the cellular contents leak and lysis occurs. Lysis is not an enzymatic process; it is thought to be due to physical damage to the membrane. The complex consisting of C5bC6C7C8C9 is referred to as the membrane attack complex (MAC).

C5a generated in the lytic pathway has several potent biological activities. It is the most potent anaphylotoxin. In addition, it is a chemotactic factor for neutrophils and stimulates the respiratory burst in them and it stimulates inflammatory cytokine production by macrophages. Its activities are controlled by inactivation by carboxypeptidase B (C3-INA).

Some of the C5b67 complex formed can dissociate from the membrane and enter the fluid phase. If this were to occur it could then bind to other nearby cells and lead to their lysis. The damage to bystander cells is prevented by Protein S (vitronectin). Protein S binds to soluble C5b67 and prevents its binding to other cells.

## BIOLOGICALLY ACTIVE PRODUCTS OF COMPLEMENT ACTIVATION

Activation of complement results in the production of several biologically active molecules which contribute to resistance,anaphylaxis and inflammation.

### Kinin production

C2b generated during the classical pathway of C activation is a prokinin which becomes biologically active following enzymatic alteration by plasmin. Excess C2b production is prevented by limiting C2 activation by C1 inhibitor (C1-INH) also known as serpin which displaces C1rs from the C1qrs complex (Figure 10). A genetic deficiency of C1-INH results in an overproduction of C2b and is the cause of hereditary angioneurotic edema. This condition can be treated with Danazol which promotes C1-INH production or with ε-amino caproic acid which decreases plasmin activity.

### Anaphylotoxins

C4a, C3a and C5a (in increasing order of activity) are all anaphylotoxins which cause basophil/mast cell degranulation and smooth muscle contraction. Undesirable effects of these peptides are controlled by carboxypeptidase B (C3a-INA).

### Chemotactic Factors

C5a and MAC (C5b67) are both chemotactic. C5a is also a potent activator of neutrophils, basophils and macrophages and causes induction of adhesion molecules on vascular endothelial cells.

### Opsonins

C3b and C4b in the surface of microorganisms attach to C-receptor (CR1) on phagocytic cells and promote phagocytosis.

### Other Biologically active products of C activation

Degradation products of C3 (iC3b, C3d and C3e) also bind to different cells by distinct receptors and modulate their functions.
In summary, the complement system takes part in both specific and non-specific resistance and generates a number of products of biological and pathophysiological significance (Table 4).
There are known genetic deficiencies of most individual C complement components, but C3 deficiency is most serious and fatal. Complement deficiencies also occur in immune complex diseases (e.g., SLE) and acute and chronic bacterial, viral and parasitic infections.

**Table 4. Activities of Complement Activation Products and their Control Factors**

| Fragment | Activity | Effect | Control Factor (s) |
|---|---|---|---|
| C2a | Prokinin, accumulation of fluids | Edema | C1-INH |
| C3a | Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction | Anaphylaxis | C3a-INA |
| C3b | Opsonin, phagocyte activation | Phagocytosis | Factors H and I |
| C4a | Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction | Anaphylaxis (least potent) | C3a-INA |
| C4b | Opsonin | Phagocytosis | C4-BP and Factor I |
| C5a | Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction | Anaphylaxis (most potent) | C3a-INA |
| | Chemotaxis, stimulation of respiratory burst, activation of phagocytes, stimulation of inflammatory cytokines | Inflammation | |
| C5bC6C7 | Chemotaxis | Inflammation | Protein S (vitronectin) |
| | Attaches to other membranes | Tissue damage | |

| Table 5. Complement deficiencies and disease | | |
|---|---|---|
| **Pathway/Component** | **Disease** | **Mechanism** |
| **Classical Pathway** | | |
| C1INH | Hereditary angioedema | Overproduction of C2b (prokinin) |
| C1, C2, C4 | Predisposition to SLE | Opsonization of immune complexes help keep them soluble, deficiency results in increased precipitation in tissues and inflammation |
| **Lectin Pathway** | | |
| MBL | Susceptibility to bacterial infections in infants or immunosuppressed | Inability to initiate the lectin pathway |
| **Alternative Pathway** | | |
| Factors B or D | Susceptibility to pyogenic (pus-forming) bacterial infections | Lack of sufficient opsonization of bacteria |
| C3 | Susceptibility to bacterial infections | Lack of opsonization and inability to utilize the membrane attack pathway |
| C5, C6, C7 C8, and C9 | Susceptibility to Gram-negative infections | Inability to attack the outer membrane of Gram-negative bacteria |
| Properdin (X-linked) | Susceptibility meningococcal meningitis | Lack of opsonization of bacteria |
| Factors H or I | C3 deficiency and susceptibility to bacterial infections | Uncontrolled activation of C3 via alternative pathway resulting in depletion of C3 |

## Autoimmunity

**Autoimmunity** is the system of immune responses of an organism against its own healthy cells and tissues. Any disease that results from such an aberrant immune response is termed an "autoimmune disease". Prominent examples include celiac disease, diabetes mellitus type 1, sarcoidosis, systemic lupus erythematosus (SLE), Sjögren's syndrome, eosinophilic granulomatosis with polyangiitis, Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura, Addison's disease, rheumatoid arthritis(RA), ankylosing spondylitis, polymyositis (PM), dermatomyositis (DM) and multiple sclerosis (MS). Autoimmune diseases are very often treated with steroids.

### Low-level autoimmunity

While a high level of autoimmunity is unhealthy, a low level of autoimmunity may actually be beneficial. Taking the experience of a beneficial factor in autoimmunity further, one might hypothesize with intent to prove that autoimmunity is always a self-defense mechanism of the mammal system to survive. The system does not randomly lose the ability to distinguish between self and non-self, the attack on cells may be the consequence of cycling metabolic processes necessary to keep the blood chemistry in homeostasis.

Second, autoimmunity may have a role in allowing a rapid immune response in the early stages of an infection when the availability of foreign antigens limits the response (i.e., when there are few pathogens present). In their study, Stefanova et al. (2002) injected an anti-MHC Class II antibody into mice expressing a single type of MHC Class II molecule ($H\text{-}2^b$) to temporarily prevent CD4+ T cell-MHC interaction. Naive CD4+ T cells (those that have not encountered any antigens before) recovered from these mice 36 hours post-anti-MHC administration showed decreased responsiveness to the antigen pigeon cytochrome C peptide, as determined by Zap-70 phosphorylation, proliferation, and Interleukin-2 production. Thus Stefanova et al. (2002) demonstrated that self-MHC recognition (which, if too strong may contribute to autoimmune disease) maintains the responsiveness of CD4+ T cells when foreign antigens are absent.[5] This idea of autoimmunity is conceptually similar to play-fighting. The play-fighting of young cubs (TCR and self-MHC) may result in a few scratches or scars (low-level-autoimmunity), but is beneficial in the long-term as it primes the young cub for proper fights in the future.

### Immunological tolerance

Pioneering work by Noel Rose and Ernst Witebsky in New York, and Roitt and Doniach at University College London provided clear evidence that, at least in terms of antibody-producing B cells (B lymphocytes), diseases such as rheumatoid arthritis and thyrotoxicosis are associated with loss of immunological tolerance, which is the ability of an individual to ignore "self", while reacting to "non-self". This breakage leads to the immune system's mounting an effective and specific immune response against self-determinants. The exact genesis of immunological tolerance is still elusive, but several theories have been proposed since the mid-twentieth century to explain its origin.

Three hypotheses have gained widespread attention among immunologists:

- **Clonal Deletion theory**, proposed by Burnet, according to which self-reactive lymphoid cells are destroyed during the development of the immune system in an individual. For their work Frank M. Burnet and Peter B. Medawar were awarded the 1960 Nobel Prize in Physiology or Medicine "for discovery of acquired immunological tolerance".
- **Clonal Anergy theory**, proposed by Nossal, in which self-reactive T- or B-cells become inactivated in the normal individual and cannot amplify the immune response.[6]
- **Idiotype Network theory**, proposed by Jerne, wherein a network of antibodies capable of neutralizing self-reactive antibodies exists naturally within the body.[7]

In addition, two other theories are under intense investigation:

- **Clonal Ignorance theory**, according to which autoreactive T cells that are not represented in the thymus will mature and migrate to the periphery, where they will not encounter the appropriate antigen because it is inaccessible tissues. Consequently, auto-reactive B cells, that escape deletion, cannot find the antigen or the specific helper T cell.[8]

- **Suppressor population** or **Regulatory T cell theory**, wherein regulatory T-lymphocytes (commonly $CD4^+FoxP3^+$ cells, among others) function to prevent, downregulate, or limit autoaggressive immune responses in the immune system.

**Tolerance can also be differentiated into "Central" and "Peripheral" tolerance, on whether or not the above-stated checking mechanisms operate in the central lymphoid organs (Thymus and Bone Marrow) or the peripheral lymphoid organs (lymph node, spleen, etc., where self-reactive B-cells may be destroyed).** It must be emphasised that these theories are not mutually exclusive, and evidence has been mounting suggesting that all of these mechanisms may actively contribute to vertebrate immunological tolerance.

A puzzling feature of the documented loss of tolerance seen in spontaneous human autoimmunity is that it is almost entirely restricted to the autoantibody responses produced by B lymphocytes. Loss of tolerance by T cells has been extremely hard to demonstrate, and where there is evidence for an abnormal T cell response it is usually not to the antigen recognised by autoantibodies. Thus, in rheumatoid arthritis there are autoantibodies to IgG Fc but apparently no corresponding T cell response. In systemic lupus there are autoantibodies to DNA, which cannot evoke a T cell response, and limited evidence for T cell responses implicates nucleoprotein antigens. In Celiac disease there are autoantibodies to tissue transglutaminase but the T cell response is to the foreign protein gliadin. This disparity has led to the idea that human autoimmune disease is in most cases (with probable exceptions including type I diabetes) based on a loss of B cell tolerance which makes use of normal T cell responses to foreign antigens in a variety of aberrant ways.[9]

## Immunodeficiency and autoimmunity

There are a large number of immunodeficiency syndromes that present clinical and laboratory characteristics of autoimmunity. The decreased ability of the immune system to clear infections in these patients may be responsible for causing autoimmunity through perpetual immune system activation.[10]

One example is common variable immunodeficiency (CVID) where multiple autoimmune diseases are seen, e.g.: inflammatory bowel disease, autoimmune thrombocytopenia and autoimmune thyroid disease.

Familial hemophagocytic lymphohistiocytosis, an autosomal recessive primary immunodeficiency, is another example. Pancytopenia, rashes, swollen lymph nodes and enlargement of the liver and spleen are commonly seen in such individuals. Presence of multiple uncleared viral infections due to lack of perforin are thought to be responsible.

In addition to chronic and/or recurrent infections many autoimmune diseases including arthritis, autoimmune hemolytic anemia, scleroderma and type 1 diabetes mellitus are also seen in X-linked agammaglobulinemia (XLA). Recurrent bacterial and fungal infections and chronic inflammation of the gut and lungs are seen in chronic granulomatous disease (CGD) as well. CGD is a caused by decreased production of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by neutrophils. Hypomorphic RAG mutations are seen in patients with midline granulomatous disease; an autoimmune disorder that is commonly seen in patients with granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis) and NK/T cell lymphomas.

Wiskott-Aldrich syndrome (WAS) patients also present with eczema, autoimmune manifestations, recurrent bacterial infections and lymphoma.

In autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) also autoimmunity and infections coexist: organ-specific autoimmune manifestations (e.g. hypoparathyroidism and adrenocortical failure) and chronic mucocutaneous candidiasis.

