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Passion, Quality and Innovation in Healthcare

Manual of TRANSFUSION MEDICINE



Ramadas Nayak
Rakshatha Nayak

JAYPEE

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CHAPTER

4

ABO Blood Grouping: Methods and Discrepancies

CHAPTER OUTLINE

- Blood banking reagents
- ABO grouping/typing
- Methods of ABO grouping
- Alternative methods to the tube test
- Factors causing discrepancies during ABO testing
- ABO discrepancies, problem encountered and their solutions

BLOOD BANKING REAGENTS

Commercial antibodies and reagent red cells are used in the blood bank to detect antigen-antibody (Ag-Ab) reactions. These reagents help to provide safe and viable blood products. It is necessary for the blood bank technologists to know the correct usage of these reagents and also the limitations of each reagent. Technologists also should be competent enough to rapidly and accurately interpret the results from patient and donor testing. The reagents used in blood banking tests are discussed here. First the blood bank reagents used in testing to detect agglutination in test tubes are discussed followed by alternative methods in which the tubes are not used in the test.

Categories of reagents used in blood bank have been shown in Table 4.1.

Table 4.1: Categories of reagents used in blood bank.

Category	*Content*
Antisera	Known red cell antibodies
Antiglobulin reagents	Anti-IgG or anti-C3d or a combination of anti-IgG and anti-C3d
Potentiators	To enhance antibodies
Reagent red cells	Known red cell antigen phenotypes

There are many manufacturers of blood bank reagents. The routine blood bank reagents available commercially are shown in Figure 4.1.

The licensing of blood bank reagents: Commercial antisera and reagent red cell products are under regulations control. Before licensing the commercial reagent, the regulatory necessitates the minimum standards relating to product specificity and potency for use in blood banks and transfusion services.

- **Specificity** reflects the unique recognition of the antigenic determinant and its corresponding antibody molecule. For example, the specificity of the commercial anti-D should be such that it should react with red cells having D antigens and should not react with red cells without D antigens.
- **Potency** means the strength of an Ag-Ab reaction. For example, potent commercial anti-A reagents should produce strong agglutination (3+ to 4+) with red cells having the A antigen.

Reagent product insert: The manufacturer of reagent provides a package or product insert to the consumer along with the reagent. Thorough understanding of the product inserts is needed for proper use of blood bank reagents. The product insert (Box 4.1)

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Fig. 4.1: Routine blood bank testing reagents (from left to right anti-A, anti-B, anti-A, B and anti-D [IgM, IgM+IgG and IgG]) *(For color version, see Plate 1)*.

Box 4.1: Usual contents of reagent product insert.

- Description
- Intended use
- Principle
- Procedure for proper use
- Interpretations
- Performance characteristics
- Limitations of the reagent
- Quality control

describes in detail the intended use, summary, principle, procedure for proper use, the specific performance characteristics, and the limitations of the reagent. Standard operating procedures (SOPs) are written in the blood bank to reflect the procedures outlined in these product inserts. Whenever new reagent lots are received in the blood bank, the product inserts must be reviewed for any procedural changes. If there are any revisions, that must be incorporated into the SOPs before using the reagent in routine testing.

Quality control (refer Chapter 22): Reagents should be tested daily for performance. The results of quality control testing are recorded and reviewed. It is mandatory to maintain records of quality control testing that includes results, interpretations, date of testing, and the personnel who performed the test. If reagents do not meet performance criteria, appropriate corrective actions should be implemented.

- **Antisera:** They **should be clear** and should be inspected daily for any evidence of bacterial contamination. Any turbidity or cloudiness in the reagent bottles indicates contamination of the product.
- **Reagent red cells:** Inspect them for any evidence of hemolysis.

Commercial Antibody Reagents

Polyclonal versus Monoclonal Antibody Products (Fig. 4.2)

Ideally, an antibody reagent product should contain a concentrated suspension of highly specific, well characterized, uniformly reactive immunoglobulin/antibody molecules. Commercially available antibody reagents can be either polyclonal antibody-based or monoclonal antibody-based products.

- **Polyclonal antibody:** If multiple clones of B cells secrete antibodies in an immunologic response to a foreign antigen, the antiserum/antibody reagent produced is called polyclonal.
- **Monoclonal antibody:** If single clones of B cells secrete antibodies in an immunologic response to a foreign antigen, the antiserum/antibody reagent produced is called monoclonal.

