

Unit 8 Pretransfusion Compatibility Testing

- A. The purpose of pretransfusion testing is to select, for each recipient, blood components that, when transfused, will have acceptable survival and will not cause clinically significant destruction of the recipient's own rbc's.
1. Prevent transfusion of incompatible donor RBCs that may result in immune-mediated hemolytic transfusion reaction.
 2. US federal government regulates pretransfusion testing by authority of CLIA, regulated tests include: ABO Group, Rh type, antibody detection, antibody identification and compatibility testing.
- B. AABB Standards states that the following procedures **must** be part of pretransfusion compatibility testing.
1. Positive identification of recipient and recipient's blood sample.
 2. Review of transfusion service records for results of previous testing on samples from the recipient.
 3. Tests on donor blood as described in the AABB Technical Manual.
 4. ABO and D typing of recipient's blood.
 5. Antibody detection tests using the recipient's serum or plasma.
 6. Selection of blood components of the **appropriate** ABO and D types.
 7. Tests with recipient's serum or plasma and donor's RBCs, ie, a crossmatch.
 8. Labeling of the components with the recipients information.
- C. Pretransfusion Testing
1. Transfusion is usually a beneficial and safe procedure but adverse affects can occur.
 - a. Donor RBCs, or less frequently, recipient's RBCs, sometimes undergo accelerated destruction.
 - b. Most **hemolytic** transfusion reactions result from **errors in patient or sample identity**.
 - c. In some cases blood group antibodies exist that were not detected by standard serological techniques. Pretransfusion testing **will not** detect **all** unexpected RBC antibodies in the recipient's serum.
 - d. Pretransfusion testing **will not** guarantee normal survival of transfused RBCs.
 2. If performed **properly**, pretransfusion tests **will**:
 - a. Ensure that a patient is issued the designated blood components.
 - b. Verify in **most** cases that blood components are ABO **compatible**.
 - c. Detect **most** clinically significant **unexpected** antibodies.
- D. Modern Blood Banking Practices
1. During the last 20 years there has been a distinct change in the attitudes about pretransfusion testing.

2. Compatibility testing has undergone considerable modification since the development of the antiglobulin test.
3. The early 1980's became a time for decisions to eliminate portions of standard testing because of the restricting economic climate. This environment stimulated the philosophy that no testing should be done unless the results are likely to influence patient care.
4. Ongoing reevaluation and prioritization has resulted in the elimination of many practices.
5. Procedures have been streamlined, both in the interests of cost effectiveness and patient safety, to the point that virtually all unnecessary practices have been discarded.
6. This intelligent approach carries with it the responsibility for precise attention, without variation, of the laboratory professional. Requires **rigid** adherence to guidelines and standards.
7. Protocols have been developed based on a balance between the following:
 - a. Patient safety.
 - b. The number of unwanted reactions eliminated (such as cold agglutinins).
 - c. The speed with which test procedures can be performed.

E. Transfusion Request Forms

1. Oral, electronic or written format acceptable.
2. Two identifiers required: Patients first and last name, unique identification number.
3. Blood request forms **must** contain sufficient information for positive patient identification.
4. Because blood is a drug, and for medical/legal reasons, the name of the physician should appear on the requisition form.
5. Additional information such as sex and age of the patient, diagnosis, and transfusion and pregnancy , special component requirements (irradiated, CMV negative) may be helpful.
6. Blood request forms lacking the required information or containing illegible information **are not** acceptable.
7. Telephoned requests should be documented by subsequent submission of a properly completed blood request form.

F. Specimen Requirements

1. The pretransfusion compatibility test is only as good as the blood sample on which it is performed.
2. The collection of a properly labeled blood sample from the **correct** patient is **critical** to safe blood transfusion.
3. The person who draws the blood sample **must** identify the patient in a positive manner using TWO PATIENT IDENTIFIERS.
 - a. It is best to only allow people adequately trained to appreciate the importance of proper identification to draw samples for compatibility testing.
 - b. Phlebotomist's identification must be on tube.
 - c. Sample must be labeled in presence of patient.