Finally, IgA deficiency is also sometimes associated with the development of autoimmune and atopic phenomena.

## Genetic factors

Certain individuals are genetically susceptible to developing autoimmune diseases. This susceptibility is associated with multiple genes plus other risk factors. Genetically predisposed individuals do not always develop autoimmune diseases.

Three main sets of genes are suspected in many autoimmune diseases. These genes are related to:

- Immunoglobulins
- T-cell receptors
- The major histocompatibility complexes (MHC).

The first two, which are involved in the recognition of antigens, are inherently variable and susceptible to recombination. These variations enable the immune system to respond to a very wide variety of invaders, but may also give rise to lymphocytes capable of self-reactivity.

Scientists such as Hugh McDevitt, G. Nepom, J. Bell and J. Todd have also provided strong evidence to suggest that certain MHC class II allotypes are strongly correlated with·

- HLA DR2 is strongly positively correlated with Systemic Lupus Erythematosus, narcolepsy[11] and multiple sclerosis, and negatively correlated with DM Type 1.
- HLA DR3 is correlated strongly with Sjögren's syndrome, myasthenia gravis, SLE, and DM Type 1.
- HLA DR4 is correlated with the genesis of rheumatoid arthritis, Type 1 diabetes mellitus, and pemphigus vulgaris.

Fewer correlations exist with MHC class I molecules. The most notable and consistent is the association between HLA B27 and spondyloarthropathies like ankylosing spondylitisand reactive arthritis. Correlations may exist between polymorphisms within class II MHC promoters and autoimmune disease.

The contributions of genes outside the MHC complex remain the subject of research, in animal models of disease (Linda Wicker's extensive genetic studies of diabetes in the NOD mouse), and in patients (Brian Kotzin's linkage analysis of susceptibility to SLE).

Recently, PTPN22 has been associated with multiple autoimmune diseases including Type I diabetes, rheumatoid arthritis, systemic lupus erythematosus, Hashimoto's thyroiditis, Graves' disease, Addison's disease, Myasthenia Gravis, vitiligo, systemic sclerosis juvenile idiopathic arthritis, and psoriatic arthritis.[12][*clarification needed*]

**Sex**

| **Ratio of female/male incidence of autoimmune diseases** | |
|---|---|
| Hashimoto's thyroiditis | 10:1[13] |
| Graves' disease | 7:1[13] |
| Multiple sclerosis (MS) | 2:1[13] |
| Myasthenia gravis | 2:1[13] |
| Systemic lupus erythematosus (SLE) | 9:1[13] |
| Rheumatoid arthritis | 5:2[13] |
| Primary sclerosing cholangitis | 1:2 |

There is some evidence that a person's sex may also have some role in the development of autoimmunity; that is, most autoimmune diseases are *sex-related*. Nearly 75% of the Americans who suffer from autoimmune disease are women,[*citation needed*] but according to the American Autoimmune Related Diseases Association (AARDA), those autoimmune diseases that do develop in men tend to be more severe. A few autoimmune diseases that men are just as or more likely to develop as women include: ankylosing spondylitis, type 1 diabetes mellitus, granulomatosis with polyangiitis, Crohn's disease, Primary sclerosing cholangitis and psoriasis.

The reasons for the sex role in autoimmunity vary. Women appear to generally mount larger inflammatory responses than men when their immune systems are triggered, increasing the risk of autoimmunity. Involvement of sex steroids is indicated by that many autoimmune diseases tend to fluctuate in accordance with hormonal changes, for example: during pregnancy, in the menstrual cycle, or when using oral contraception. A history of pregnancy also appears to leave a persistent increased risk for autoimmune disease. It has been suggested that the slight, direct exchange of cells between mothers and their children during pregnancy may induce autoimmunity.[14] This would tip the gender balance in the direction of the female.

Another theory suggests the female high tendency to get autoimmunity is due to an imbalanced X chromosome inactivation.[15] The X-inactivation skew theory, proposed by Princeton University's Jeff Stewart, has recently been confirmed experimentally in scleroderma and autoimmune thyroiditis.[16] Other complex X-linked genetic susceptibility mechanisms are proposed and under investigation.

## Environmental factors

An interesting inverse relationship exists between infectious diseases and autoimmune diseases. In areas where multiple infectious diseases are endemic, autoimmune diseases are quite rarely seen. The reverse, to some extent, seems to hold true. The hygiene hypothesis attributes these correlations to the immune manipulating strategies of pathogens. While such an observation has been variously termed as spurious and ineffective, according to some studies, parasite infection is associated with reduced activity of autoimmune disease.[17][18][19]

The putative mechanism is that the parasite attenuates the host immune response in order to protect itself. This may provide a serendipitous benefit to a host that also suffers from autoimmune disease. The details of parasite immune modulation are not yet known, but may include secretion of anti-inflammatory agents or interference with the host immune signaling.

A paradoxical observation has been the strong association of certain microbial organisms with autoimmune diseases. For example, *Klebsiella pneumoniae* and coxsackievirus Bhave been strongly correlated with ankylosing spondylitis and diabetes mellitus type 1, respectively. This has been explained by the tendency of the infecting organism to produce super-antigens that are capable of polyclonal activation of B-lymphocytes, and production of large amounts of antibodies of varying specificities, some of which may be self-reactive (see below).

Certain chemical agents and drugs can also be associated with the genesis of autoimmune conditions, or conditions that simulate autoimmune diseases. The most striking of these is the drug-induced lupus erythematosus. Usually, withdrawal of the offending drug cures the symptoms in a patient.

Cigarette smoking is now established as a major risk factor for both incidence and severity of rheumatoid arthritis. This may relate to abnormal citrullination of proteins, since the effects of smoking correlate with the presence of antibodies to citrullinated peptides.

## Pathogenesis of autoimmunity

Several mechanisms are thought to be operative in the pathogenesis of autoimmune diseases, against a backdrop of genetic predisposition and environmental modulation. It is beyond the scope of this article to discuss each of these mechanisms exhaustively, but a summary of some of the important mechanisms have been described:

- **T-Cell Bypass** – A normal immune system requires the activation of B-cells by T-cells before the former can undergo differentiation into plasma B-cells and subsequently produce antibodies in large quantities. This requirement of a T-cell can be bypassed in rare instances, such as infection by organisms producing super-antigens, which are capable of initiating polyclonal activation of B-cells, or even of T-cells, by directly binding to the β-subunit of T-cell receptors in a non-specific fashion.
- **T-Cell-B-Cell discordance** – A normal immune response is assumed to involve B and T cell responses to the same antigen, even if we know that B cells and T cells recognise very different things: conformations on the surface of a molecule for B cells and pre-processed peptide fragments of proteins for T cells. However, there is nothing as far as we know that requires this. All that is required is that a B cell recognising antigen X endocytoses and processes a protein Y (normally =X) and presents it to a T cell. Roosnek and Lanzavecchia showed that B cells recognising IgGFc could get help from any T cell responding to an antigen co-endocytosed with IgG by the B cell as part of an immune complex. In coeliac disease it seems likely that B cells recognising tissue transglutamine are helped by T cells recognising gliadin.
- **Aberrant B cell receptor-mediated feedback** – A feature of human autoimmune disease is that it is largely restricted to a small group of antigens, several of which have known signaling roles in the immune response (DNA, C1q, IgGFc, Ro, Con. A receptor, Peanut agglutinin receptor(PNAR)). This fact gave rise to the idea that spontaneous autoimmunity may result when the binding of antibody to certain antigens leads to aberrant signals being fed back to parent B cells through membrane bound ligands. These ligands include B cell receptor (for antigen), IgG Fc receptors, CD21, which binds

complement C3d, Toll-like receptors 9 and 7 (which can bind DNA and nucleoproteins) and PNAR. More indirect aberrant activation of B cells can also be envisaged with autoantibodies to acetyl choline receptor (on thymic myoid cells) and hormone and hormone binding proteins. Together with the concept of T-cell-B-cell discordance this idea forms the basis of the hypothesis of self-perpetuating autoreactive B cells.[20] Autoreactive B cells in spontaneous autoimmunity are seen as surviving because of subversion both of the T cell help pathway and of the feedback signal through B cell receptor, thereby overcoming the negative signals responsible for B cell self-tolerance without necessarily requiring loss of T cell self-tolerance.

- **Molecular Mimicry** – An exogenous antigen may share structural similarities with certain host antigens; thus, any antibody produced against this antigen (which mimics the self-antigens) can also, in theory, bind to the host antigens, and amplify the immune response. The idea of molecular mimicry arose in the context of Rheumatic Fever, which follows infection with Group A beta-haemolytic streptococci. Although rheumatic fever has been attributed to molecular mimicry for half a century no antigen has been formally identified (if anything too many have been proposed). Moreover, the complex tissue distribution of the disease (heart, joint, skin, basal ganglia) argues against a cardiac specific antigen. It remains entirely possible that the disease is due to e.g. an unusual interaction between immune complexes, complement components and endothelium.
- **Idiotype Cross-Reaction** – Idiotypes are antigenic epitopes found in the antigen-binding portion (Fab) of the immunoglobulin molecule. Plotz and Oldstone presented evidence that autoimmunity can arise as a result of a cross-reaction between the idiotype on an antiviral antibody and a host cell receptor for the virus in question. In this case, the host-cell receptor is envisioned as an internal image of the virus, and the anti-idiotype antibodies can react with the host cells.
- **Cytokine Dysregulation** – Cytokines have been recently divided into two groups according to the population of cells whose functions they promote: Helper T-cells type 1 or type 2. The second category of cytokines, which include IL-4, IL-10 and TGF-β (to name a few), seem to have a role in prevention of exaggeration of pro-inflammatory immune responses.
- **Dendritic cell apoptosis** – immune system cells called dendritic cells present antigens to active lymphocytes. Dendritic cells that are defective in apoptosis can lead to inappropriate systemic lymphocyte activation and consequent decline in self-tolerance.[21]
- **Epitope spreading** or **epitope drift** – when the immune reaction changes from targeting the primary epitope to also targeting other epitopes.[22] In contrast to molecular mimicry, the other epitopes need not be structurally similar to the primary one.
- **Epitope modification** or **Cryptic epitope exposure** – this mechanism of autoimmune disease is unique in that it does not result from a defect in the hematopoietic system. Instead, disease results from the exposure of cryptic N-glycan (polysaccharide) linkages common to lower eukaryotes and prokaryotes on the glycoproteins of mammalian non-hematopoietic cells and organs[23] This exposure of phylogenically primitive glycans activates one or more mammalian innate immune cell receptors to induce a chronic sterile inflammatory state. In the presence of chronic and inflammatory cell damage, the adaptive immune system is recruited and self–tolerance is lost with increased autoantibody production. In this form of the disease, the absence of lymphocytes can accelerate organ damage, and intravenous IgG administration can be therapeutic. Although this route to autoimmune disease may underlie various degenerative disease states, no diagnostics for this disease mechanism exist at present, and thus its role in human autoimmunity is currently unknown.

The roles of specialized immunoregulatory cell types, such as regulatory T cells, NKT cells, γδ T-cells in the pathogenesis of autoimmune disease are under investigation.

## Classification

Autoimmune diseases can be broadly divided into systemic and organ-specific or localised autoimmune disorders, depending on the principal clinico-pathologic features of each disease.

- **Systemic autoimmune diseases** include SLE, Sjögren's syndrome, sarcoidosis, scleroderma, rheumatoid arthritis, cryoglobulinemic vasculitis,

and dermatomyositis. These conditions tend to be associated with autoantibodies to antigens which are not tissue specific. Thus although polymyositis is more or less tissue specific in presentation, it may be included in this group because the autoantigens are often ubiquitous t-RNA synthetases.

