The blood bank "black box" debunked: pretransfusion testing explained

An ABO-incompatible red blood cell (RBC) transfusion (i.e., transfusion of RBCs with A or B antigens to a recipient with the corresponding antibodies) is fatal in 10% of cases[1] and is the main cause of death from transfusion. Errors leading to an ABO-incompatible transfusion can occur at any point in specimen collection, testing and patient identification. Fastidious adherence to proper patient identification and labelling procedures is required of all people involved in the transfusion process. Given the importance of ABO typing in ensuring a safe transfusion, I describe the testing performed by blood banks when a "type and screen" is ordered.

Type and screen

When a sample of a recipient's blood and requisition form arrive at the blood bank, they are checked against each other to ensure that the information is consistent and complete, as required by the standards for pretransfusion testing established by the American Association of Blood Banks, local hospital policy and national standards. If there is any discrepancy or if information is missing, the specimen will be rejected and the ward notified that a recollection is necessary. Even the most seemingly minor labelling error can be associated with an extremely high risk of recipient misidentification, hence these specimens are discarded.

If the specimen is acceptable for pretransfusion testing, the first step in ensuring serologic compatibility between the donor and recipient is to perform a type and screen, which takes about 45 minutes (Table 1). It consists of 2 distinct tests. The "type" (or "group") test determines which ABO antigens are present on the the patient's RBCs. It also usually features a test for the RhD antigen. The type test is divided into 2 steps. The first step uses commercially available antibodies that will react with either the A or B antigens, if present, on the patient's RBCs and cause them to agglutinate. This is known as forward (cell) typing. RBCs from a person with type AB blood will react with both anti-A and anti-B antibodies, whereas those from a person with type O blood will not react with either antibody. The RhD antigen is tested in the same manner, with commercially available anti-D antibodies mixed with the RBCs (Box 1). The second step of the type test uses commercially available A_1 and B cells that will react with antibodies, if present, in the recipient's plasma. This is called reverse (serum) typing. Almost everyone has naturally occurring antibodies to the ABO antigens they lack — a person with type O blood will have both anti-A and anti-B antibodies in their plasma, whereas a person with type AB blood will have neither of these antibodies in their plasma. These forward and reverse typing tests are used together to establish a patient's ABO type.

The "screen" test is done to determine whether the recipient has formed what are known as "unexpected" RBC antibodies. About 3%–10% of recipients who have received multiple RBC transfusions will have antibodies to non-ABO antigens.[2,3] This is in contrast to the regular, predictable occurrence of antibodies to the ABO antigens that the recipient lacks; hence the moniker "unexpected" (see Box 1). The screen is performed by using 2 or 3 commercially available type O cells that, between them, express essentially all of the approximately 20 clinically significant RBC antigens. By incubating the recipient's plasma with these cells and looking for agglutination of the RBCs or hemolysis caused by antigen–antibody interactions, unexpected antibodies can be detected and the identification process begun. (The risk of overt hemolysis due to abbreviated pretransfusion testing is estimated in Table 1). If unexpected antibodies are found, additional testing, sometimes taking several hours, is required to identify them and to locate antigen-

Table 1: Estimated turnaround times for various procedures involved in pretransfusion testing

Procedure	Estimated turnaround time,* min	Risk of acute hemolysis, %†
Emergency issue of uncrossmatched red blood cells (RBCs)	5	5
ABO and RhD type only	15	5
Type and screen	45	< 0.1
Type and screen plus computer crossmatch‡	60	< 0.1
Type and screen plus serologic crossmatch	> 120§	< 0.1

*Time from receipt of an acceptable specimen and requisition form at the blood bank to issuance of RBCs or completion of test. Turnaround times will vary between institutions.
†The estimated probability that the recipient will experience an overt acute hemolytic reaction if RBCs are administered after the procedure is completed. Overall, about 5% of recipients will have an unexpected antibody; the risk of an acute hemolytic reaction is diminished only when antibody screening methods are performed.
‡This procedure can be performed when no unexpected antibodies are identified. The time listed represents the fastest time that crossmatched blood can be provided to a ward.
§Antibody identification can take several hours depending on the nature of the antibody. Locating rare RBC units that lack a high-incidence antigen, or combination of antigens, can take days and may require consultation of a rare-donor registry or the use of frozen RBCs.