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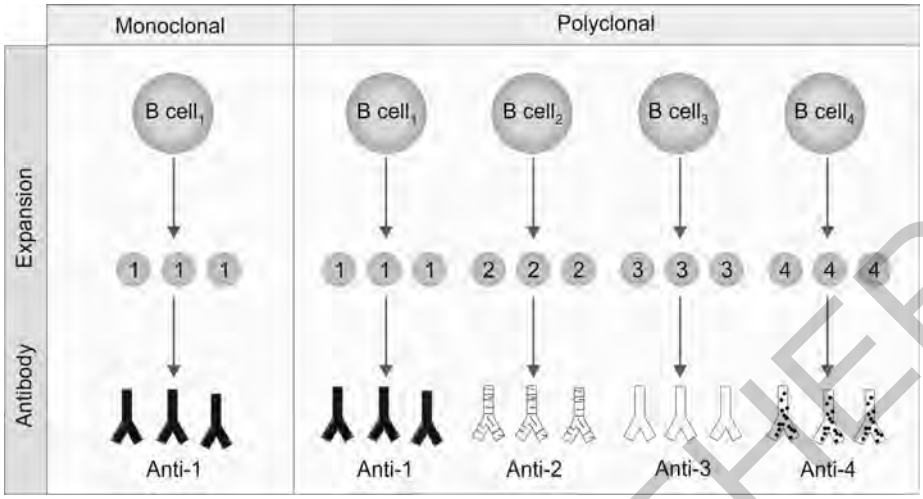

Fig. 4.2: Comparison of monoclonal versus polyclonal immune responses. In monoclonal immune responses, there is activation of single clone of B cells by the immune response and it secretes an antibody with one specificity (anti-1 in left part). In polyclonal immune responses, after exposure to the antigen, there is activation of multiple clones of B cells by the immune response and they secrete antibodies of different specificities. In the right part, these response to the antigen are named as anti-1, anti-2, anti-3, and anti-4. These were secreted by different clones of B-cell.

Polyclonal antibody reagents

In the early 1990s, the of monoclonal antibody-based blood banking reagents were introduced into transfusion medicine. Before this, commercial antisera used in blood bank were derived from polyclonal sources. These polygonal reagents were obtained from the immunization of animals and humans with purified antigens. The separation techniques used to produce the polyclonal antisera were time-consuming.

Polyclonal immune response: B cells produce antibodies that are specific for the multiple epitopes of the injected antigen. This produces a heterogeneous population of antibodies which can recognize different epitopes (epitopes are single antigenic determinants; functionally, parts of the antigen that combine with the antibody) on a single antigen. Polyclonal antiserum is prepared from several different clones of B cells that secrete antibodies of different specificities. Examples for polyclonal antiserum used in blood bank are antihuman globulin (AHG) reagents. Polyclonal antihuman immunoglobulin G (IgG) serum is produced by immunizing rabbits with purified human IgG molecules. This activates multiple clones of B cell in rabbits. Each clone of B cell produces an antibody directed at a specific epitope of the IgG molecule. The combination of the multiple clones of B cell secreting many antibodies in the rabbit serum makes a polyclonal antibody reagent (Fig. 4.2). (For details refer Chapter 9 page 175)

Monoclonal antibody reagents

Monoclonal antibodies are the products of a single clone of B cells that secrete antibodies of the same specificity. The monoclonal antibody technique is useful in producing high-titer antibodies with well-defined specificities. Monoclonal antibodies are produced by an immortal clone that manufactures antibodies of a defined specificity (Fig. 4.2). In vitro, the monoclonal antibodies are produced using hybridoma technology.

Hybridoma technology (Fig. 4.3): Hybridomas are hybrid cells formed by the fusion of myeloma cells and antibody-producing B lymphocytes and are used in the production of monoclonal antibodies. Monoclonal antibody is produced by immunizing the laboratory

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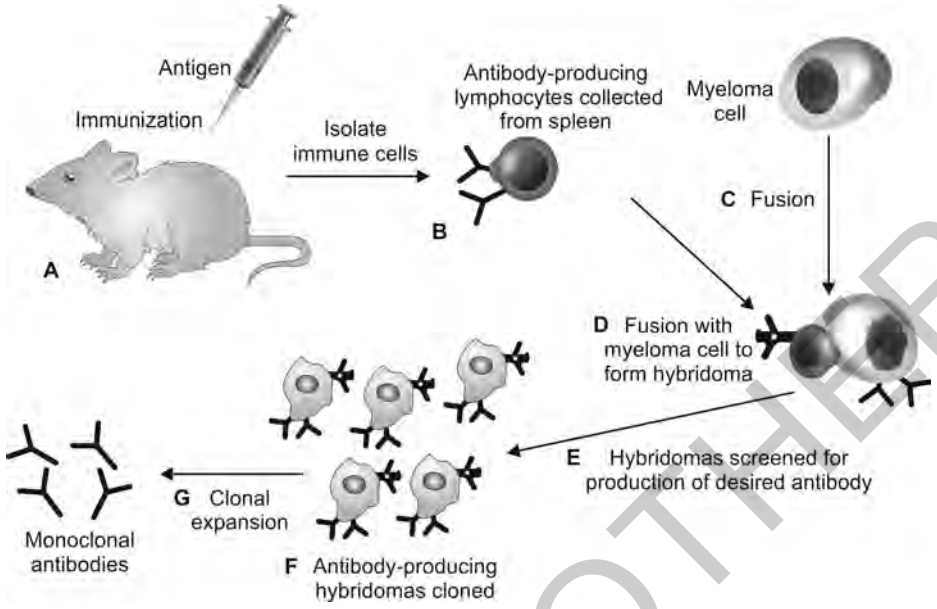