4. The lab requisition must be compared to the information on the patient's hospital armband. If the patient does not have an armband on **the specimen must not be drawn** until the patient is banded or identified in an acceptable manner according to institution policies.
5. In an emergency situation, when the patient's identity is unknown, an emergency identification number or a temporary band is attached to the patient in accordance with the institution's SOP.
6. Preadmission testing (PAT) poses a special problem, patient's may forget their wristband at home. If this happens, the patient must be redrawn and all work repeated with the new sample.
7. Blood samples must be drawn into stoppered tubes and **labeled in the presence of the patient using information from the patient's armband**. Manual method or imprinted labels may be used. Minimum information required includes: patient's full name, identification number, date, time and identity of the individual drawing the specimen.
8. Many institutions utilize commercial blood bank armband systems.
9. ***Serum or EDTA plasma*** may be used for pretransfusion testing. Serum is preferred because with plasma, small fibrin clots may sometimes form which may make it difficult to distinguish agglutination.
 - a. Antibodies demonstrable only through complement activation cannot be detected if plasma is used. Anticoagulants, such as EDTA, **chelate calcium and prevent complement activation**.
 - b. It is permissible to collect blood from an IV line as long as proper protocol is followed.
 - c. Hemolyzed samples **should not** be used.
10. Compatibility tests **must** be performed on blood samples collected within **three days** before red cell transfusions when the patient has been transfused or pregnant within the preceding 3 months **or** if the history is uncertain or unavailable, day of collection is day 0, can be used until 11:59pm day 3.
 - a. The sample used for serological testing must represent the patient's current immunological status.
 - b. It is not possible to predict when such antibodies will be demonstrable, so a 3 day limit has been arbitrarily selected.
 - c. Many blood banks prefer to set a 3-day limit on all specimens used for pretransfusion testing.
 - d. Exceptions may be approved when a patient has not been recently transfused or pregnant.
11. Requirement for samples from **infants less than 4 months old are different** and will be discussed in detail later. But they do have major exceptions.
 - a. If there are no unexpected antibodies detected by initial tests **and** the infant receives no blood components containing clinically significant antibodies, **antibody detection and crossmatching tests can be omitted throughout the neonatal period**.
 - b. After the infant's ABO and D types have been determined, ABO and D typing **may be omitted, provided the baby receives only RBCs that are of the infant's ABO or group O, and are either of the infant's D type or D negative**.

12. When a sample is received in the laboratory, a qualified member of the staff must confirm that the information on the label and on the transfusion request form **are identical**.
 - a. If there is any discrepancy or any doubt about the identity of the patient, a new sample **must** be obtained.
 - b. It is **unacceptable** for anyone to correct an incorrectly labeled sample.
 - c. Study found mislabeled samples 40 times more likely to have blood group discrepancy.
13. The recipient's blood specimen and a sample of the donor RBCs must be sealed or stoppered and kept at 1-6 C for at least **seven days after transfusion**.
 - a. Donor RBCs may be from the remainder of the segment actually used in the crossmatch and must be placed in a sealed or stoppered tube.
 - b. A donor segment removed just before issuing the blood may be saved.
 - c. Keeping the patient's and donor's samples makes it possible to do repeat or additional testing if the patient experiences an unfavorable response to the blood transfusion.

G. Previous Records

1. Compatibility testing **must** include checking previous transfusion service records for the recipient's serological history.
2. If the patient has been tested previously, results of current testing **must** be compared with interpretation of previous testing.
3. Discrepancies between current and previous testing **must** be resolved before blood is issued.
4. The most significant information to be obtained from the records is the existence of clinically significant antibodies reactive at 37 C or AHG.
 - a. The specificity (identity) of previously identified antibodies should be compared with that of antibodies detectable currently.
 - b. If an individual has ever had a clinically significant antibody identified, antigen negative blood must be selected, even if the present antibody screen is negative.

H. Serological Testing - *current mandated tests* for pretransfusion samples include the following:

1. ABO Grouping
 - a. Testing unknown RBCs with reagent anti-A and -B.
 - b. Testing unknown serum with reagent A₁ and B RBCs
 - c. Discrepancies must be resolved before blood is given.
 - d. In an emergency situation give the universal red cell donor group O or if plasma is needed AB.
2. D Typing
 - a. Testing patient/donor cells with reagent anti-D by direct agglutination. NOTE: D typing of D positive donor units not required by transfusion service.
 - b. Weak D test not required for patients or retyping donors by transfusion service.
 - b. Tests with anti-D **must be controlled** to avoid incorrect interpretation.
 - c. If a problem arises in the interpretation transfuse with D negative until problem is resolved.

- d. It is not necessary to perform the weak D test on recipients. Many facilities will perform this test one time to determine if the patient is a weak D.
3. Antibody Detection Tests
- a. Standards states that the serum or plasma of a recipient must be tested against single-donor suspension of cells selected to carry those blood group antigens necessary for the detection of the most important clinically significant unexpected antibodies.
 - b. ***Unexpected antibodies are those other than anti-A or anti-B.***
 - c. ***An antibody is considered clinically significant if:***
 - 1) it has caused hemolytic disease of the fetus and newborn (HFDN)
 - 2) it has caused an acute hemolytic transfusion reaction
 - 3) it has caused unacceptably shortened survival of transfused RBCs.
 - 4) it is reactive at 37 C and/or AHG.
 - d. IgG-coated RBCs must be used to detect false-negative antiglobulin tests due to inactivation of the Coomb's serum.
 - e. The reagent red blood cells suitable for antibody screening are available commercially and are offered as a set of two or three vials, each containing RBCs from a single group 0 donor.
 - 1) The following antigens **must** be present: D, C, E, c, e, M, N, S, s, P¹, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a and Jk^b.
 - 2) No requirements that other low incidence antigens be present, such as Lu^a, V or C^w.
 - 3) No requirements that homozygous cells be present.
 - 4) Must not be used beyond expiration as some antigens deteriorate during storage.
4. Type and Screen (T&S) Protocol
- a. The T&S procedure has emerged as an acceptable alternative to crossmatching blood.
 - b. The protocol consists of performing an ABO, D and indirect antiglobulin test (IAT or antibody screen) for the presence of alloantibodies.
 - c. When performed correctly the Type and Screen will detect **99.9%** of all unexpected antibodies.
 - 1) When antibodies **are** detected in a patient serum it is identified and appropriate antigen negative units are crossmatched.
 - 2) If the antibody screen is negative blood **is not** crossmatched but can be available immediately if needed.
 - d. This protocol has been established as a **safe and cost-effective** measure in promoting effective use of the blood supply by reducing unnecessary crossmatching of blood.
 - e. Patients who are candidates for this protocol are *obstetrical and pre-operative*. Procedures selected are those for which the risk of excessive bleeding are minimal but possible.
 - f. If unusual complications produce an immediate need for blood the patient can receive ABO and D type specific blood, IS crossmatch compatible within 10 minutes.
 - g. The transfusion service must have appropriate donor blood available for all patients undergoing operations on a Type and Screen basis.