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negative RBC units for transfusion.

A type and screen is valid for up to 3 days if the recipient has received a transfusion or has been pregnant in the past 3 months. A pregnant woman can form antibodies to foreign antigens (i.e., paternally derived) on fetal cells should a feto-maternal hemorrhage occur; thus, it is important to repeat the type and screen if a woman requires a transfusion within 3 months of delivery. No standard exists for how long a type and screen is valid in patients who have not been transfused or pregnant in the preceding 3 months; for an exact duration, consult your local blood bank.

Box 1: Notes on the Rh blood group system and anti-RBC antigens

Rh blood group system

- Next to the ABO system, Rh is the most clinically significant blood group system
- Antibodies in the Rh system will cause hemolysis of antigen-positive transfused red blood cells (RBCs)
- There are over 45 antigens in this system. The RhD antigen is the most immunogenic (i.e., an Rh-negative RBC recipient or pregnant woman is highly likely to produce an anti-D antibody if exposed to even small quantities of RhD-positive blood)
- Testing for the RhD antigen is routinely included in pretransfusion testing. The presence of other Rh antigens is not routinely tested
- The designation "Rh positive" indicates that the RhD antigen is present.
- Unlike the anti-ABO antibodies, the anti-D antibody is not naturally occurring but is formed after exposure to Rh-positive RBCs

Anti-RBC antigens

- There are over 250 antigens on the surface of a typical RBC
- Only a few antigens elicit the production of antibodies that are considered clinically significant because they cause hemolysis of antigen-positive transfused RBCs. Some of these antigens include Kell (K), Duffy (Fy), Kidd (Jk) and Rhesus (Rh)
- Other antibodies are not usually considered clinically significant because they do not cause hemolysis of antigen-positive transfused RBCs at normal body temperature. Some of these antibodies include anti-Lewis (Le), anti-H, anti-I/i and anti-M/N

Crossmatch

A crossmatch is performed to ensure compatibility between the donor's RBCs and the recipient's plasma. It can be done serologically to ensure compatibility with both anti-ABO and non-ABO antibodies or by computer as a check on ABO compatibility (Fig. 1). If the recipient has a negative antibody screen (has not formed unexpected antibodies), the computer can be used to electronically match the ABO type of the recipient with a compatible donor unit using laser wands and bar-code technology. The computer system must have the logic to recognize and allow an RBC unit to be issued if the ABO match between a donor and a recipient is compatible and to reject units that are incompatible. In the absence of computer crossmatch technology, a serologic crossmatch is required to ensure ABO compatibility (Fig. 2).

If a recipient has formed an unexpected antibody, the blood bank cannot use the computer crossmatch system (Fig. 2). Instead, a serologic crossmatch is required. It involves mixing the recipient's plasma with a potential donor RBC unit (selected by immunologic testing to be antigen negative) and inspecting for agglutination or hemolysis to ensure that the RBC unit

