BACKGROUND

Pre-transfusion testing refers to the laboratory testing required to ensure compatibility between the blood of the transfusion recipient and the blood product intended for transfusion. This process includes proper completion of the requisition, proper patient identification, collection and labelling of the blood sample from the patient, laboratory testing to determine the patient’s blood group and to identify the presence of red blood cell alloantibodies, and compatibility testing. Pre-transfusion testing is completed when a compatible blood product is identified for transfusion to the intended recipient. This chapter provides an overview of the pre-transfusion tests that are routinely performed.

PATIENT IDENTIFICATION AND SAMPLE COLLECTION AND LABELLING

It is essential that hospitals and clinics collecting samples for pre-transfusion testing have a specific policy and procedure for positive patient identification and appropriate labelling of pre-transfusion test samples. The first step in pre-transfusion testing is the preparation of the testing requisition. Patient identification should be verified at this time. The second step is to ensure the blood sample is collected from the intended transfusion recipient. Samples must be labelled with two unique identifiers. Unique identifiers include first and last name and most commonly, either the hospital number or provincial health insurance number. The sample must be labelled immediately following collection in the presence of the patient. Some standards require the date of collection on the tube and all require a method to identify the phlebotomist. Patients should be asked to state their first and last name and date of birth since they may be wearing the wrong identification bracelet.

A non-hemolyzed sample of blood is required for testing. The samples used for patient testing may be anticoagulated in EDTA (preferred) or may be clotted blood and serum, depending on local policy. The Canadian Society for Transfusion Medicine Standards for Hospital Services recommends that the original sample may be used up to 96 hours after collection, and may be used to crossmatch any number of units as required clinically (CSA 15 10.4.3). After 96 hours, re-collection of a new sample is necessary to ensure ongoing availability of compatible red cell units because of the possibility of antibody development in patients exposed to red blood cell antigens by transfusion or pregnancy. If the patient has not been recently transfused or is not currently pregnant, samples for pre-transfusion testing may be tested in advance and the validity of compatibility testing results extended beyond the normal 96 hour expiry. Local policies may vary; this extension of the expiry of a crossmatch is sometimes used for surgical pre-admission patients who may have their blood drawn for compatibility testing several days or weeks before the planned surgical date, provided they have not been transfused and have not become pregnant in the interim period. The results are considered valid and compatible blood can be available on the anticipated surgical date on the basis of this prior testing. Pregnancy or recent transfusion could induce antibody formation which would invalidate the crossmatch.

PRE-TRANSFUSION TESTING

Pre-transfusion tests include ABO and Rh (D) typing of the recipient's red blood cells and an antibody screen. The latter is a method to detect clinically significant non-ABO antibodies in the recipient's plasma. Most clinically significant antibodies are IgG and develop following exposure to foreign red blood cell antigens during transfusion or pregnancy.

Before a non-emergent transfusion, compatibility testing is performed in addition to blood grouping and antibody screening. The compatibility test may be a serological crossmatch or may involve the use of a validated computer program to ensure that an appropriate component has been selected for the intended
recipient. The latter is known as an electronic crossmatch.

**ABO typing**

ABO typing involves testing the recipient’s red blood cells for the presence of A and B antigens using anti-A and anti-B antisera (forward grouping). Testing of the recipient plasma for the presence of anti-A and anti-B using known Type A and Type B red blood cells (reverse grouping) is also part of routine ABO blood group testing. Reverse grouping is not performed on infants under four months.

**Rh typing**

The Rh (D) type of the transfusion recipient is determined by testing recipient red blood cells with anti-D. A significant proportion of the population lack the Rh (D) antigen on their red blood cells. Eighty percent of Rh (D) negative recipients may develop antibodies to the D antigen if exposed to Rh (D) positive red blood cells. It is, therefore, preferable to provide Rh-negative (D negative) blood products to any Rh (D) negative individual. In particular, Rh (D) negative women of child-bearing potential should not be given Rh (D) positive blood products because development of antibodies to the D antigen could contribute to hemolytic disease of the fetus and newborn (HDFN) in future pregnancies. For more information about HDFN, please see [Chapter 12](https://professionaleducation.blood.ca/en/transfusion/guide-clinique/pre-transfusion-testing).

**Antibody screening**

Alloantibodies to antigens that are not present on an individual's red blood cells may develop in anyone who has been exposed to foreign red blood cell antigens through pregnancy or transfusion. To detect antibodies to non-group A or B antigens, a sample of the patient's plasma or serum is tested against selected commercial Type O red blood cells that express the majority of clinically significant antigens, other than A and B. This screening test usually takes 30 to 60 minutes to complete. For infants under four months of age, the screen may be performed on either infant plasma, or maternal plasma.