5. Limitations of Antibody Detection Tests - Antibody screening tests *cannot* detect all antibodies of potential clinical significance.
 - a. Antibody may be reactive with low incidence antigen absent on screen cells.
 - b. If antibody is exhibiting "dosage" it may be missed. Duffy (Fy), Kidd (Jk) and Rh antibodies may only be detected with homozygous cells. Will influence decision to use 2 or 3 cell screen.
 - c. Antibody may have dropped below the level of detectability.
6. Compatibility Testing - History
 - a. In the early 1980's questions arose about the following: the routine use of anti-A,B antisera and A² cells in ABO grouping, repeat D typing of D positive donor units, weak D testing, repeat alloantibody screening of donor units, DAT testing and the performance of elution.
 - b. Questions were raised as to the clinical significance of antibodies reactive at RT or below, the usefulness of albumin in the reaction mixture, and the appropriateness of using AHG in both the antibody screen and the crossmatch.
 - c. During 1984-85, the FDA and AABB allowed the AHG phase of the crossmatch to be deleted **if the patient's antibody screen was negative**.
 - d. In 1984, Judd recommended deleting the autocontrol as a part of routine transfusion testing.
 - e. By 1986, the minor (testing donor plasma with patient cells) crossmatch was of historic interest only.
7. Compatibility Testing - **the Coomb's Crossmatch**
 - a. Patient's who are experiencing clinical signs and symptoms of anemia, actively bleeding patients and patients who are having surgical procedures with anticipated blood loss must have blood crossmatched.
 - b. In the major crossmatch, patient serum is added to each potential donor cell (as in an antibody screen) and read after three phases: IS, Albumin 37 C, and AHG. These tubes are set up and read along with the antibody screen.
 - c. Reactivity at any phase was evaluated. Donor cells reactive at 37 C, AHG or causing hemolysis would require an interpretation of "**incompatible**" and **could not** be issued for transfusion.
8. Compatibility Testing - **the Immediate Spin Crossmatch or Abbreviated Crossmatch**
 - a. When no clinically significant antibodies are detected in the antibody screen, and there is no history of such antibodies, the antiglobulin phase of the crossmatch is not required.
 - b. Rarely is a clinically significant unexpected antibody detected by the AHG phase of the crossmatch when the antibody screen is negative.
 - c. Tests must be done to demonstrate ABO incompatibility **are required** which is documented by performing the IS phase of the crossmatch and discard the tubes after recording.
 - d. The policy to omit the AHG phase of the crossmatch must be made by the **medical director**.

- e. The decision to omit the AHG phase should be based on the following:
 - 1) Incidence of incompatible crossmatches when the antibody screen is negative and the reasons.
 - 2) Sensitivity of antibody detection procedure used.
 - 3) Potential benefits of omitting the AHG phase in the laboratory.
 - 4) Expertise of the individuals working in the transfusion service.
- f. If clinically significant antibodies are detected in the antibody screen, **the AHG phase of the crossmatch is required.**

9. Compatibility Testing - **the Computerized Crossmatch.**

- a. If a computer system has been validated on site to prevent the release of ABO-incompatible blood components, then it may be used prior to transfusion to detect ABO incompatibility instead of a serologic crossmatch **when the antibody screen is negative.**
- b. The following conditions **must** be met:
 - 1) There have been 2 determinations of the recipient's ABO group. One determination must be made on a current sample. The second determination may be made on the same sample, a second current sample or with previous records.
 - 2) The computer system contains the donor unit number, the component name, ABO and D types of the component, blood confirmatory donor type, the ABO and D types of the recipient, two unique recipient identifiers and antibody screen results.
 - 3) There is a method to ensure correct data entry.
 - 4) The system contains logic to alert the user to discrepancies between donor unit labeling and blood group confirmatory test interpretation and to ABO incompatibilities between recipient and donor unit.

I. Optional Pretransfusion Testing - testing of patient blood for transfusion may **optionally** include the following:

1. ABO Grouping

- a. Red cells tested with anti-A,B
- b. Serum/plasma tested with A² cells.

2. D Typing

- a. Weak D tests on patients.
- b. Rh control with chemically modified reagents unless patient is AB positive.

3. Antibody Screening (IAT)

- a. RT incubation
- b. Additives, such as albumin, LISS
- c. Enzymes
- d. Polyspecific AHG in the IAT

4. Autocontrol or DAT

- a. Data published by Judd et al indicate that performing an auto control or DAT as part of routine pretransfusion testing may be of limited value, even when the patient has been recently transfused.