Fig. 4.3: Method of production of monoclonal murine antibody. Monoclonal antibodies are the products of a single clone of B lymphocytes. (A) **Immunization:** First mice are immunized with antigen. (B) **Harvesting:** The spleen B lymphocytes of mice are harvested. (C) **Fusion:** The antibody-forming B lymphocytes are fused with a myeloma cell from a mouse. (D) **Hybrid cells (hybridomas):** The resulting hybrid cells are cultured. (E) **Screening:** The hybridomas are screened for the production of desired antibody. (F) **Cloning:** Each hybridoma is descended from a single B cell clone. (G) **Monoclonal antibodies:** The hybridoma cell line is expanded and they produce the same antibody molecule.

animals, usually mice, with antigen. After a suitable immune response, antibody-secreting lymphocytes are obtained from spleen of the mice immunized with antigen. These antibody-secreting lymphocytes (B lymphocytes) from spleen are harvested and are fused with myeloma cells (i.e. abnormal cell from a mouse that has malignant tumor of plasma cells called myeloma). This results in fused hybrid cells called as "hybridomas". Each hybridoma is descended from a single clone of B cell. The hybridoma cells are propagated in tissue culture and they grow rapidly. They are screened for the production of antibody of required specificity and affinity. All cells of the hybridoma cell line produce the same antibody molecule, called a monoclonal antibody. These antibodies are identical in terms of antibody structure and antigen specificity. In contrast to polyclonal antibodies, monoclonal antibodies recognize one specific epitope of the antigen. In routine blood typing, murine (mouse) monoclonal antibodies have replaced polyclonal reagents for A and B antigens and complement proteins.

Human B lymphocytes transformed with Epstein-Barr virus (EBV): Human monoclonal antibodies also can be produced with blood group specificity. For this, human B lymphocytes are transformed with EBV. These transformed cells are grown in tissue culture and they secrete monoclonal antibodies. It was first derived by using B lymphocytes from D-immunized donors. Both IgG and IgM anti-D were obtained from the EBV-transformed cells.

Heterohybridomas: These are hybrid cells formed by the fusion of B lymphocyte of one species with the myeloma cell of a different species. Heterohybridoma is another method for the production of stable lines of human monoclonal antibodies. In this method, human B lymphocytes are fused with murine myeloma cells which form heterohybridomas. A number of human monoclonal antibodies

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are now available in the market for use in blood typing.

Use of monoclonal antibody-based reagents in blood bank in place of polyclonal antibody-based reagents has increased the accuracy and there is no need to depend on reagents prepared from human blood donated by volunteers. The approved monoclonal antibodies available for use in blood bank include anti-A, anti-B, anti-A,B, anti-D, anti-C, anti-E, anti-c, anti-e, anti-IgG, anti-C3b, anti-C3d. Desired monoclonal antibody reagents can be produced in large quantities with lot-to-lot consistency of a single specificity.

Disadvantage of monoclonal antibody reagents: In routine testing, discrepant reactions may be observed between human- or animal-source reagents and the monoclonal antibody reagents.

Differences between monoclonal and polyclonal antibody are presented in Table 4.2.

Monoclonal and polyclonal antibody reagents

Blending of monoclonal and polyclonal antibodies produces reagents with specific advantages in blood bank testing. Examples of monoclonal and polyclonal blend antibodies are antiglobulin reagents (discussed in Chapter 9 page 174) in which there is a combination of rabbit polyclonal antibodies and a murine (mouse) monoclonal antibody.

Reagents for ABO antigen typing are discussed on pages 70, 71. Reagents for D antigen typing are discussed in Chapter 5 on pages 105-6.

Low-Protein Reagent Control

To ensure that the ABO and Rh typing results were interpreted correctly, a reagent control is used. The reagent control should not show any agglutination (a negative result). The ABO and D typing reagents are produced with protein concentrations similar to human serum (approximately 6% bovine albumin).