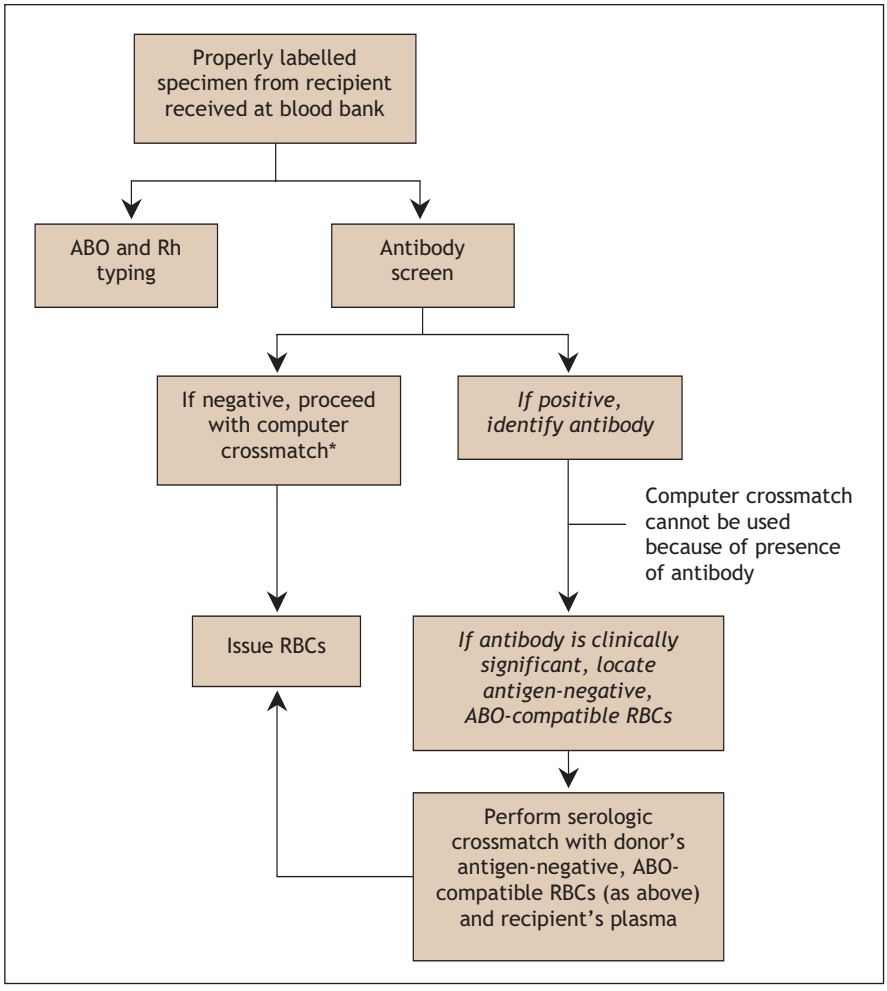

Fig. 1: Steps involved before a unit of red blood cells (RBCs) can be issued for transfusion. Steps in italics represent the main time-consuming procedures in pretransfusion testing. *If a computer crossmatch system is unavailable, a second verification of ABO compatibility between donor RBCs and recipient plasma is required (see Fig. 2).

truly lacks the antigen corresponding to the patient's antibody.

Uncrossmatched RBC units are always type O (universal donor) and often RhD negative, thus they can be safely transfused to virtually any recipient in an emergency situation when the typically short delay caused by pretransfusion testing to find ABO-matched units would compromise the patient's life.

Compatibility requirements for plasma, platelets and cryoprecipitate

Plasma: Plasma is transfused to correct the dilutional coagulopathy associated with massive transfusion of RBCs, to correct a complex coagulopathy (e.g., liver failure), to immediately reverse the effects of oral anticoagulant therapy and to replace clotting factors for which a sterile concentrate is not available. It is not used for volume replacement. Because of anti-ABO antibodies in units of plasma, only ABO-compatible units can be used (Table 2). Thus, the recipient's ABO type is needed for plasma transfusion. Because plasma from donors with type AB blood does not contain anti-ABO antibodies, it can be given to any patient and is used for emergency transfusion in patients with unknown blood types. Crossmatching of plasma is not required, since there are no RBCs in these products.

Table 2: Compatible blood products for transfusion according to recipient's ABO type

Recipient's ABO type	Compatible plasma	Compatible RBCs	Compatible platelets	
			First choice	Second choice
A	A, AB	A, O	A, AB	B, O
B	B, AB	B, O	B, AB	A, O
O	O, A, B, AB	O	O*	A, B, AB
AB	AB	O, A, B, AB	AB	A, B, O

*Although type O red blood cells (RBCs) do not have A or B antigens, the recipient's plasma contains anti-A and anti-B antibodies, which could reduce the recovery of transfused platelets bearing A or B antigens.