- b. Standards does not require an auto control or DAT.
5. Microscopic reading of tests, a magnifier viewing lamp is adequate.
 6. Crossmatch
 - a. 37 C and AHG testing when antibody screen is negative, with no previous history of clinically significant alloantibodies.
 - b. RT incubation
 - c. Enzyme tests
 - d. AHG with polyspecific AHG
 - e. Minor crossmatch.

J. Selection of Units

1. Whenever possible patients should receive blood components of their own **ABO group**. When this is not possible, components of alternative ABO groups may be selected. The following is a list of acceptable alternatives **in the order of selection**:

ABO GROUP OF RECIPIENT	ABO GROUP OF DONOR
O	O only
B	B, O
A	A, O
AB	AB, A, O, B

2. **D positive blood** should be selected for D positive recipients. D negative blood is acceptable but, except for the following circumstances, should be reserved for D negative recipients. Some exceptions are:
 - a. D negative donor unit which will expire soon can be given to D pos "sure give".
 - b. Multiple antibodies present in the patient serum which the D negative unit lacks.
3. **D negative components** should be selected for D negative recipients to avoid immunizing the patient to the D antigen. If ABO compatible D negative components are not available there should be consultation with the blood bank medical director and the patient's physician, who may prefer postponing the transfusion.
 - a. If transfusion is urgently required first utilize ABO compatible D negative.
 - b. If there is no alternative, give D positive, the risk of immunization must be weighed against the potential loss of the patient's life.
 - c. Depending on the child bearing potential of the patient it may be appropriate to administer Rh Immune Globulin to recipients of D pos components (ie, platelets).
4. **Blood administered after non group specific transfusion** cannot be done until the following criteria is met:
 - a. Status of anti-A and/or anti-B in current sample of the recipient's blood.
 - b. When serum from a freshly drawn sample is compatible at the AHG phase of the crossmatch with the recipient's own ABO group, group specific blood may be issued.
 - c. If the AHG crossmatch is incompatible due to ABO antibodies, continue transfusing with RBCs of the alternative compatible ABO group.

- d. If the change involved only the D type, change back to D type specific

5. Other blood groups

- a. It is not necessary to select units on the basis of other blood groups *unless the recipient has a clinically significant unexpected antibody*.
- b. If the antibody is strongly reactive, screen donor units with recipient serum, confirm with reagent antisera.
- c. If the antibody is weakly reactive use commercially prepared antisera to confirm that compatible units lack the antigen(s) in question.
- d. When commercially prepared reagents are not available, raw patient serum or donor antisera can be used.

K. Antibody Detection Techniques

1. Most common technique is to test serum of potential transfusion recipient with 2 or 3 commercially prepared group O cells.
 - a. Reagent screen cells contain a preservative but expire within a few weeks of preparation.
 - b. With each set of screen (or panel) cells an analysis of the antigens present (antigen profile or "antigram") is included.
 - c. Care must be taken that the lot number of the antigram correspond with the lot number on the screen cells.
 - d. Patient serum is added to screen cells and observed for agglutination and/or hemolysis at 3 phases:
 - 1) RT IS,
 - 2) after incubation at 37 C with enhancement media, and
 - 3) after washing and addition of AHG reagent.
 - e. If agglutination and/or hemolysis is observed in the screen cells, the temperature and mode of reactivity suggest which blood group system(s) are the most likely to be involved.
2. Before deciding upon routine procedures for antibody detection the blood bank director must decide which antibodies are considered "significant".
 - a. This decision must be given careful consideration if the AHG phase of the crossmatch is not routinely done.
 - b. Once a procedure has been adopted, the method must be described in the Standard Operating Procedure (SOP) manual and each staff member **must know and follow the directions as written**.
3. The antibody detection method selected to base donor selection on should:
 - a. Detect as many clinically significant antibodies as possible.
 - b. Not detect clinically insignificant antibodies.
 - c. Allow prompt delivery of blood to the recipient.

4. The antibody detection technique should be of sufficient sensitivity to detect very low levels of antibody in the recipient's serum or plasma.
 - a. Undetected low levels of antibody in a recipient specimen may result in rapid production of antibody if antigen positive cells are transfused.
 - b. Antibody present in **donor** plasma will not harm a recipient.
5. Methods selected for antibody detection and crossmatching tests may be the same or they may differ, for example:
 - a. RT tests such as IS crossmatch may be preferred to detect ABO incompatibilities, but may not be included in antibody screening tests.
 - b. The antibody detection method **must** demonstrate clinically significant unexpected antibodies and **must** include the AHG test.
 - c. The crossmatch **must** demonstrate ABO incompatibility but the AHG is not required.
6. All personnel in a laboratory should use the same interpretations and notations and be consistent in grading reactions.
 - a. Consistency in grading is especially important.
 - b. Hemolysis or agglutination constitutes the visible endpoint of an RBC antigen-antibody interaction and must be observed accurately and consistently.
 - c. Using a light source and optical aid enhances the sensitivity and consistency.
 - d. Since both hemolysis and agglutination are possible, the supernatant fluid should always be observed for free hemoglobin **immediately** after centrifugation, then **gently** disperse the RBCs and observe for agglutination.
 - 1) The manner in which the RBCs is dislodged from the bottom of the tube affects detection of agglutination.
 - 2) The tube should be held at an angle so that the fluid cuts across the cell button as the tube is gently tilted.
 - 3) The reaction strength (or grade) should be determined when **all** cells have been resuspended.
 - 4) Over shaking may break up large agglutinates or disperse weakly cohesive agglutinates.
 - 5) The strength of agglutination or degree of hemolysis observed with each cell sample should be **RECORDED AS EACH TUBE IS READ.**
 - e. Microscopic observation is useful in distinguishing rouleaux from true agglutination and detecting specific patterns of agglutination characteristic of some antibodies, such as MF pattern seen with anti-Sd^a. Routine microscopic observation is not required.