- **Spontaneous agglutination** of red cells occurs less frequently at this low-protein concentration than with **reagents containing higher concentrations of protein**.
- **False-positive result during typing:** It can be due to (1) spontaneous agglutination of red cells during typing and (2) if blood specimen contains strong cold autoantibodies (antibodies to self-antigens) or has protein abnormalities.
- **Reagent control:**
  - **No need of reagent control:** A **negative result using ABO low-protein reagents can act as a reagent control**. An **additional reagent control** in ABO and D typing is **not needed when the patient or donor red cells show no agglutination with anti-A, anti-B, or anti-D in red cell testing.**
  - **Need for reagent control:** A reagent control is needed to interpret the results, **if red cells are agglutinated with anti-A, anti-B, and anti-D**. The reagent control should be performed according to the reagent manufacturer, example of which is presented in Table 4.3.

Reagent red cells: A_1 and B red cells for ABO serum testing (discussed on page 71)

Screening cells (discussed in Chapter 10 on page 191)

Antibody identification panel cells (discussed in Chapter 10 on page 198)

Antiglobulin reagents (discussed in Chapter 9 on page 174)

Table 4.2: Differences between monoclonal and polyclonal antibody.

	Monoclonal antibody	*Polyclonal antibody*
Production/secretion by clone of B lymphocytes	Production by a single clone of antibody-producing B lymphocytes	Production by several different clones of antibody-producing B lymphocytes
Immunoglobulins secreted	Single immunoglobulin class (IgG or IgM)	Mixture of IgM and IgG antibodies
Features of immunoglobulin	Unique specificity for a particular epitope	Mixture of antibodies that may be directed at different epitopes of the same antigen

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Table 4.3: Example of situation where low-protein reagent control is necessary.

Reaction with			*Red cell antigens present*	*Interpretation*	*Reagent control*
Anti-A	*Anti-B*	*Anti-D*			
+	0	+	A and D	No agglutination with anti-B	Not needed
0	+	0	B	No agglutination with anti-A	Not needed
0	+	+	B and D	No agglutination with anti-A	Not needed
+	+	0	A and B	No agglutination with anti-D	Not needed
+	+	+	Typing cannot be interpreted	Agglutination with anti-A, anti-B and anti-D	Reagent control needed to determine the ABO and D typing

(+: agglutination, 0: no agglutination)

Antibody Potentiators or Enhancement Media/Reagents

Red blood cells (RBCs) have a negative charge at this surface which makes RBCs to repel each other. This natural repulsive force which holds the RBC apart is called zeta potential (refer page 14 of Chapter 1). This is protective and keeps RBCs from adhering to each other in the peripheral blood. In a physiologic saline solution, the zeta potential or the force of repulsion between red cells influences the agglutination reaction.

- **IgM antibody molecules** are larger in size, pentameric in shape (refer Fig. 1.3), and have multivalent properties. The **agglutination is facilitated** when the red blood cells have attached **IgM antibodies** and IgM antibodies can easily react in a saline medium.
- **IgG antibody molecules** are smaller in size (refer Fig. 1.4) and less able to span the distance between adjacent red cells generated by the zeta potential. IgG molecules may attach to red cells, but **visible agglutination may not be produced**.

The test methods in transfusion medicine use serum or plasma and red cells (either red cells provided by the manufacturer or saline-suspended red cells). To increase the sensitivity of the test methods/system, various enhancement reagents, or potentiators may be used. These enhancement reagents are commercially available which enhance or speed up the antibody-antigen reaction (Ag-Ab complex formation) in routine tests. They **help in the detection of IgG antibodies by increasing their reactivity**. Enhancement media can reduce the zeta potential of the red cell membrane (Fig. 4.4) in vitro test and promote agglutination. They may enhance antibody uptake (first stage of agglutination) or promote direct agglutination (second stage of agglutination), or serve both functions. They are added to the cell/serum mixture before the 37°C incubation phase. These reagents shorten the incubation time.

Types of potentiators: The major types of potentiators used in the blood bank laboratory are low ionic strength saline (LISS), polyethylene glycol (PEG), and proteolytic enzymes (ficin and papain).

Uses of potentiators: They are commonly used in both antibody screening and identification procedures. They increase the speed and sensitivity of the antibody attachment to the red cell antigen.