Platelets: Platelets are transfused in patients with thrombocytopenia if they are bleeding, if prophylaxis against spontaneous bleeding is required or if a platelet count threshold needs to be surpassed before an invasive procedure. Patients without thrombocytopenia may require platelet transfusions if they have acquired platelet functional defects (e.g., due to medications, or after cardiac bypass surgery). ABO compatibility for platelet transfusion is desirable but not required because of the small amount of plasma present in a standard dose of platelets (each unit contains about 60 mL of plasma, and 5 or 6 units make up a standard dose). However, platelets have a short shelf life (5 days) and ABO-matched pools may not be available for the recipient; therefore, most institutions will cross ABO boundaries when issuing platelets (e.g., issuing type A platelets to a type B recipient). Typically the only adverse event of crossing ABO lines is that the recipient may have a positive direct antiglobulin test result, but significant hemolysis is very rare. It is important to remember that platelets themselves have ABO antigens on their surface, and thus a donor–recipient ABO mismatch may result in poor recovery of platelets after transfusion.

Cryoprecipitate: Each unit of cryoprecipitate contains a small amount of plasma (15 mL/unit) and thus does not require ABO compatibility. This product contains at least 150 mg of fibrinogen and significant amounts of clotting factor VIII and von Willebrand's factor; sterile preparations are recommended for the treatment of hemophilia A and von Willebrand's disease, respectively.

The steps in pretransfusion testing are relatively simple, but complete accuracy is required at each step. ABO compatibility between donor and recipient is of paramount importance, and un-

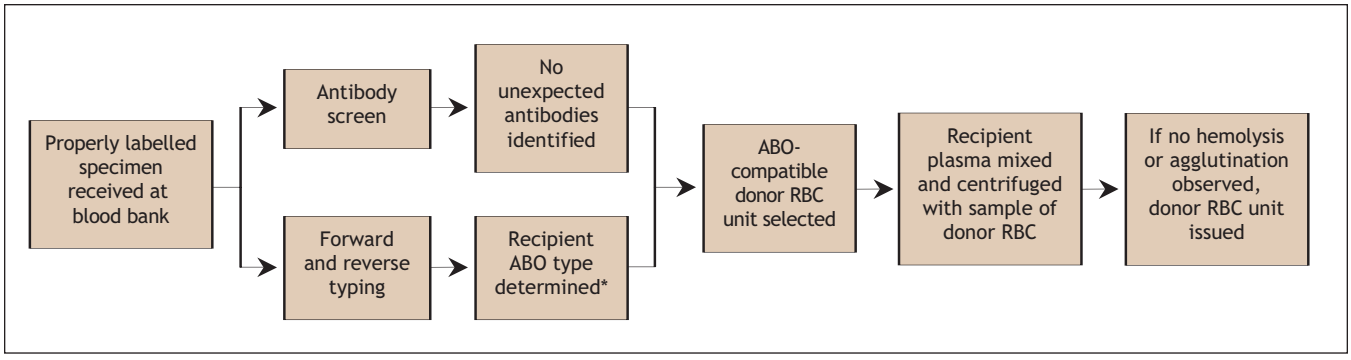

Fig. 2: In the absence of a computer crossmatch system, a second verification of ABO compatibility must occur before a unit of RBCs can be issued. If no unexpected antibodies are detected after the type and screen tests, an ABO-compatible RBC unit is selected and mixed with the recipient's plasma. This mixture is briefly centrifuged and inspected for hemolysis and agglutination; if both are absent, ABO compatibility is verified and the RBC unit issued. This procedure must be repeated for each donor RBC unit. If unexpected antibodies are detected in the recipient's plasma, a similar procedure is used to verify ABO compatibility, but the donor RBC units are selected to be compatible with the recipient's antibody. *This step represents the first verification of ABO compatibility.

derstanding the relation between the ABO antigens and their reciprocal antibodies is the key to safe transfusions.

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