L. General Considerations

1. Labeling Tubes

- a. **Each tube for serological testing must be labeled *before use*.**
 - b. Use the recipient's initials (or other identifying information) and the donor unit number or reagent RBC identification.
2. You should **never** rely on the position of a tube in a rack or centrifuge head to identify the contents of a tube.
- a. It **is** good technique to put tubes in the serofuge in the order in which you will read and record reactions.
 - b. It is important to use the same organizational technique when arranging tubes in the rack to improve organization and speed.

3. Volume of Serum or plasma

- a. Most serologic procedure call for 2 drops of serum or plasma.
- b. Some researchers have proven that 2 drops is insufficient to provide an optimum ratio of antibody to antigen in some cases.
- c. Some alloantibodies were detectable only when the volume of serum was increased to 3 or 4 drops. Antibody present in low concentration will be picked up when the serum to cell ratio is increased.
- d. There is tremendous variability in the delivery volume of pipettes and reagent dropper (pipette drops small and highly variable, dropper drops tend to be too large).
- e. It is important to standardize the volume of serum and RBCs used in routine test systems based on the pipettes and reagents utilized in **your** particular laboratory especially if low-ionic reagents requiring equal volumes of serum and reagent are used.

4. Cell Suspension

- a. RBCs used for crosssmatching should be obtained from sealed segment of original tubing attached to blood container.
- b. Wash once and prepare 2 - 4%. Many workers prefer a 2% cells suspension.
- c. Best to use weakest cell suspension that can be observed for agglutination.
- d. If cell suspension is too heavy weak antibodies will be missed.

M. Testing Techniques

1. **Saline tube tests** are the simplest serological techniques.

- a. Recipient serum or plasma mixed with saline suspended RBCs. Centrifuged immediately (IS) or incubated at RT or 37 C. May set up 2 sets of tubes, one for RT and one for 37 C.
- b. In crossmatching test is used to detect ABO incompatibility.

- c. In antibody tests is used to detect IgM antibodies which react predominantly at RT:
anti-M, -N, -Pⁱ, -Le and -I.
 - d. Rare examples of antibodies of other specificities may be observed, but more often will be reactive at 37 C or AHG as well.
2. **Bovine albumin tube test** has been utilized to enhance direct agglutination of RBCs by antibodies of the IgG class since 1945.
- a. Albumin is added to the serum-cell mixture prior to incubation at 37 C.
 - b. Decreases amount of time required for incubation.
 - c. There is still controversy as to **how** it increases the uptake, whether it reduces the zeta potential (electrical charge between cells), or whether it is due to a function of the ionic strength of the albumin **diluent**.
 - d. Many antibodies have enhanced reactivity when albumin is added to the test system.
3. **Low Ionic Strength Saline (LISS) tube test** conditions shortens the incubation time required for the detection of most antibodies.
- a. Antibody uptake and degree of agglutination are enhanced.
 - b. Several important factors must be considered when using LISS reagent or additives.
 - 1) The shortened incubation period and enhanced sensitivity in subsequent AHG tests depend upon attainment of the desired ionic conditions.
 - 2) Adding additional serum to the test system will **increase** the ionic strength of the mixture.
 - 3) It is of **critical importance** to adhere to the procedure recommended by the manufacturer.
4. **Polyethylene Glycol Test (PEG) tube test**
- a. Water soluble, neutral polymer that has been shown to be an effective potentiator of antigen-antibody reactions.
 - b. Advantages over albumin include:
 - 1) increases the rate of detection of clinically significant RBC antibodies.
 - 2) Decreases detection of insignificant antibodies.
 - 3) May decrease the need for other serologic enhancement techniques.
 - c. The test is read at IS saline, PEG is added, and immediately after incubation, the test is washed. There is no 37 C reading.
5. **Enzyme techniques** (tube) are more appropriately used for antibody identification than for routine antibody detection during pretransfusion testing.
- a. They are especially useful when increased sensitivity is desired, as in investigation of delayed hemolytic transfusion reactions when antibodies are not detected by other methods.
 - b. **Enzymes greatly enhance the reactivity of Rh antibodies.**
 - c. Enzymes **cannot** be the only detection method utilized because **M, N, S, Fy** and certain other antigens are usually destroyed so that antibodies to those antigens would not be detected.

- d. The enzymes utilized in the blood bank include: *papain, bromelain, trypsin and ficin*. Ficin treated cells are available commercially.