Low Ionic Strength Solution (LISS)

The incubation of serum and red cells in a reduced/lower ionic environment **increases the rate of binding of antibody to specific antigen receptor sites on red cells**. In physiologic saline, sodium and chloride ions cluster around the antigen and antibody molecules. Formation of Ag-Ab complex is influenced by the attraction of opposite charges. The clustering of sodium and chloride

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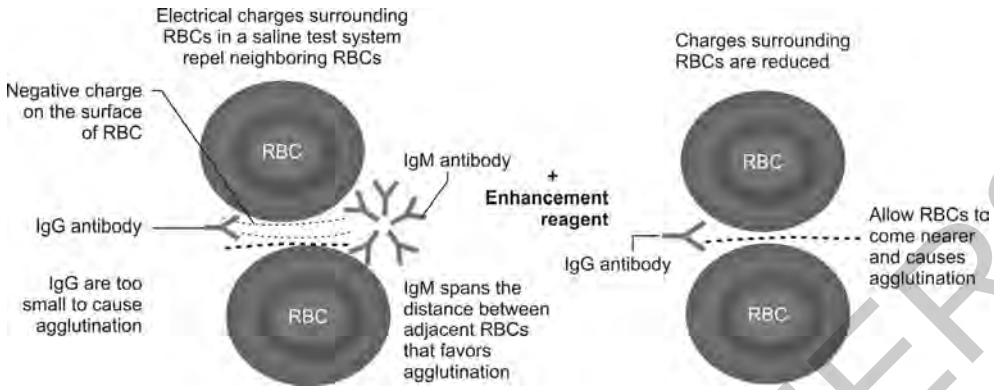

Fig. 4.4: Addition of enhancement reagents can lower the zeta potential and allows better interaction between RBCs and increasing the possibility of agglutination.

ions around Ag and Ab hinders the Ag-Ab complex formation. If the ionic strength is reduced/lowered, the antigen and antibody molecules can combine at a faster rate. The advantage of LISS is that it **increases the antibody uptake and reduces the incubation time and time needed for the result**. LISS reagent **contains sodium chloride, glycine and salt-poor albumin** (approximately 0.03 M) ionic strength compared with saline (approximately 0.17 M). It is formulated to prevent hemolysis of red cells, which is a concern when using LISS. It not only lowers the zeta potential, but also increases the uptake of antibody onto the RBC during the sensitization phase. This increases the possibility of agglutination.

Uses of LISS: It may be used to suspend test red cells for use in tube or column agglutination tests or as an additive medium for tube or solid phase tests. The LISS is **used mainly to enhance antibody uptake and improve detection at antiglobulin phases of testing**, with the antibody screen (speeds the agglutination, economical, and provides good sensitivity).

Disadvantages of LISS: These include (1) increasing serum in the test alters the ionic strength of a LISS procedure, decreasing the sensitivity of the test system and (2) enhances the cold autoantibodies (especially if the tubes are centrifuged at immediate-spin and microscopic evaluations).

Polyethylene Glycol

PEG is **water-soluble polymer** and **used as an additive in a low ionic saline medium**. In LISS solution, the **PEG removes water molecules** from the test system/environment and **allows a greater probability of collision between antigen and antibody molecules**. Thus, it effectively concentrates antibody in the test mixture, while creating a low ionic environment that enhances the rate of antibody uptake. This will concentrate any antibodies present, thereby increases the degree of RBC sensitization. Usually, PEG test systems are more sensitive than LISS, albumin, or saline systems. However, in patients with raised levels of plasma protein (e.g. multiple myeloma), PEG is **not used due to increased precipitation of proteins**. LISS and PEG enhance autoantibodies. Hence, their use in the blood samples containing both autoantibodies and alloantibodies makes their identification difficult.

Uses

- Polyethylene glycol can directly affect the aggregation of red cells. Hence, it can be **used only in indirect antiglobulin testing** (IAT) where it increases the sensitivity. Since, PEG can cause nonspecific aggregation of cells, the tubes used in the test should not be centrifuged after the 37°C incubation and they should be read before washing. Only monospecific anti-

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IgG AHG reagent is suitable for use with PEG because polyspecific AHG reagents can cause nonspecific agglutination.

- Polyethylene glycol **increases the sensitivity of antibody detection and identification**. It often detects the antibodies which are not detected using bovine serum albumin (BSA) or LISS.

Antibodies usually considered as of little clinical significance (IgM type) and do not react well or at all with PEG. PEG can enhance warm autoantibodies.

Enzymes

Proteolytic enzymes are enzymes that **breakdown/denature certain protein molecules. Commonly used** proteolytic enzymes in the immunohematology laboratory that are commercially available are **papain, ficin, and bromelain**. Proteolytic enzymes originate from plants—ficin comes from figs, bromelain used in solid-phase red cell adherence assay (refer page 82) from pineapple, and papain from papaya. These enzymes can **remove the negatively charged molecules from the red cell membrane** and denature certain antigenic determinants. The loss of these negatively charged molecules from the red cell membrane reduces the zeta potential and **enhances the agglutination** of some antigens to their corresponding antibodies. Depending on the red cell antibody, the enzymes used in testing may enhance, depress, or inhibit entirely the Ag-Ab complex formation. Hence, it is necessary to have a good knowledge of the blood group systems in the interpretation of these enzyme tests.