- 
- **Local** syndromes which affect a specific organ or tissue:
  - Endocrinologic: Diabetes mellitus type 1, Hashimoto's thyroiditis, Addison's disease
  - Gastrointestinal: Coeliac disease, Crohn's disease, Pernicious anaemia
  - Dermatologic: Pemphigus vulgaris, Vitiligo
  - Haematologic: Autoimmune haemolytic anaemia, Idiopathic thrombocytopenic purpura
  - Neurological: Multiple sclerosis, Myasthenia gravis, Encephalitis

Using the traditional “organ specific” and “non-organ specific” classification scheme, many diseases have been lumped together under the autoimmune disease umbrella. However, many chronic inflammatory human disorders lack the telltale associations of B and T cell driven immunopathology. In the last decade it has been firmly established that tissue "inflammation against self" does not necessarily rely on abnormal T and B cell responses.

This has led to the recent proposal that the spectrum of autoimmunity should be viewed along an “immunological disease continuum,” with classical autoimmune diseases at one extreme and diseases driven by the innate immune system at the other extreme. Within this scheme, the full spectrum of autoimmunity can be included. Many common human autoimmune diseases can be seen to have a substantial innate immune mediated immunopathology using this new scheme. This new classification scheme has implications for understanding disease mechanisms and for therapy development.[24]

## Diagnosis

Diagnosis of autoimmune disorders largely rests on accurate history and physical examination of the patient, and high index of suspicion against a backdrop of certain abnormalities in routine laboratory tests (example, elevated C-reactive protein). In several systemic disorders, serological assays which can detect specific autoantibodies can be employed. Localised disorders are best diagnosed by immunofluorescence of biopsy specimens. Autoantibodies are used to diagnose many autoimmune diseases. The levels of autoantibodies are measured to determine the progress of the disease.

## Treatments

Treatments for autoimmune disease have traditionally been immunosuppressive, anti-inflammatory, or palliative.[8] Managing inflammation is critical in autoimmune diseases.[25]Non-immunological therapies, such as hormone replacement in Hashimoto's thyroiditis or Type 1 diabetes mellitus treat outcomes of the autoaggressive response, thus these are palliative treatments. Dietary manipulation limits the severity of celiac disease. Steroidal or NSAID treatment limits inflammatory symptoms of many diseases. IVIG is used for CIDP and GBS. Specific immunomodulatory therapies, such as the TNFα antagonists (e.g. etanercept), the B cell depleting agent rituximab, the anti-IL-6 receptor tocilizumaband the costimulation blocker abatacept have been shown to be useful in treating RA. Some of these immunotherapies may be associated with increased risk of adverse effects, such as susceptibility to infection.

Helminthic therapy is an experimental approach that involves inoculation of the patient with specific parasitic intestinal nematodes (helminths). There are currently two closely related treatments available, inoculation with either Necator americanus, commonly known as hookworms, or Trichuris Suis Ova, commonly known as Pig Whipworm Eggs.[26][26][27][28][29][30]

T cell vaccination is also being explored as a possible future therapy for autoimmune disorders.

### Nutrition and autoimmunity

### Vitamin D/Sunlight

- Because most human cells and tissues have receptors for vitamin D, including T and B cells, adequate levels of vitamin D can aid in the regulation of the immune system.[31] Vitamin D plays a role in immune function by acting on T cells and natural killer cells.[32] Research has demonstrated an association between low serum vitamin D and autoimmune diseases, including multiple sclerosis, type 1 diabetes, and Systemic Lupus Erythematosus (commonly

referred to simply as lupus).[32][33][34] However, since photosensitivity occurs in lupus, patients are advised to avoid sunlight which may be responsible for vitamin D deficiency seen in this disease.[32][33][34] Polymorphisms in the vitamin D receptor gene are commonly found in people with autoimmune diseases, giving one potential mechanism for vitamin D's role in autoimmunity.[32][33] There is mixed evidence on the effect of vitamin D supplementation in type 1 diabetes, lupus, and multiple sclerosis.[32][33][34]

**Omega-3 Fatty Acids**

- Studies have shown that adequate consumption of omega-3 fatty acids counteracts the effects of arachidonic acids, which contribute to symptoms of autoimmune diseases. Human and animal trials suggest that omega-3 is an effective treatment modality for many cases of Rheumatoid Arthritis, Inflammatory Bowel Disease, Asthma, and Psoriasis.[35]
- While major depression is not necessarily an autoimmune disease, some of its physiological symptoms are inflammatory and autoimmune in nature. Omega-3 may inhibit production of interferon gamma and other cytokines which cause the physiological symptoms of depression. This may be due to the fact that an imbalance in omega-3 and omega-6 fatty acids, which have opposing effects, is instrumental in the etiology of major depression.[35]

**Probiotics/Microflora**

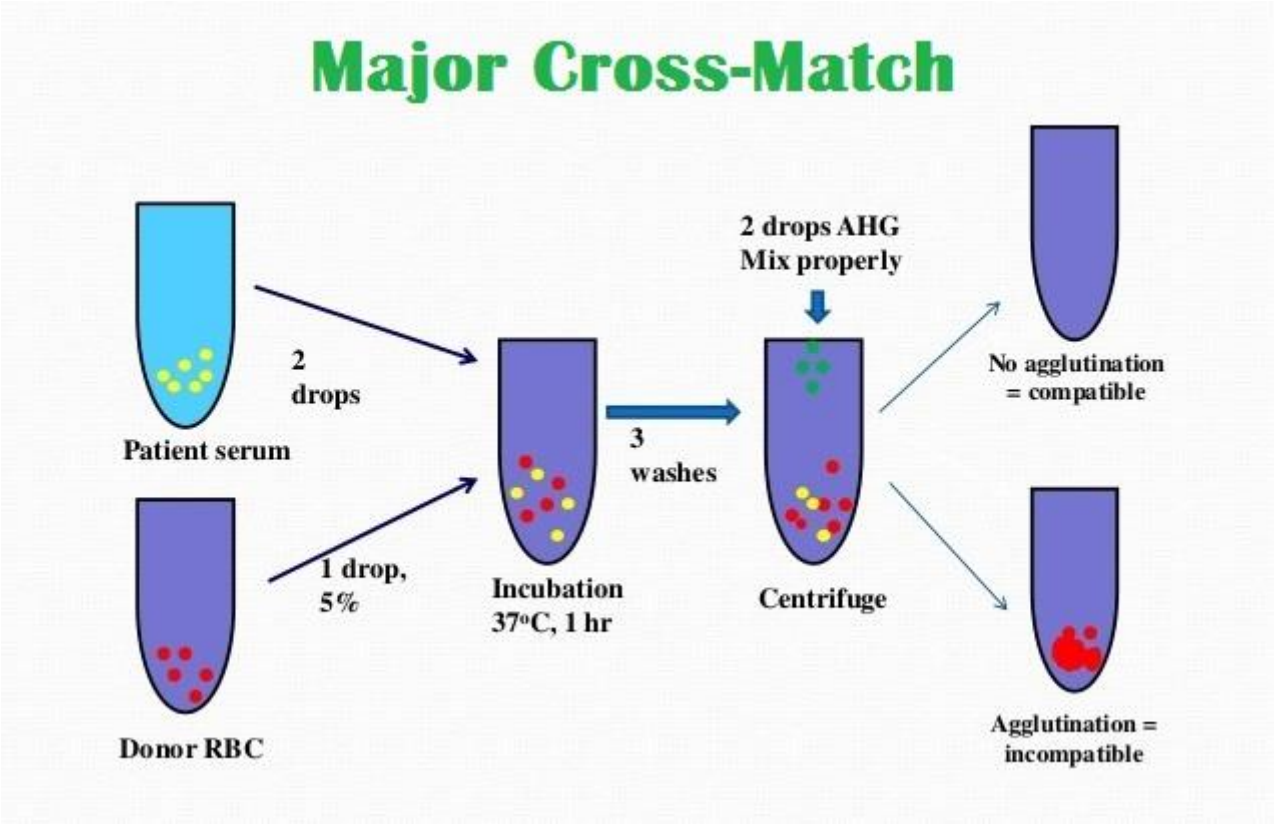
- Various types of bacteria and microflora present in fermented dairy products, especially *Lactobacillus casei*, have been shown to both stimulate immune response to tumors in mice and to regulate immune function, delaying or preventing the onset of nonobese diabetes. This is particularly true of the Shirota strain of *L. casei* (LcS). The LcS strain is mainly found in yogurt and similar products in Europe and Japan, and rarely elsewhere.[36]

**Antioxidants**

- It has been theorized that free radicals contribute to the onset of type-1 diabetes in infants and young children, and therefore that the risk could be reduced by high intake of antioxidant substances during pregnancy. However, a study conducted in a hospital in Finland from 1997-2002 concluded that there was no statistically significant correlation between antioxidant intake and diabetes risk.[37] This study involved monitoring of food intake through questionnaires, and estimated antioxidant intake on this basis, rather than by exact measurements or use of supplements.

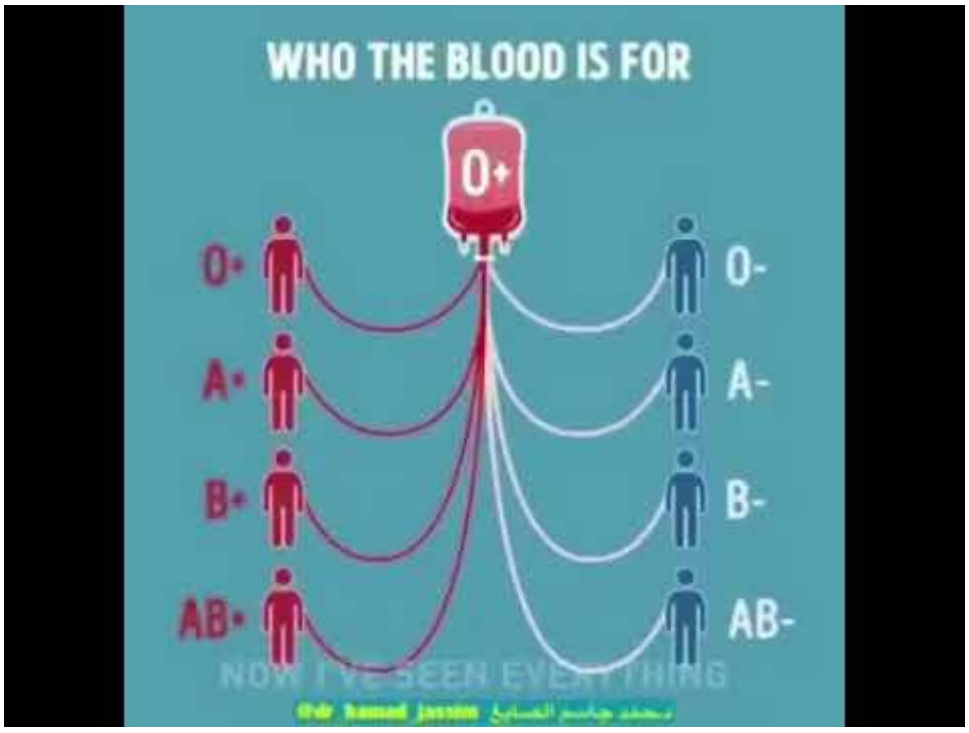
| Blood Group | Antigens | Antibodies | Can give blood (RBC) to | Can receive blood (RBC) from |
|---|---|---|---|---|
| AB | A and B | None | AB | AB, A, B, O |
| A | A | B | A and AB | A and O |
| B | B | A | B and AB | B and O |
| O | None | A and B | AB, A, B, O | O |