6. Gel Technology

- a. Gel technology is based on the principle of controlled centrifugation of red cells through a dextran-acrylamide gel.
- b. Testing is performed in a prefilled card containing dextran acrylamide gel particles combined with diluent or reagent.
- c. Card or strip of several microtubes are filled with a mixture of gel, buffer, and reagent .
- d. Depending on the test to be carried out, either a neutral gel containing no reagents or specific gel containing reagents (eg, antiglobulin or anti-A, B, D, etc) may be used.
- e. At the top of the gel the suspension of patient red cells or a mixture of patient or commercial red cells with patient or reagent serum (for reverse ABO typing or antibody screening/identification) is added, followed by centrifugation through the gel under precise conditions.
- f. In negative reactions, there will be no attachment of antibodies to red cells, and these will freely pass through the gel and pellet down in the bottom of the microtube.
- g. Conversely in positive reactions, the red cells are trapped in the gel in various degrees because of either the size of the aggregates or other physical forces, with the strongest reaction giving minimal to no observable migration with most or all red cells remain at the top of the gel microcolumn (4+). The picture below, from left to right, would be graded 4+, 3+, 2+, 1+ and negative.



- h. Simply pipette, incubate, spin and read- no washing or tube shaking.
- i. Sensitivity of 98% when compared to LISS tube method.
- j. Results can be saved for peer review or documented with a photo
- k. Simplifies cross-training, improves productivity and workflow efficiency.
- l. Used for antibody detection and identification as well as ABO, Rh and typing for other blood group antigens.

7. Microplate

- a. For routine processing of DONOR blood.
- b. Microtiter plate with 96 wells.
- c. Adapted to RBC or serum reverse testing.

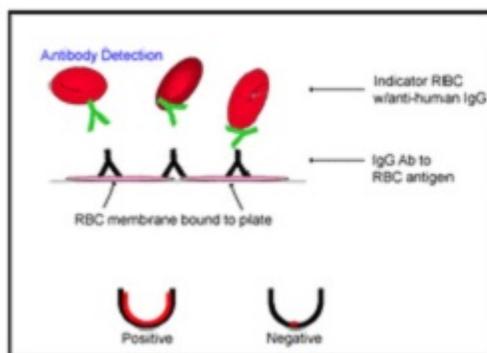
- d. Add appropriate sample, centrifuge, dislodge button and inspect for agglutination.



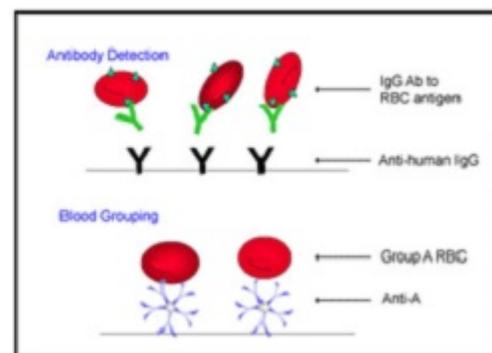
8. Solid Phase adherence

- a. RBC antigens or antibodies are immobilized (coated) to microplate wells to detect antigen-antibody interactions.
- b. Intact RBCs or RBC membranes of known phenotype.
- c. Add patient serum or plasma.
- d. Incubate and wash.
- e. Antibody coating antigens, if present, will attach to IgG on coated cells added to each well.
- f. Centrifuge, agglutinates will coat well, NO agglutination, cells pellet at bottom.

Solid Phase Adherence Test-1



Solid Phase Adherence Test-2



9. Automated Testing Platforms

- a. Perform multiple tests using solid phase, column agglutination, microtiter plate or other technologies.
- b. All testing processes from pipetting to result interpretation, performed under computerized control using barcode technology.
- c. Usually interfaces with laboratory information system for reporting results.

10. **The Antiglobulin technique** is **required** when testing samples from recipients for the presence of unexpected antibodies and, in some cases discussed previously, for serological compatibility with RBCs from donors.

- a. Either anti-IgG or polyspecific AHG reagent may be used for the AHG phase.
- b. The use of polyspecific has the advantage of detecting the rare clinically significant antibodies which may only be detectable because they activate complement, *these have Kidd (Jk) blood group specificity*.
- c. The disadvantage of using poly routinely is that clinically insignificant "nuisance" cold reactive auto-antibodies such as anti-I or -IH are detected.

- d. The director of the transfusion service may consider that the potential benefits of polyspecific reagents in detecting rare complement-dependent antibodies are not worth the amount of time expended in resolving problems caused by these antibodies.
- e. The decision on which AHG reagent to use is based on the incidence at which clinically significant antibodies are detected.

N. Interpretation of Antibody Screening and Crossmatch Results.

1. **NEGATIVE ANTIBODY SCREEN, COMPATIBLE CROSSMATCHES**

- a. The vast majority of samples tested will have a negative antibody screen and the crossmatches with donor's RBCs are compatible.
- b. **This does not guarantee absence of antibody or normal survival of transfused cells.**
- c. A negative antibody screen simply means that there are no antibodies in the serum directed at antigens present on these particular screen cells, a compatible crossmatch should also be interpreted in a similar fashion.

2. **POSITIVE ANTIBODY SCREEN, INCOMPATIBLE CROSSMATCHES** may be due to alloantibodies, autoantibodies, problems with reagents or rouleaux formation.