- In Rh, Kidd, and Lewis blood group systems, the enzymes enhance the reaction.
- In red cell antigens such as M, N, S, Xga, Fya, and Fy, the proteolytic enzymes denature the red cell antigens.

Uses: Proteolytic enzymes (ficin or papain) are usually not used as potentiators in the antibody screen because they remove/eliminate some antigens from the red cells. They should not be used as the sole method in antibody screening. However, these enzymes **can be used as additional tools for investigating complex antibody problems**. In a mixture of antibodies, enzymes may enhance the reactions of one antibody or abolish the reactions of another antibody. Thus, it may provide important information in the solution of the problem. **Enzymes enhance cold and warm autoantibodies**.

Bovine Serum Albumin (BSA)

BSA is prepared from bovine serum or plasma. It is available commercially either in 22% or 30% concentration. It is **less commonly used** as a potentiator than other enhancement media. LISS influences both the stages of agglutination (refer page 11 of Chapter 1). In contrast, BSA influences the second stage of agglutination by allowing antibody-sensitized RBCs to come nearer to each other than in a saline medium without additives. BSA's enhancement action is by reducing the zeta potential and disperses the charges.

- **Enhances the sensitivity of the IAT:** The addition of BSA to reaction tubes causes the direct agglutination of Rh antibodies and increases the sensitivity of the IAT (indirect antiglobulin test) for a wide range of antibody specificities. For better results, incubation time may be increased.
- **No enhancement of warm autoantibodies:** BSA does not enhance warm autoantibodies. Thus, it is useful when dealing samples from patients with autoantibodies in their serum.
- **Reduces the repulsion between red cells:** BSA reduces the repulsion between red cells. However, it does not reduce the required incubation time.

Various mechanisms of antibody potentiators are mentioned in Table 4.4.

Lectins

Lectins were first discovered as plant seed extracts but were also found in bacteria to mammals. They are **useful as blood banking reagents because of their specificity toward certain red cell antigens**. Lectins are **used as alternatives to antisera for**

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Table 4.4: Mechanism of antibody potentiators.

Type of antibody potentiator	*Mechanism of action*
Low ionic strength saline (LISS)	Rate of antibody uptake is increased
Polyethylene glycol (PEG)	Concentrates the antibody in the test environment in LISS
Proteolytic enzymes (papain and ficin)	Removes negative charges from the red cell membrane thereby reduces the zeta potential. Denatures some red cell antigens
Bovine serum albumin (BSA)	Reduces the repulsion between cells but the incubation time is not shortened

blood typing. Lectins are sugar-binding proteins of nonimmune origin. They behave **similar to that of antibodies but they are not immunoglobulin** (antibodies) in nature. For example, a lectin from the eel (a snake-like, catadromous fish) *Anguilla anguilla is* a useful anti-H reagent. Lectins bind specifically to the carbohydrate portions/determinants of certain red cell antigens and cause red cells agglutination. Although it does not produce antibodies, they can be used for identifying antigens present on patient or donor red cells. Refer Table 3.5 for the major lectins used in blood group serology.

ABO GROUPING/TYPING

It is advisable to use fully automated systems (where possible) for blood grouping to reduce the risks of interpretation and transcription errors. Blood grouping must be done by a validated technique using appropriate controls. All new batches of grouping reagents must be checked for reliability by the techniques used in the laboratory. Grouping reagents must be stored as per the manufacturer's instructions.

ABO grouping is the single most important serological test done in blood bank during compatibility testing. Hence, it is necessary that there should not be any compromise in the sensitivity and security of the test system. When the **ABO group** of an individual is to be determined, **it is necessary to do both cell grouping and serum grouping**. The naturally occurring antibodies namely anti-A and anti-B in the plasma should be tested against known A and B cells. This process is called "reverse" grouping (refer pages 75, 76). This acts as an excellent built-in check.

Landsteiner's Rule (Law)

It exists **only in the ABO system**.

- **Corresponding antigens and antibodies cannot normally coexist in the same individual's RBCs**.
  - Individuals with group A have A antigen on their RBCs will not normally have anti-A antibodies in their serum.
  - Individuals with group B have B antigen on their RBCs will not normally have anti-B antibodies in their serum.
  - Individuals with group AB with AB antigens on their RBCs will not normally have anti-A and anti-B antibodies in their serum.
- In the ABO system (unlike other blood group systems), **if an individual lacks A or B antigen on the red cell, the corresponding antibody will be found in the serum**. These are the so-called isoantibodies (naturally occurring) usually of IgM type. These antibodies are capable of causing intravascular hemolysis if incompatible blood is transfused.
  - Adults lacking group A antigen will have anti-A antibody in their sera.
  - Adults lacking group B antigen will have anti-B antibody in their sera.
  - Adults lacking group both A and B antigen (O group) will have anti-A or anti-B antibody.