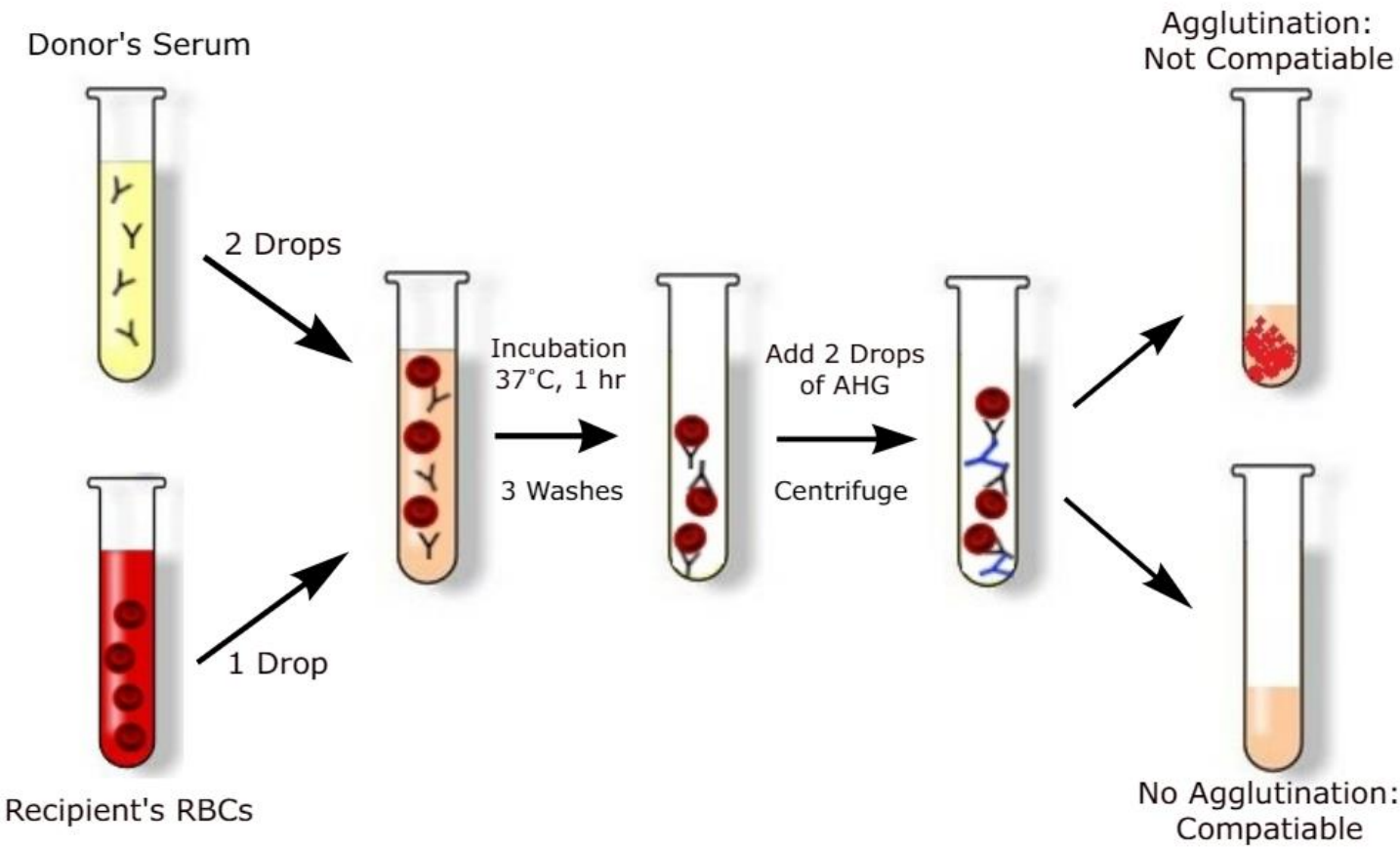
Most **blood group** antibodies are found either on **immunoglobulin** G (IgG) or **immunoglobulin** M (IgM) molecules, but occasionally the **immunoglobulin** A (IgA) class may exhibit **blood group** specificity.

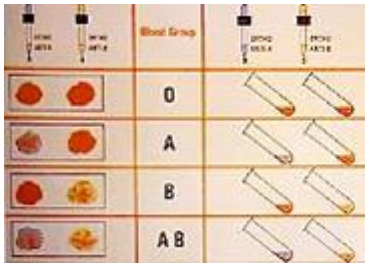


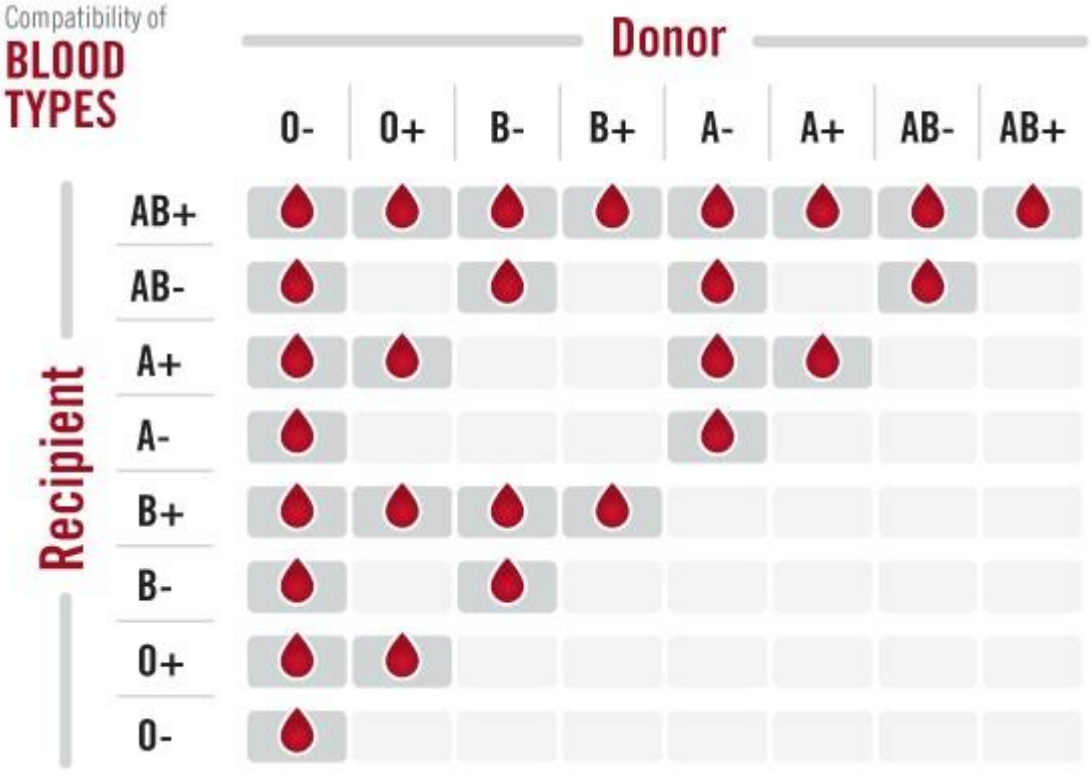
# CROSS MATCHING

- Also known as compatibility testing.
- It is the most important test before a blood transfusion is given.
- The primary purpose of cross matching is to detect ABO incompatibilities between donor and recipient.
- This is carried out to prevent transfusion reactions by detecting Abs in recipient's serum.




Donor's Serum
2 Drops
Incubation
37˚C, 1 hr
3 Washes
Add 2 Drops
of AHG
Centrifuge
Agglutination:
Not Compatiable
1 Drop
Recipient's RBCs
No Agglutination:
Compatiable

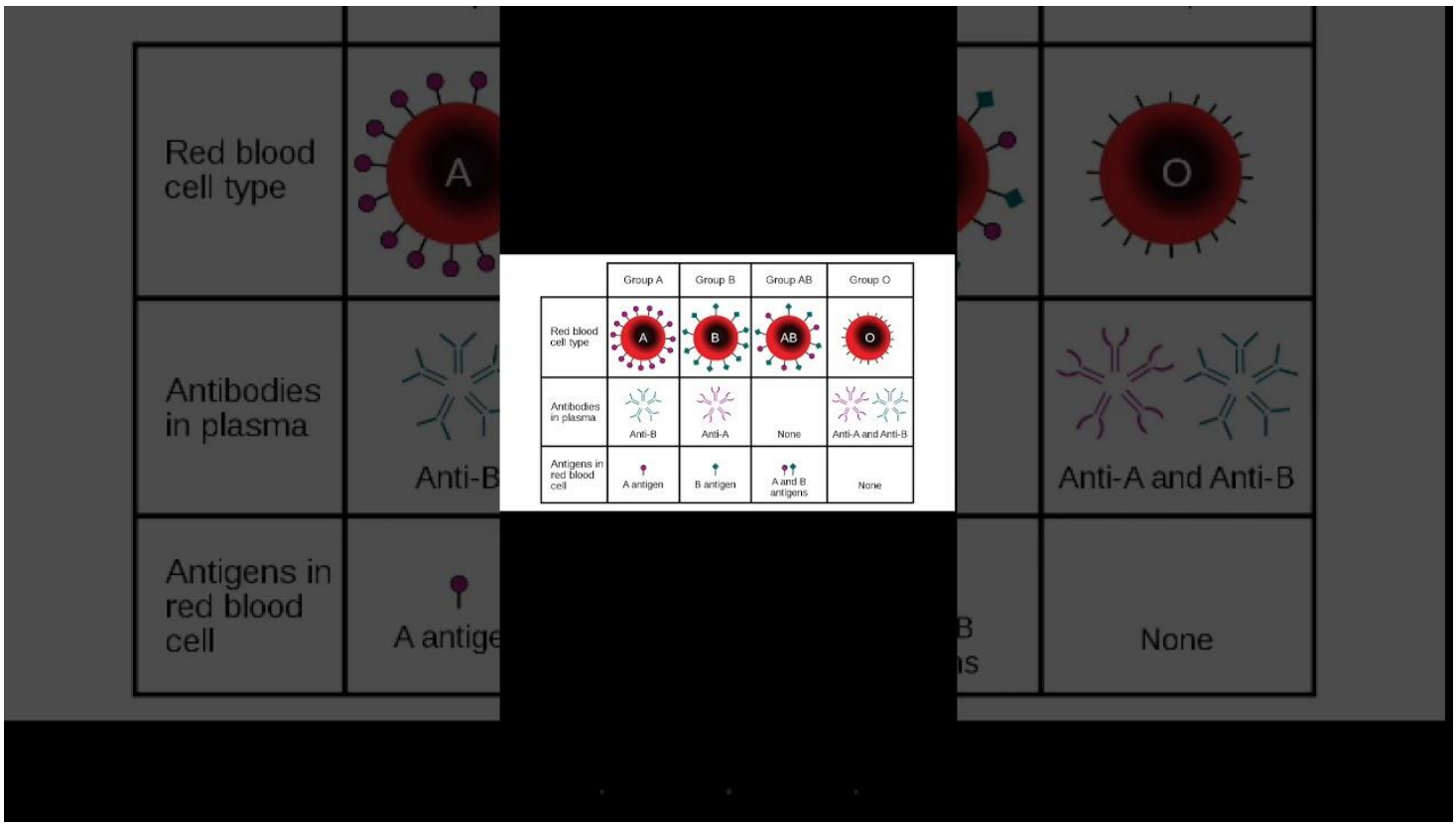

O
A
B
AB


Compatibility of
BLOOD
TYPES
Donor
O-
O+
B-
B+
A-
A+
AB-
AB+
Recipient
AB+
AB-
A+
A-
B+
B-
O+
O-

| Recipient's blood | | | Reactions with donor's red blood cells | | | |
|---|---|---|---|---|---|---|
| ABO antigens | ABO antibodies | ABO blood type | Donor type O cells | Donor type A cells | Donor type B cells | Donor type AB cells |
| None | Anti-A Anti-B | O | | | | |
| A | Anti-B | A | | | | |
| B | Anti-A | B | | | | |
| A & B | None | AB | | | | |