- a. **Must identify the problem prior to issuing blood for transfusion, unless the need for blood is urgent.** If there is not time:
 - 1) The blood bank physician should advise the patient's physician of the potential risks involved.
 - 2) Often the risk of death due to transfusing incompatible blood **may be less** than the risk of death due to depriving the patient of O₂ carrying capacity.
- b. Positive reactions usually due to presence of **alloantibodies**.
 - 1) Perform a panel and identify antibody specificity.
 - 2) Estimate the likelihood of finding compatible, antigen negative blood in the available inventory.
 - 3) Use appropriate reagent antiserum to confirm that units are negative for antigen that antibody is directed against.
 - 4) The Technical Manual state that it is not necessary to confirm that blood is antigen negative for antibodies directed against the M, N, P¹ or Le antigens. Just issue crossmatch compatible. **You would not make this decision on your own, rather consult with your supervisor.**
 - 5) If multiple antibodies are present and you are unable to identify all specificities send a sample to a reference lab.
 - 6) If the antibody is directed against a high incidence antigen the most promising source will be the patient's siblings or AABB rare donor blood.
- c. If, when performing the antibody work up, you obtain a **positive reaction with the autocontrol**, obtain the patient's history.

- 1) If patient has been transfused within the preceding 2-3 months alloantibody in patient serum may be reacting with transfused donor cells.
 - 2) *Mixed field agglutination* is usually noted, as only donor cells positive for the antigen are coated with antibody.
 - 3) Perform a procedure to remove bound antibody from coated cells (**elution**) into solution and identify the specificity.
- d. **Potent cold-reactive antibodies** may cause problems with ABO, D typing as well as antibody detection and crossmatching tests.
- 1) **Most common specificity is anti-I.**
 - 2) Important to determine if cold autoantibody may be masking a clinically significant alloantibody.
 - 3) Use prewarmed technique - prewarm cells, serum and saline. No IS, wash with warm saline immediately after incubation and use anti-IgG AHG serum.
 - 4) Test patient serum with cord blood RBCs, should get 0/1+ reaction.
 - 5) Cold autoabsorption may be necessary. Will be discussed in detail later.
 - 6) To determine correct ABO/D type keep specimen warm (or rewarm), use cells washed with warm saline.
- e. **Warm reactive autoantibodies** rarely cause problems with ABO typing but frequently cause problems with D typing using a high protein typing sera and antibody detection and crossmatch tests.
- 1) Use low protein anti-D reagent with appropriate control.
 - 2) Warm autoantibodies will cause positive antibody screens and incompatible crossmatches. These are **very** difficult to work up and will be discussed in detail later.
- f. **Rouleaux formation** is a property of serum that causes all cells tested to appear agglutinated at RT and 37 C, *does not affect the AHG test because serum is removed.*
- 1) Appearance microscopically is described as "stacked coins" appearance.
 - 2) To confirm that the pseudoagglutination is rouleaux use the **saline replacement technique.**
 - a) Spin tubes down again and remove serum.
 - b) Add 1 - 3 drops of saline and respin.
 - c) Rouleaux formation will disperse with the addition of saline, true agglutination will not.
- g. **Reagent related problems** may be due to a variety of drugs and additives present in reagents and can cause **false** positive results.
- 1) If all reagent RBCs are positive but crossmatches appear compatible suspect an antibody reactive with a substance in the preservative solution. Will be eliminated by washing reagent cells prior to use.
 - 2) If there is uniform agglutination in all tubes consider an antibody reactive with a substance in the enhancement media. Resolve by using a different enhancement media or doing saline testing.

3. POSITIVE ANTIBODY SCREEN, COMPATIBLE CROSSMATCHES

- a. Patient has an antibody against antigen on screen cells which is not present on donor cells.
- b. Patient has a weak antibody reacting with homozygous screen cells, donor cells are either heterozygous or negative for antigen.
- c. Perform antibody identification (panel study) and test donors for antigen. Crossmatch antigen negative blood for patient.

4. NEGATIVE ANTIBODY SCREEN, INCOMPATIBLE CROSSMATCH

- a. The most common cause is the unit having a positive DAT. *Whenever you have negative screens and only 1 unit is incompatible perform a DAT on the donor unit.*
- b. Rarely, positive reactions in the crossmatch may be due to antibodies to **low incidence** antigens present on the donor cells which are absent on the screen cells. Perform the DAT first, if negative, perform a panel.
- c. Repeat ABO grouping on donor cell sample.
- d. A negative antibody screen and incompatible crossmatches at **RT only** with may be due to:
 - 1) Donor RBCs are ABO incompatible, retype donor.
 - 2) Anti-A¹ in the serum of A² or A²B individuals.
 - 3) Other alloantibodies reactive at RT. Perform a panel, include RT incubation.
- e. Crossmatch incompatible at the **AHG phase only** may be due to:
 - 1) Donor RBCs have a positive DAT.
 - 2) Antibody reactive only with cells having a strong expression of a particular antigen. Screen cells may not possess as strong an expression of the antigen. *Perform a panel.*
 - 3) Antibody reactive with low frequency antigen. *Perform a panel.*

O. Labeling and Release of Blood

1. A tag or label must be securely attached to blood container indicating:
 - a. Recipient's two independent identifiers.
 - b. Donor unit number.
 - c. Interpretation of compatibility testing.
2. The final check of records maintained in the transfusion service for each unit of blood or component must include the following:
 - a. Two independent identifiers, of which is the patient's name..
 - b. Recipient's ABO/D types.
 - c. Donor unit or pool ID number.
 - d. Donors ABO group and, if required, D type.
 - e. Interpretation of the crossmatch if performed.
 - f. The date and time of issue.
 - g. Special transfusion requirements (CMV reduced risk, irradiated or antigen negative).