Grouping Procedures

ABO typing procedures consist of two parts:

1. **Forward typing/grouping** (red cell grouping or front type) is defined as **using known sources of commercial antisera** (anti-A, anti-B) **to detect antigens on**

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an individual's RBCs. In this technique, undetermined (unknown) test RBCs (antigen) is typed against known potent and specific antibody or antisera (namely specific anti-A and anti-B).

2. **Reverse typing/grouping** (serum grouping or back type) is defined as **detecting ABO antibodies in the patient's serum by using known reagent RBCs**, namely A_1 and B cells. In this technique, the undetermined/unknown (patient or donor) serum is tested against known group RBC antigens (namely A_1 and group B red cells). It is used to test for the expected ABO antibodies in the patient's serum.

The manufacturer's instructions must be followed for all commercial reagents. Both forward and reverse typing results must match to confirm the true ABO type of an individual. This is an excellent way to guard against errors in ABO grouping. Any discrepancy between the forward and reverse groups should be investigated further.

Interpretation of results of ABO blood grouping is presented in Table 4.5.

Reagents for ABO Antigen Typing/ Blood Grouping

Monoclonal ABO Antibody Reagents

- ABO antibody reagents needed for ABO typing include monoclonal anti-A, anti-B and anti-A,B (ABO antibody). These commercial antisera reagents contain monoclonal antibodies obtained from cultures of cells secreting antibodies called hybridomas (refer pages 63, 64).
- Before this technology, polygonal ABO grouping reagents were used that are obtained from human donors with or without deliberate immunization.
- The advantages of monoclonal ABO reagents over polyclonal reagents include: (1) more specific, (2) more potent, (3) more batch to batch consistency, (4) absence of nonspecific antibodies, and (5) free from viruses (e.g. HIV and hepatitis virus).
- Anti-A and anti-B reagents are used to determine whether an individual's red cells have the A and B antigens of the ABO blood group system. These reagent ABO antibodies are suspended in a diluent with a concentration not exceed a 6% bovine albumin concentration and is considered a low-protein medium.
- ABO monoclonal antibody reagents are prepared in such a manner that they produce strong reactions with antigen-positive red cells and can detect weak expressions of the A and B antigens. The anti-A and anti-B red cell typing reagents produce strong agglutination (refer Table 1.4) reactions (3+ to 4+) for most antigen-positive red cells.
- Test is performed in immediate-spin phases (refer page 74) and it is recommended that test should be confirmed by checking for expected ABO antibodies using reagent red cells. In immediate-spin phases, the source antigen and source antibody used in immunohematologic testing are combined. It is immediately centrifuged, and observed for agglutination.
- **Coloring dyes for reagents:** Dyes were added to these ABO antibody reagents in order to reduce potential errors in testing.
  - Anti-A always contains a blue dye
  - Anti-B contains a yellow dye

Table 4.5: Interpretation of results of ABO blood grouping.

Forward typing (red cell grouping)		*Interpretation of ABO group*	*Reverse typing (red cell grouping)*	
Anti-A	*Anti-B*		*A_1 cells*	*B cells*
++++	–ve	A	–ve	++++
–ve	++++	B	++++	–ve
++++	++++	AB	–ve	–ve
–ve	–ve	O	++++	++++

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  - Anti-A,B is a blending of clones for A and B antigen recognition and the reagent is clear.
- **Reagent labels:** ABO typing reagents are labeled with the antibody specificity and specify how it can be used. For example, a reagent bottle of anti-A may contain the phrase "**for slide, tube, and microplate testing**".
- **Product insert:** The detailed methods are provided in the product insert.

Known ABO Blood Group Red Cells

A_1 and B cells are used for reverse grouping; group O cells or an "autocontrol" may be included to ensure that reactions with A and B cells are not due to the presence of cold autoantibodies. Known red cells used in ABO grouping are obtained from pooled A cells, B cells, and O cells. The red cells are washed in saline to remove serum, plasma, hemolyzed cells and small clots which may give rise to false-positive reactions. The supernatant of final wash in saline should be clear. A 2–4% red cell suspension for tube, microplate typing techniques and 30–40% for slide technique is used. A diluent control should be included where recommended by the manufacturer.

Supplementary Reagents

With routine use of monoclonal reagents for blood grouping, the necessity of some of supplementary reagents has been reduced. The supplementary reagents are mainly used for testing the suspected subgroups. The main supplementary reagents used in ABO grouping include: anti-A_1 lectin, anti-H, monoclonal anti-A, and anti-B. Anti-A,B may be used to distinguish O group from A subgroups.