Compatible

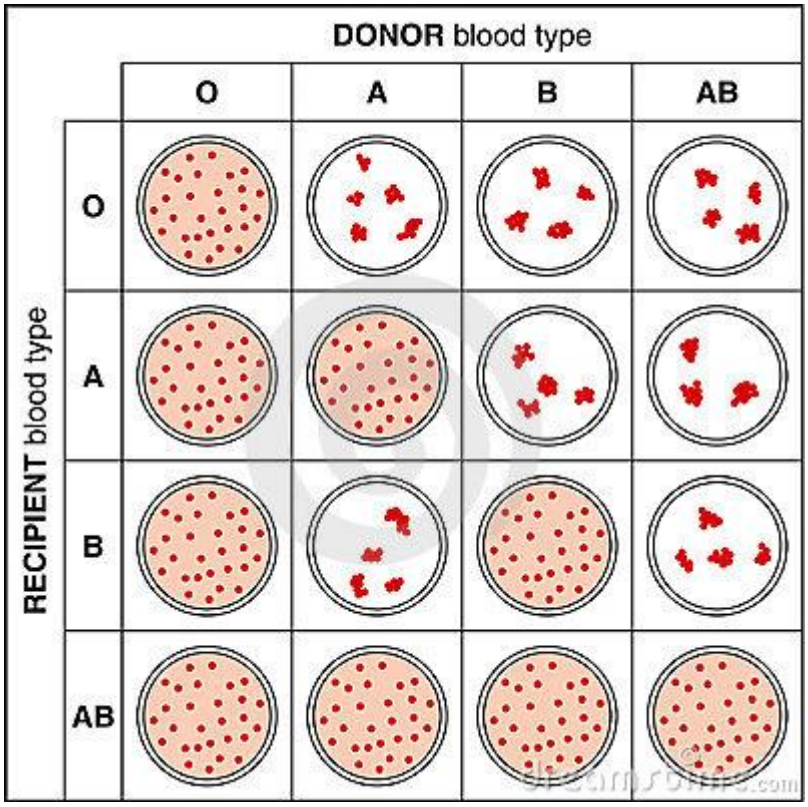
Not compatible

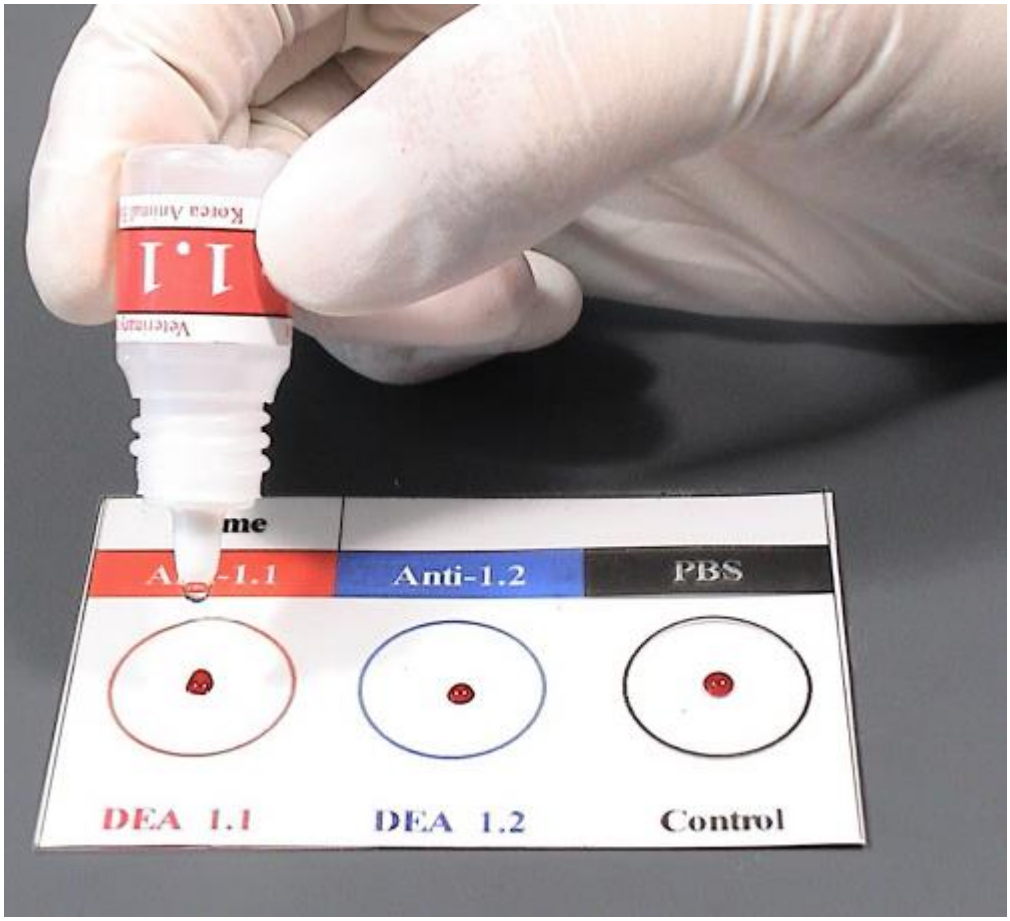


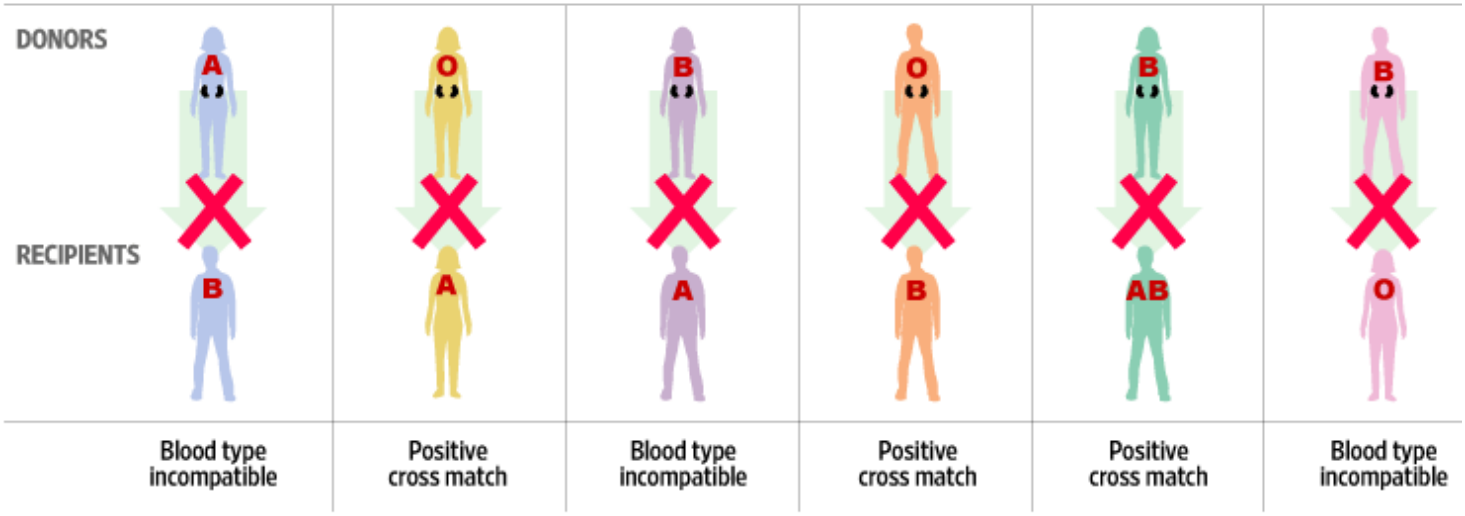
| Blood group | Antigen on red cells | Antibody in plasma | Rhesus factor |
|---|---|---|---|
| **A +ve** | A | B | Present |
| **A –ve** | A | B | Absent |
| **B +ve** | B | A | Present |
| **B –ve** | B | A | Absent |
| **AB +ve** | A and B | None | Present |
| **AB –ve** | A and B | None | Absent |
| **O +ve** | None | A and B | Present |
| **O –ve** | None | A and B | Absent |

# Blood Type, screen and crossmatch

- Screen and type blood from all trauma patients with suspected blunt abdominal injury
  - Initial crossmatch on a minimum of 4 units
    - If clear evidence of abdominal injury
    - And/or hemodynamic instability
  - Until crossmatch blood available use O-negative or type specific blood
- **An indication for immediate transfusion is hemodynamic instability despite administration of 2 L of fluid to adult patients**


DONOR blood type
O
A
B
AB
RECIPIENT blood type
O
A
B
AB

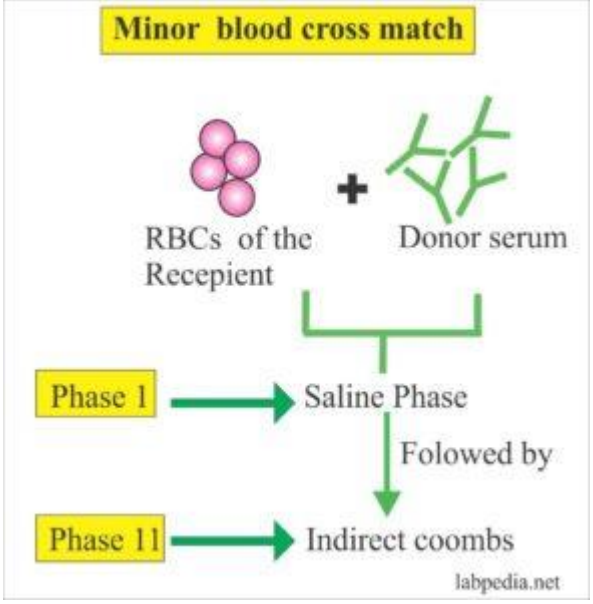

Anti-1.2
PBS
DEA 1.1
DEA 1.2
Control


Imagine these 6 incompatible pairs:
Blood Types A, B, AB, O
DONORS
RECIPIENTS
A
B
O
A
B
A
O
B
B
AB
B
O
Blood type incompatible
Positive cross match
Blood type incompatible
Positive cross match
Positive cross match
Blood type incompatible


Cross Matching
II. Cross Matching-
Major cross matching -Donor's RBCs are mixed with recepient's plasma.
Minor cross matching- Recepients RBC's are mixed with donor's plasma.
When no agglutination then only donors blood can be transfused.