3. Prior to issuing a unit of blood, blood bank personnel must:
 - a. Check the expiration date of the blood to avoid issuing an outdated component.
 - b. Inspect the unit for abnormal appearance.
 - c. Indicate on an appropriate form the:
 - 1) Name of the individual issuing the blood.
 - 2) Date and time of issue.
 - 3) Name of person to whom blood was issued or destination.
 - d. Final identification of the recipient prior to transfusion rests with the transfusionist, who must identify the patient and donor unit and certify that identifying forms, tags and labels are in agreement.

P. Massive Transfusion

1. *Defined* as infusion within 24-hours of a volume of blood exceeding the recipient's total blood volume. **OR** transfusion of 8 to 10 RBC units in 1 hour which may occur due to:
 - a. unexpectedly in surgical and medical emergencies.
 - b. planned circumstances such as cardiac and vascular surgery.
 - c. exchange transfusion of an infant or adult.
2. Such a small volume of the patient's blood is left that complete crossmatching has limited benefit.
 - a. **Pretransfusion sample no longer represents currently circulating transfused blood.**
 - b. Only important to confirm ABO compatibility of subsequently transfused blood.
 - 1) Perform IS crossmatch only.
 - 2) Computer crossmatch
 - c. When unexpected alloantibody is present in the patient's pretransfusion specimen, abbreviating the crossmatch is acceptable following massive transfusion so long as antigen negative, ABO compatible blood is transfused.
3. Massive transfusion protocol (MTP)
 - a. Studies have been done to document that in addition to RBCs transfusion of other components during this time are equally critical.
 - b. Must take into consideration "dilutional coagulopathy. Giving RBCs plus IV solutions will NOT replace clotting factors or platelets.
 - c. Trauma protocol for massively bleeding patients may involve: 4 units group O negative RBCs, 4 AB FFP and 1 AB platelet. Repeat until bleeding is controlled.

Q. Release of Blood in Urgent Situations.

1. When there is a desperate need for blood, the patient's physician must weigh the hazard of transfusing uncrossmatched or partially crossmatched blood against the risk of waiting while testing is completed.
2. When blood is released before the crossmatch is completed, the records **must** contain a statement of the requesting physician indicating that the clinical situation was sufficiently urgent to require release of blood.

- a. This does not absolve the blood bank personnel from their responsibility to issue properly labeled donor blood of an ABO group compatible with the patient.
- b. Compatibility testing must be started as soon as the specimen reaches the transfusion service.
3. When urgent release is requested issue uncrossmatched blood and immediately begin compatibility testing procedures. Blood released should be:
 - a. ABO and D specific, if there has been time to perform ABO and D testing on the patient's current blood specimen. **Previous records must not be used to determine which blood group to issue.**
 - b. Group O D negative RBCs must be given when the patient's ABO and D type is unknown.
4. **Indicate in a conspicuous fashion** on the blood bag or component that compatibility testing has not been completed at the time of issue.
5. Complete crossmatches **promptly**. If incompatibility is detected at any state of testing, **immediately notify the patient's physician and blood bank physician.**
6. If the patient dies from a medical problem unrelated to the blood transfusion, it is not necessary to complete compatibility testing that may be pending. **This decision rests with the physician responsible for the transfusion service.**
7. If there is **any** reason to suspect that transfusion aggravated the original problem or contributed to death, all testing should be completed, including panels and antigen testing.

R. Effective Blood Utilization, Routine Surgical Blood Orders

1. Increasing blood demands and limited blood resources have made blood banking increasingly conscious of the need to use blood efficiently.
 - a. In many ORs it had been standard practice to have blood crossmatched and reserved for every individual patient as a "standby" precaution.
 - b. Since most of these units were not used, the crossmatches and the expanded inventory needed to meet these demands were wasted resources.
2. Routine surgical blood orders involves ordering blood for common elective procedures and are based on previous records of blood usage.
 - a. The transfusion service crossmatches an agreed upon number of blood units based on procedure.
 - b. Since surgical requirements vary among institutions, routine blood orders should be based on local transfusion utilization patterns as determined by the blood bank medical director, staff surgeons and anesthesiologists.
 - c. Routine orders must be modified for patients with anemia, bleeding disorders or other conditions in which increased use is anticipated.
 - d. Blood bank must be prepared to provide addition blood if an unexpected problem arises.
 - e. Ordering levels should be reviewed periodically to monitor whether the minimum number of units should be increased or decreased.

- f. The Type and Screen protocol is best for procedures such as hysterectomy, thyroidectomy, OB patient and others where blood is seldom needed.
 - a) Uncrossmatched ABO/D identical blood can be released with 99.9% assurance of safety.
 - b) Patients with detectable unexpected antibodies are not eligible and will have antigen negative units crossmatched.