- **Anti-A_1 lectin (Fig. 4.5):** It is obtained from seeds of *Dolichos biflorus*. It reacts strongly only with A_1 (even if found in small quantities) individuals and is **used to differentiate between A_1 and A_2 subgroups**.
- **Anti-H (Fig. 4.5):** It reacts selectively with ABO group individuals with H substance. **Group O individuals** which contain only H **react strongly with anti-H**, whereas a group A_1B individual contains very little H and thus reacts very weakly or negatively with anti-H.



Fig. 4.5: Anti-A_1 lectin and anti-H reagents.

- **Group "O" reagent screen cells:** They consist of two or three group O red cells with various antigen specificities. They are used in some donors **to detect antibodies other than anti-A or anti-B**. These antibodies do not occur naturally and are termed as **irregular antibodies**. They occur due to immunization either by prior transfusion or pregnancy. They help on ruling out ABO typing discrepancy caused by cold antibodies.
- **Group A_2 and group A_2B red cells:** These may be used to resolve discrepancies of ABO groping. A_2 red cells are used when suspected A subgroup has develops anti-A_1. Agglutination of reagent A_1 cells by patient serum, but without agglutination of A_2 cells suggests presence of anti-A_1.

METHODS OF ABO GROUPING

In ABO grouping technique (method or procedures), donor or patient red blood cells (unknown antigen) are combined with commercial antisera (known antibodies) and observed for the presence or absence of agglutination. Presence of agglutination indicates the presence of corresponding antigen and absence of agglutination indicates the absence of corresponding antigen on the red cells tested (Tables 4.5 and 4.6).

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Table 4.6: Interpretation of ABO grouping.

Reaction with antisera added			*Interpretation*
Anti-A	*Anti-B*	*Anti-A,B*	*ABO blood group antigen and phenotype*
0	0	0	O
+	0	+	A
0	+	+	B
+	+	+	AB

(+: agglutination; 0: no agglutination)

Box 4.2: Various methods for ABO blood grouping.

- Manual methods
  - Traditional methods: Slide or tile technique
  - Tube technique
  - Microplate method
  - Column agglutination technique
    - Gel technology (ID Micro Typing System)
    - Glass microbead technology
  - Solid phase red cell adherence assay
  - Erythrocytes magnetized technology
- Automated or semiautomated method
- Molecular blood grouping and genotyping

The **procedure to determine ABO blood group system is called as ABO grouping**, ABO forward grouping, front typing, and ABO red cell testing. The Technical Manual of the AABB refers the procedure to the determination of an individual's ABO blood group as ABO typing (phenotype) and not an ABO grouping. However, many other references retain the terminology of ABO grouping and the FDA name for these reagents remains ABO grouping reagents.

Methods: Different methods available for routine ABO blood grouping are listed in Box 4.2. It is necessary to use the appropriate reagent, because not all reagents have been validated by the manufacturer for all techniques. Molecular techniques for ABO blood grouping are not used routinely since serology is superior, quicker, cheaper and accurate.

Forward Typing/Grouping

The basic principles of all the methods are same (except molecular blood grouping and genotyping). They are always performed at room temperature and agglutination is observed against well-lighted background. ABO grouping is carried out by making a 2% saline suspension of red cells and adding anti-A, anti-B, and anti-A,B sera. The slide and tube techniques are described here.

Slide or Tile Technique

Slide or tile grouping is **not very sensitive method**. It is performed only for emergency ABO grouping especially in outdoor blood camps and **used in under-resourced or developing countries**. The technique is satisfactory if potent grouping reagents are used.

Disadvantages: (1) Insensitive, (2) cannot detect weak antigens in forward typing and low-titer antibodies in reverse typing, (3) it dries up due to evaporation (hence must be read within about 5 minutes) and (4) needs confirmation either by tube or microplate technique.

Materials required:

- Clean glass slide or white tile
- Reagent antibody namely anti-A, anti-B, and anti-A,B sera
- Test red cells (30–40% suspension)
- Applicator sticks.

Method (Fig. 4.6)

- Take a slide/white tile and label to identify the cells used.
- Put one drop each of anti-A, anti-B, and anti-A,B sera on the marked slide/tile.
- Add one drop of 30–40% red cell suspension to each of anti-A, anti-B, and anti-A,B sera (not shown in Figures 4.6 and 4.7) on the marked slide/plate.
- Mix the cells and reagent antisera with clean applicator stick and spread the mixture over an area of 2 cm.
- Rock the slide or tile gently and look for "agglutination".
- Record the results within 2 to 5 minutes.

Interpretation: ABO blood is interpreted depending on the agglutination in the antisera

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