# IMPORTANCE OF BLOOD GROUPING AND Rh TYPING:

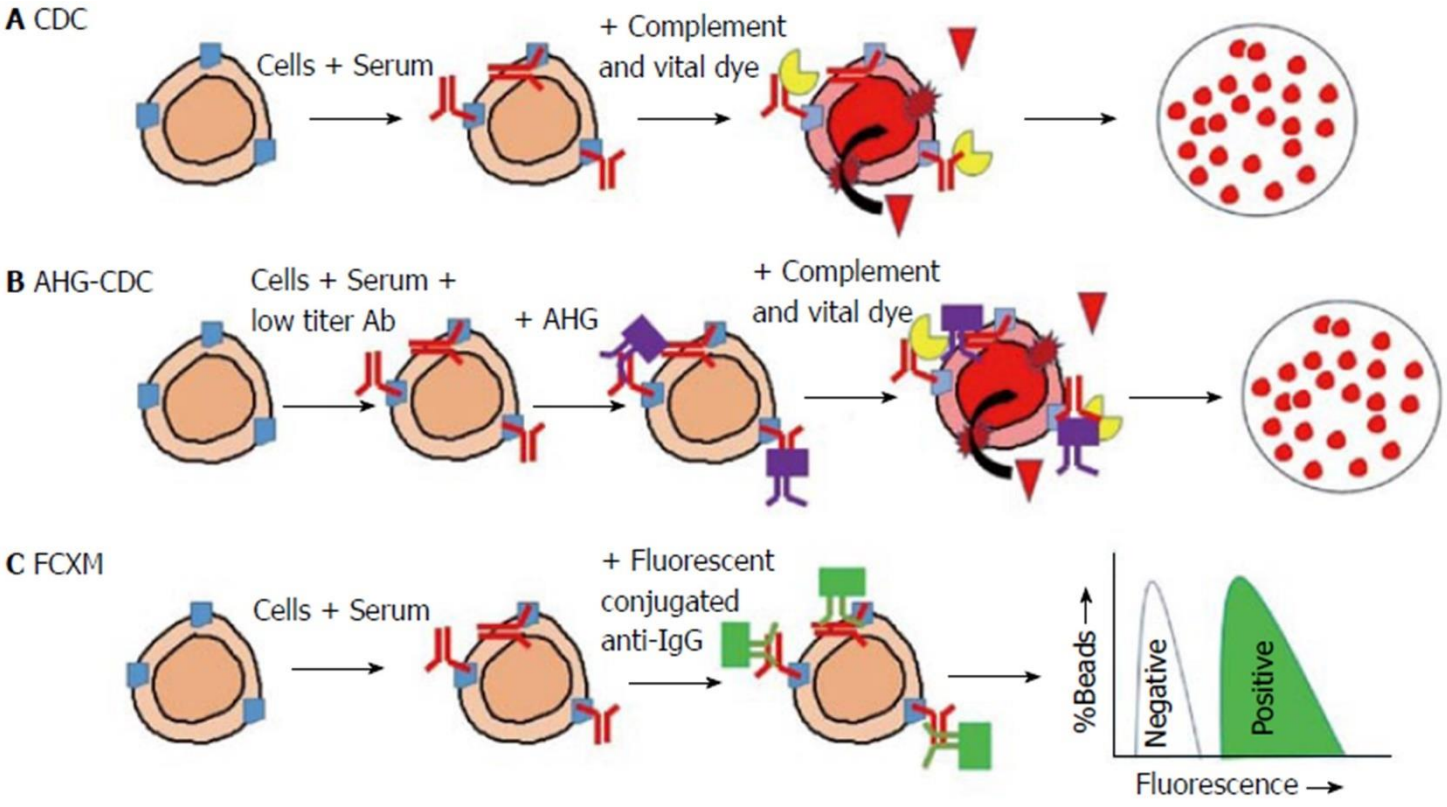
- In blood transfusion
- Haemolytic disease of newborn.
- Paternity dispute
- Medicolegal issues
- Immunology,genetics,anthropology
- Susceptibility to various disease(blood group O – peptic ulcer Blood group A – gastric ulcer)



- Two main functions of cross matching test:

  1)It is a confirm ABO compatibility between donor and recipient.

  2)It may detect presence of irregular Ab in patient's serum that will react with donor RBCs.



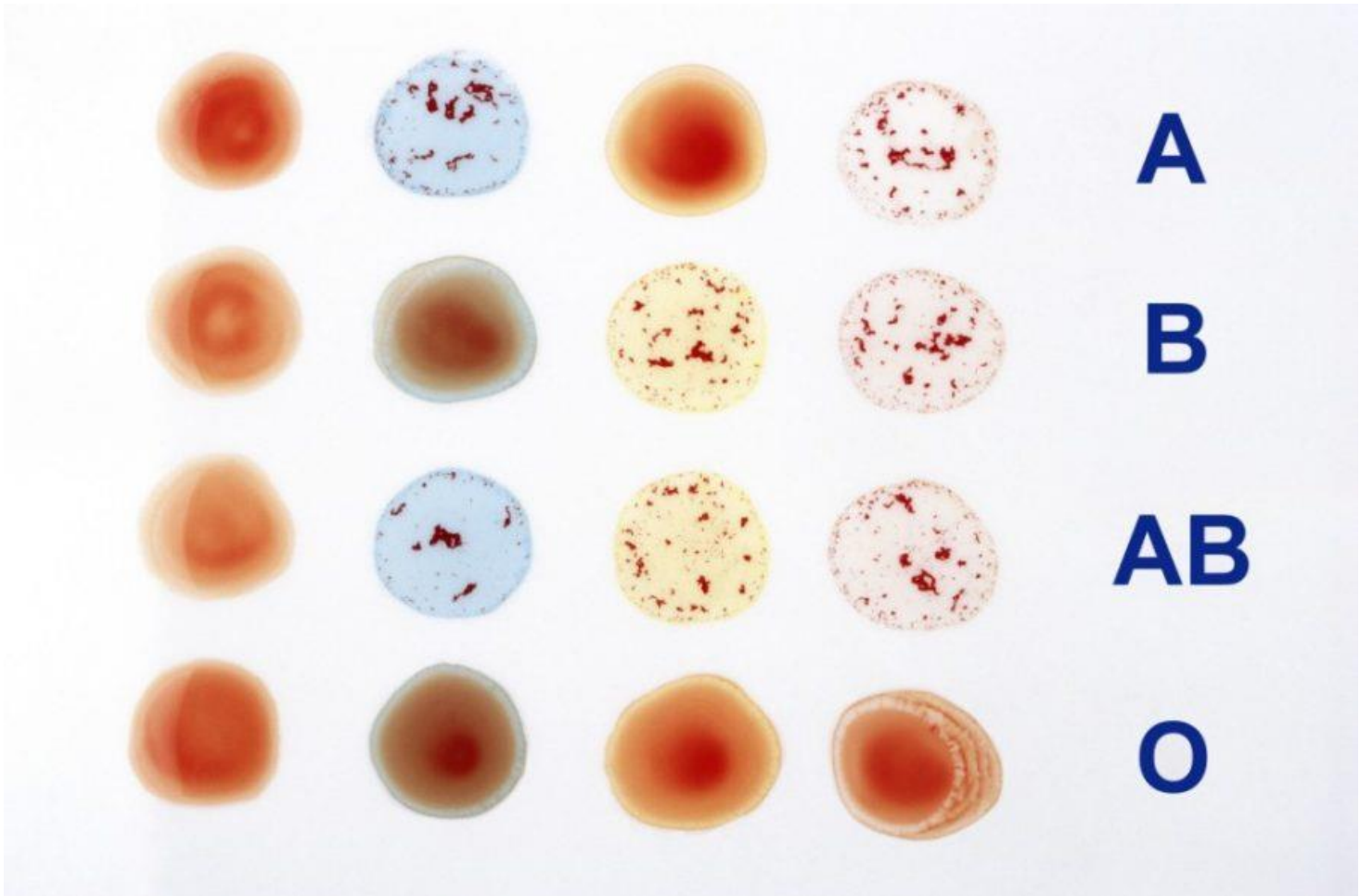
# Blood Crossmatching Procedure

**Step 1**

- MAJOR crossmatch
- Donor Blood + Recipient Plasma
- Positive Test=Macro or Microagglutination present **(Incompatible)**
- Negative Test=Macro or Microagglutination Absent (Compatible)

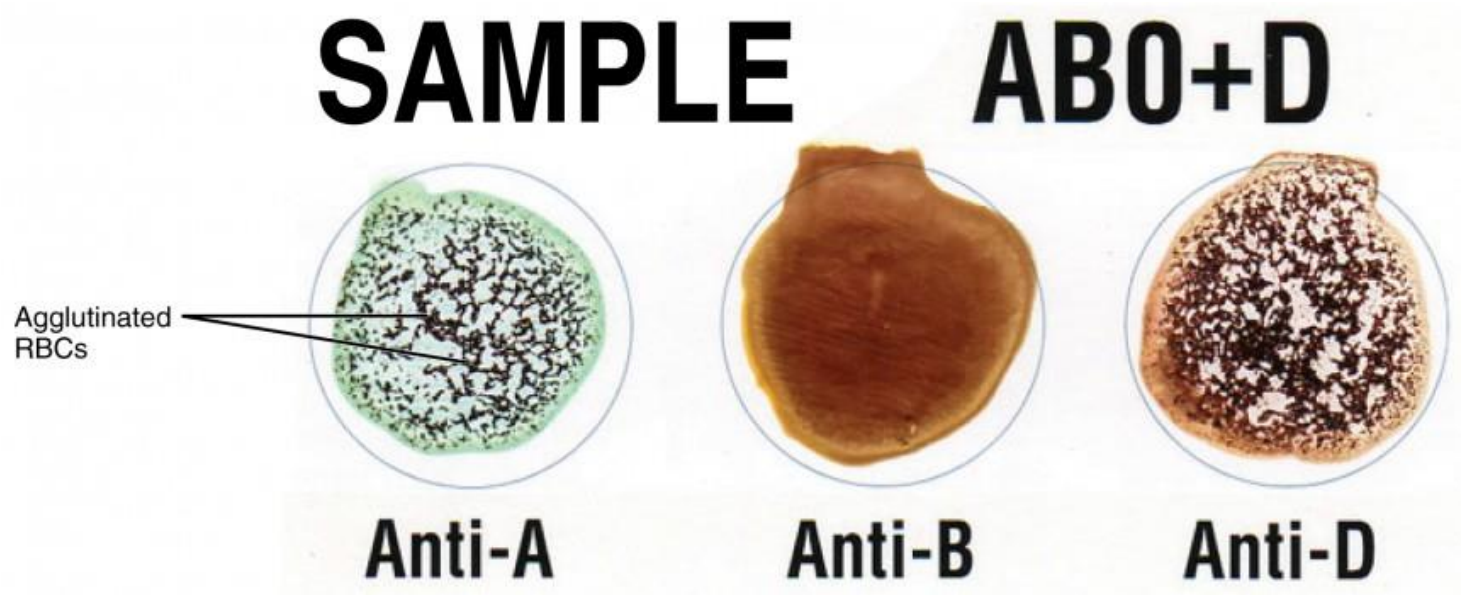
**Step 2**

- MINOR crossmatch
- Recipient Blood + Donor Plasma
- Positive Test=Macro or Microagglutination present **(Incompatible)**
- Negative Test=Macro or Microagglutination Absent (Compatible)



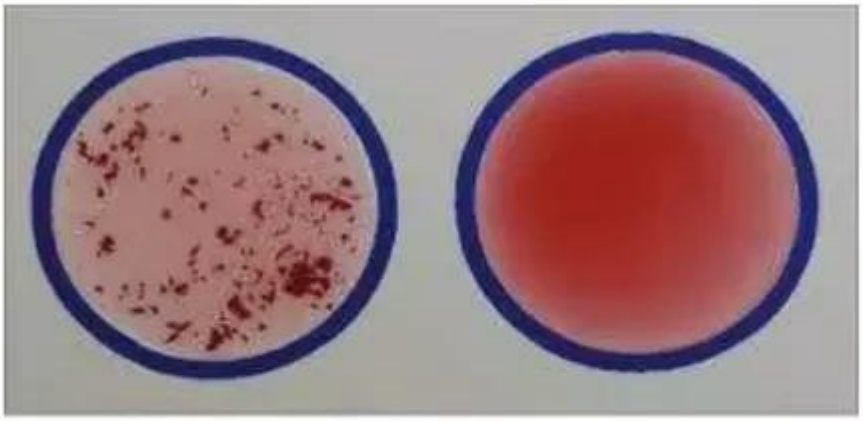
# Blood Grouping and Crossmatch

| | | | |
|---|---|---|---|
| O+ | O+ is the most common blood type. It can be given to patients with O+, A+, B+, and AB+ blood types. Patients who are type O+ can receive both O+ and O- blood. | 1 in 3 | 39.0% |
| A+ | A+ is the second most common blood type. It is given to A+ and AB+ patients. Patients who are type A+ can receive from A+, A-, O+, and O- blood types. | 1 in 3 | 34.0% |
| B+ | B+ can be given to an B+, or AB+ patient. Patients who are type B+ can receive blood from donors with B+, B-, O+, and O- blood types. | 1 in 12 | 8.5% |
| AB+ | AB+ donors are the universal recipient, able to receive any other blood type. AB+ red cells can go only to AB+ patients. However, AB+ is a universal plasma donor. | 1 in 29 | 3.5% |
| O- | O- is the universal donor. O- blood can be used by patients of any blood type. However, patients who are type O- can only receive O- blood. | 1 in 15 | 6.6% |
| A- | A- blood can be given to patients with A-, A+, AB+, and AB- blood types. Patients who are type A- can receive A- and O- blood. | 1 in 16 | 6.3% |
| B- | B- blood can be given to patients with B-, B+, AB+, and AB- blood types. Patients who are type B- can only receive B- and O- blood. | 1 in 67 | 1.5% |
| AB- | AB- is the rarest blood type. It can be given to AB- and AB+ blood types. Type AB- is also the universal blood type for plasma. Patients who are AB- can receive AB-, O-, A-, and B- blood. | 1 in 167 | 1.0% |



# AGGLUTINATION REACTION

✓Visible effect – formation of a precipitate in the form of flakes.




## Rh negative:

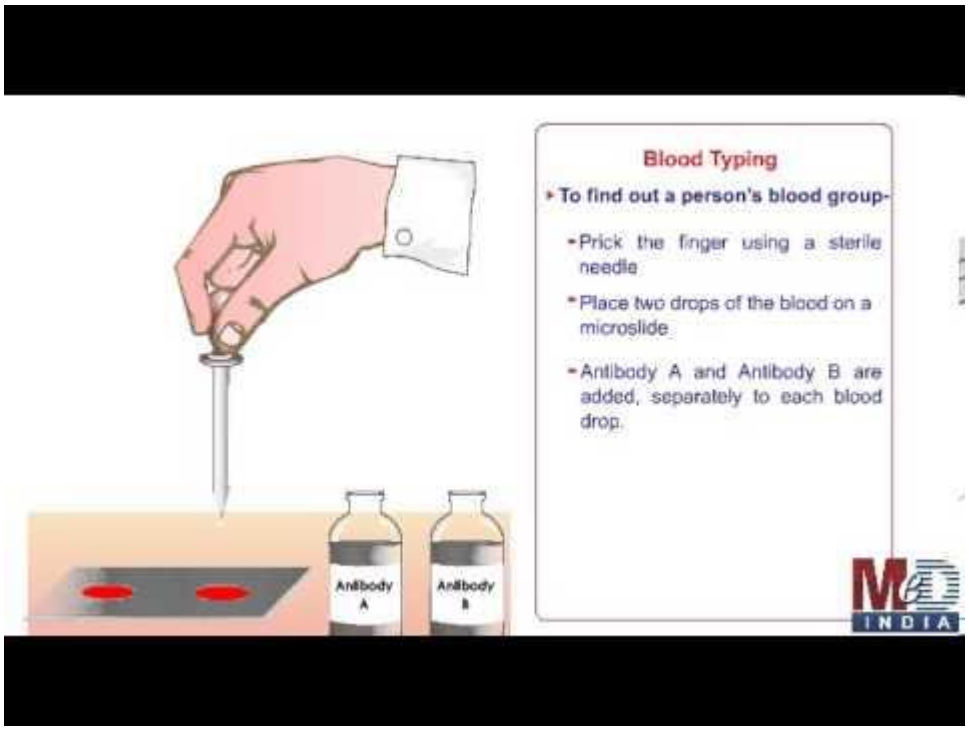
There is absence of D antigen.These individuals constitute 17% of population.

## Cc and Ee antigen:

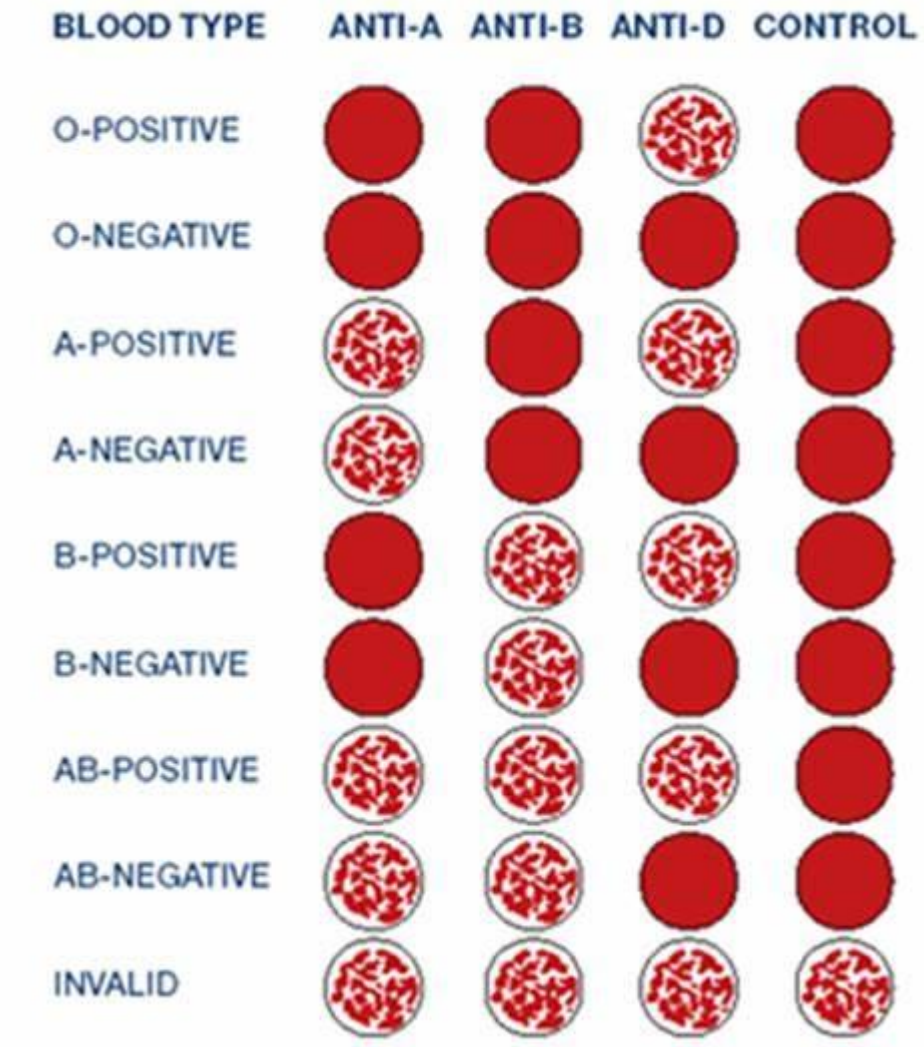
These are weak antigens and therefore risk of sensitisation is less than that of D antigen.

Donor's Blood Type

| Patient's Blood Type | O- | O+ | B- | B+ | A- | A+ | AB- | AB+ |
|---|---|---|---|---|---|---|---|---|
| AB+ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| AB- | ✓ | | ✓ | | ✓ | | ✓ | |
| A+ | ✓ | ✓ | | | ✓ | ✓ | | |
| A- | ✓ | | | | ✓ | | | |
| B+ | ✓ | ✓ | ✓ | ✓ | | | | |
| B- | ✓ | | ✓ | | | | | |
| O+ | ✓ | ✓ | | | | | | |
| O- | ✓ | | | | | | | |

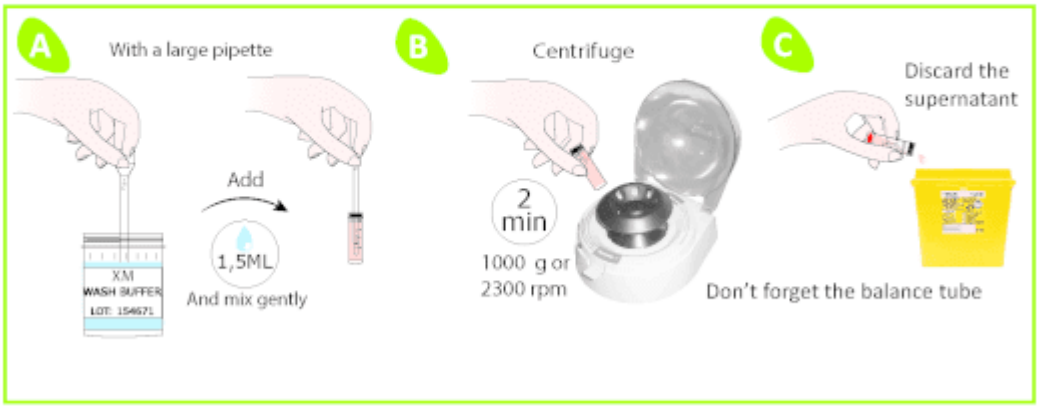


# HOW TO READ YOUR RESULTS




5 Suspension washing procedure

x3 STEP 5 MUST BE DONE 3 TIMES



# Importance of cross matching

- Routine blood grouping involves only ABO and Rh.
- Other clinically significant blood group systems not matched routinely
- Though, antibodies to minor antigens are of rare occurrence, they can cause transfusion reactions
- Cross matching between patient's serum and donor's cells will detect antibodies to other blood groups, if present.

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---|---|---|---|---|
| Anti-A |  |  |  |  |
| Anti-B |  |  |  |  |
| Result | No agglutination when either anti-A or anti-B is added | Agglutination only when anti-A added | Agglutination only when anti-B added | Agglutination when both anti-A and anti-B are added |
| Interpretation of result | **Group O** | **Group A** | **Group B** | **Group AB** |
| Per cent frequency in UK population | 47 | 42 | 8 | 3 |

 No agglutination

 Agglutination - cells clumped together

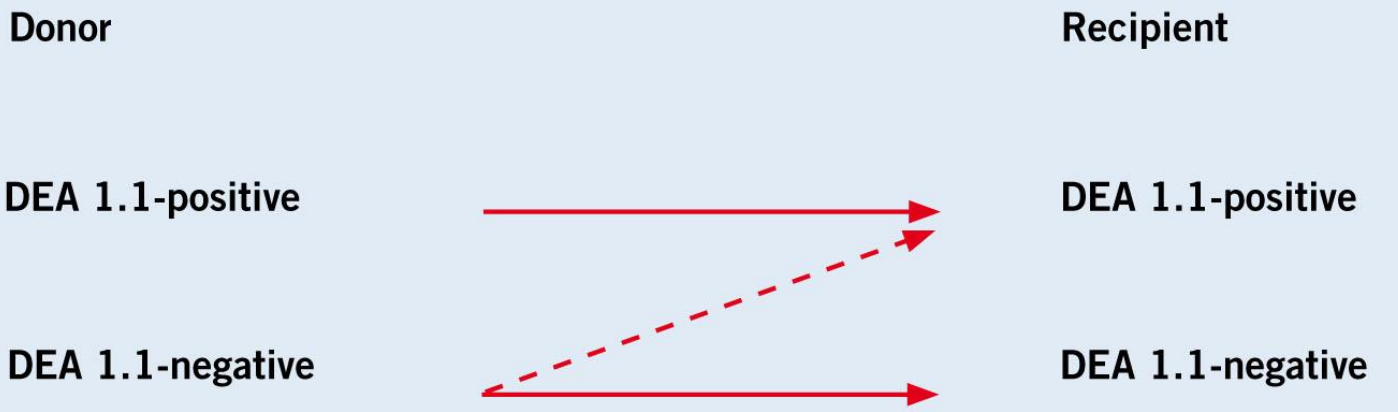
PERSON TO RECEIVE TRANSFUSION IS TYPE:

| The Person Who Donates the Blood is Type: | A | B | AB | O |
|---|---|---|---|---|
| A | yes | no | yes | no |
| B | no | yes | yes | no |
| AB | no | no | yes | no |
| O | yes | yes | yes | yes |

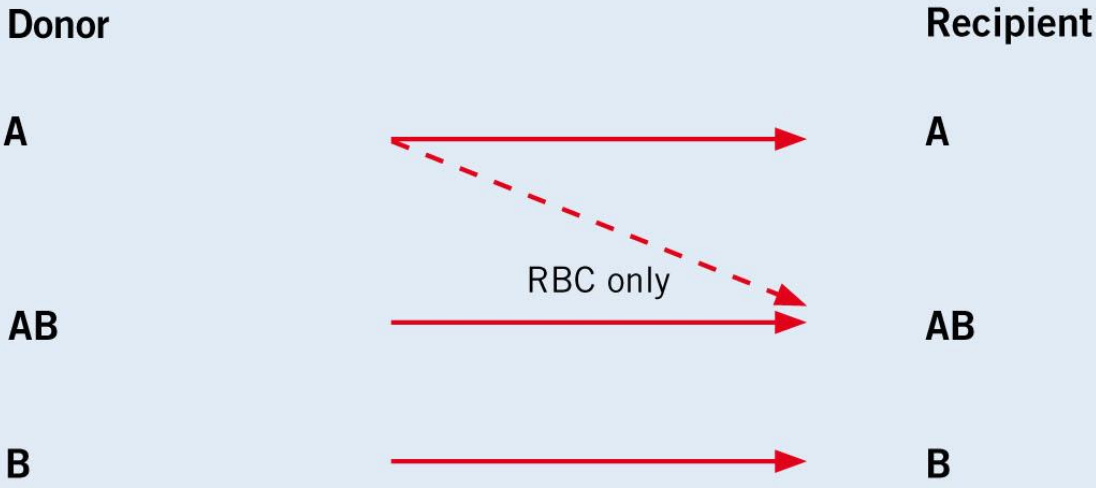
| Donor blood group | Recipient (patient) blood group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **O RhD Pos** | **O RhD Neg** | **A RhD Pos** | **A RhD Neg** | **B RhD Pos** | **B RhD Neg** | **AB RhD Neg** | **AB RhD Neg** |
| O RhD Pos | ✓ | × | ✓ | × | ✓ | × | ✓ | × |
| O RhD Neg | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| A RhD Pos | × | × | ✓ | × | × | × | ✓ | × |
| A RhD Neg | × | × | ✓ | ✓ | × | × | ✓ | ✓ |
| B RhD Pos | × | × | × | × | ✓ | × | ✓ | × |
| B RhD Neg | × | × | × | × | × | ✓ | ✓ | ✓ |
| AB RhD Pos | × | × | × | × | × | × | ✓ | × |
| AB RhD Neg | × | × | × | × | × | × | ✓ | ✓ |

✓ABO compatible. No risk of Rh sensitisation. Safe to transfuse if patient antibody screen is negative.
×ABO incompatible or risk of Rh sensitisation. Not safe to transfuse.

## Dogs




## Cats




Determination of blood groups in dogs and cats:

- If the RBCs are A group, a red line will appear on the membrane, in front of the letter noted A on the kit.
- If the RBCs are B group, a red line will appear on the membrane, in front of the letter noted B on the kit.
- If the RBCs are AB groups, two red lines will appear on the membrane, in front of the letters A and B on the kit.

DEA, dog erythrocyte antigen; RBC, red blood cell

# Rh TYPING

## HISTORY:

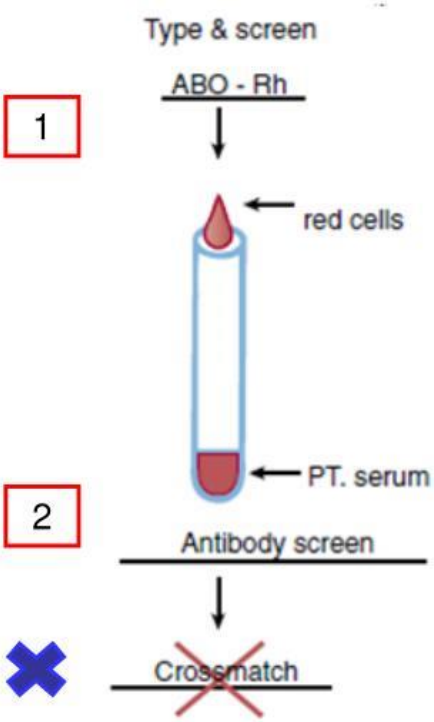
- 1939 – Levine and Stetson defined D antigen(Rh factor)

- 1949 – Landsteiner and Weiner discovered anti Rh (named after Rhesus monkey)

**RELATIONSHIPS BETWEEN BLOOD TYPES AND ANTIBODIES**

| Blood Type | Antigens on Red Blood Cell | Can Donate Blood To | Antibodies in Cerum | Can Recieve Blood From |
|---|---|---|---|---|
| A | A | A, AB | Anti-B | A, O |
| B | B | B, AB | Anti-A | B, O |
| AB | A and B | AB | None | AB, O |
| O | None | A, B, AB, O | Anti-A and Anti-B | O |

# Type and Screen




The patient's serum is screened for the presence of **unexpected antibodies** by incubating it with selected reagent RBCs (i.e., **screen cells**). These cells contain all antigens capable of inducing **clinically significant** RBC antibody reactions

For those few patients in whom the antibody screen **reveals** the presence of unexpected antibody, the antibody is subsequently **identified** in the blood bank and **units of blood lacking the corresponding antigen** are set aside for surgery.

# Type and Crossmatching

- Crossmatching

  -Trial transfusion within a test tube between donor RBCs and recipient serum to detect a potential for serious transfusion reaction

  - 3 Phases:

    -Reconfirm ABO-Rh typing

    - Detect antibodies that are incomplete or do not agglutinate easily

    - Detect antibodies in other blood group systems (ie. Rh, Kell, Kidd, Duffy)

- Antibody screening
  - Trial transfusion between the recipient's serum and commercially supplied RBCs with antigens that will react with antibodies commonly implicated in non-ABO hemolytic transfusion reactions
  - Donor's serum also screened for unexpected antibodies to prevent their introduction to the recipient's serum
  - Otherwise known as the Coomb's test.

# SLIDE METHOD

## REQUIREMENTS:

1)3 slides
2)Antisera A , B
3) Blood samples

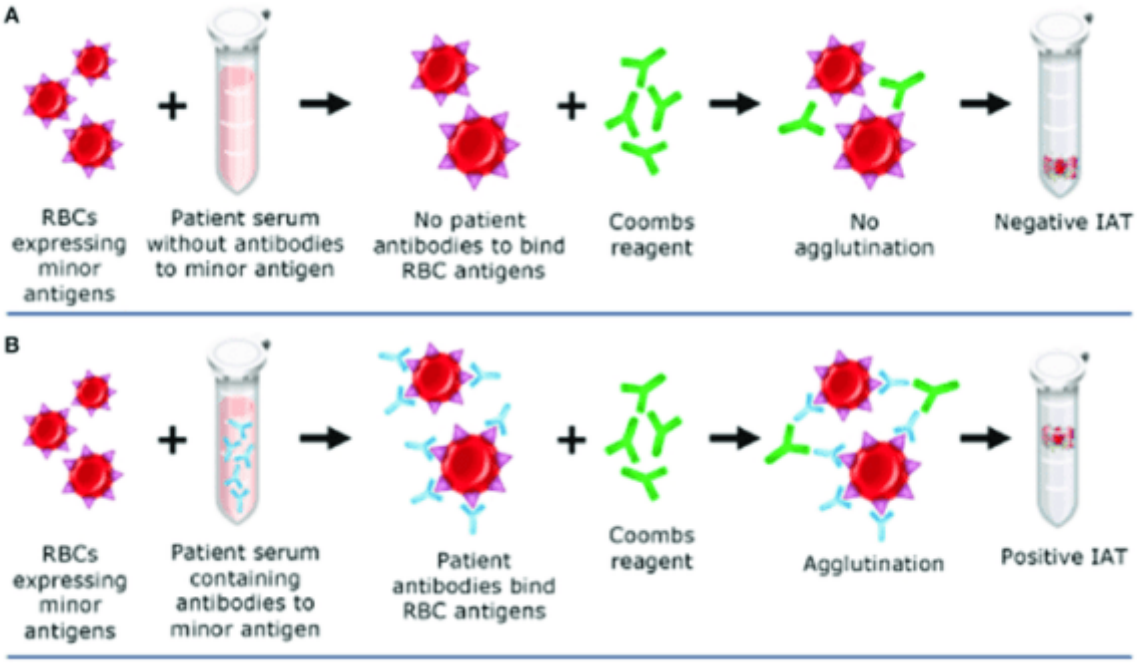
- Rh positive

There is presence of D antigen.

These individuals constitute 80% of population.

| Blood Group | Gives to these groups | Receives from these groups |
|---|---|---|
| $O^-$ | All | O- only |
| $O^+$ | AB+, A+, B+, O+ | O- and O+ |
| $A^-$ | AB-, AB+, A+, A- | O- and A- |
| $A^+$ | AB+ and A+ | O-, O+, A-, A+ |
| $B^-$ | B-, B+, AB-, AB+ | O- and B- |
| $B^+$ | B+ and AB+ | O-, O+, B-, B+ |
| $AB^-$ | AB- and AB+ | O-, A-, B-, AB- |
| $AB^+$ | AB+ only | All |

| Compatible Blood Type Donors | | |
|---|---|---|
| **Blood Type** | **Donate Blood To** | **Receive Blood From** |
| **A+** | A+ AB+ | A+ A- O+ O- |
| **O+** | O+ A+ B+ AB+ | O+ O- |
| **B+** | B+ AB+ | B+ B- O+ O- |
| **AB+** | AB+ | Everyone |
| **A-** | A+ A- AB+ AB- | A- O- |
| **O-** | Everyone | O- |
| **B-** | B+ B- AB+ AB- | B- O- |
| **AB-** | AB+ AB- | AB- A- B- O- |


A
RBCs expressing minor antigens
Patient serum without antibodies to minor antigen
No patient antibodies to bind RBC antigens
Coombs reagent
No agglutination
Negative IAT
B
RBCs expressing minor antigens
Patient serum containing antibodies to minor antigen
Patient antibodies bind RBC antigens
Coombs reagent
Agglutination
Positive IAT