Many techniques are currently available for the detection of antibodies (Figure 1). Some methods involve addition of testing reagents such as saline, albumin, low ionic strength saline (LlSS), or polyethylene glycol (PEG). Some use gel column agglutination technique with anti IgG antibody in the column (gel card) or microtiter well plates with bound red blood cell antigens (solid phase) to perform antibody detection.
The ID-Micro Typing System™ incorporates gel technology to provide easy-to-use and easy-to-read testing.

1) Pipet  
2) Incubate  
3) Centrifuge  
4) Read

**Figure 1.** Examples of commercial kits for antibody screening. Top panels: the ID-Micro Typing system from Ortho Clinical Diagnostics. Bottom panels: Immucor Capture-R.

**Antibody identification**

If a clinically significant antibody is detected in a recipient’s plasma by screening technique, further testing is usually done to identify the antibody. This process, known as antibody identification, involves testing the plasma with a panel of commercial Type O red blood cells with known antigen expression. Antibody identification involves multiple steps designed to exclude particular antibodies, determine the optimal temperature of antibody reactivity and determine the presence of autologous reactivity of the antibody. Antigen
typing of the recipient red blood cells may also assist in the antibody identification process, by allowing determination of which red blood cell antibodies an individual is likely to develop. Following antibody identification, donor red blood cell units are screened to identify those that lack the antigens corresponding to the antibody identified in the recipient. Donor phenotyping information may be printed on the red blood cell unit label in some cases; in other cases, donor units may be phenotyped by the hospital blood bank or requested from Canadian Blood Services. See Chapter 6 of this Guide for more information on red blood cell phenotyping.

CROSSMATCH

The term crossmatch is used to describe a method of confirming compatibility between the patient’s blood (plasma) and the donor red blood cells. The crossmatch is meant primarily to detect and prevent ABO incompatibility. A crossmatch may involve either the direct mixing of donor red blood cells with recipient plasma (serological crossmatch) or using a computer system to ensure that the recipient and donor testing have been completed and that the donor units selected for a particular recipient are compatible. This is known as the electronic or computer-assisted crossmatch. The latter may be used only in the setting of a computer system that has been validated to prevent release of incompatible units, and only for a recipient with a negative antibody screen.

TYPE AND SCREEN, OR CROSSMATCH?

For patients who are unlikely to require blood transfusion in a given medical or surgical setting, “type and screen” is a common approach (determine the recipient’s ABO and Rh type and perform an antibody screen). If this screen is negative, no further testing would occur, but a crossmatch could be performed and blood products provided quickly in the event that they were needed. This practice of performing type and screen only allows better inventory management of red blood cell supply, as there are more units left available in inventory, and few units being held for a specific patient, unavailable to others. For patients where a positive antibody screen is detected on type and screen request, further testing as outlined above together with a crossmatch would be undertaken to ensure timely availability of compatible blood products.

A crossmatch should be requested for those patients for whom a blood transfusion is intended or definitely anticipated. This order should include the number of red blood cell units required. In the laboratory a crossmatch order results in blood grouping and antibody screening as well as compatibility testing with preparation and labelling of the red blood cell units for transfusion to the particular recipient.

Crossmatch is the final step in procuring red cell units suitable for transfusion. In a patient who has a clinically significant antibody (or antibodies), the units selected for crossmatch will be antigen negative. If crossmatch-compatible red blood cells cannot be found, the transfusion service physician may authorize the release of incompatible units if the need for transfusion outweighs the risk of transfusing incompatible blood. Depending on the number and complexity of the antibodies present in recipient plasma, a variable amount of additional time may be required to find compatible red blood cells. In most cases, compatible allogeneic red blood cells safe for transfusion can be identified. Consultation with a reference laboratory experienced in antibody investigation may be necessary in some cases, which could contribute to a potential delay in transfusion. When compatible blood is very difficult to obtain, rare units or frozen units may be accessed from the blood supplier. In rare cases, either autologous donation or directed donations from close family members may be required to ensure that adequate amounts of compatible blood are available.

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Published: Friday, September 8, 2017

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Verify patient identification
- Positive patient identification following hospital/clinic policy

Collect and label patient sample
- Collect sample according to hospital/clinic policy
- Label in the presence of the patient immediately after collection
- Label with two unique patient identifiers, following hospital/clinic policy
- Label with phlebotomist identifier

ABO and Rh typing
- Forward grouping: use anti-A, anti-B and anti-D to detect A, B and Rh(D) antigens on recipient red blood cells
- Reverse grouping: use Type A and B red blood cells to detect anti-A and anti-B antibodies in recipient plasma

Antibody screening
- Use Type O red blood cells expressing most clinically significant antigens to detect antibodies in recipient plasma
- If antibodies are detected, use a panel of Type O red blood cells with known antigens to identify the recipient antibodies, then provide red blood cell units which lack the corresponding antigen(s).

Compatibility testing
- Serological crossmatch: mix donor red blood cells with recipient plasma to ensure compatibility
- Electronic crossmatch: use validated computer algorithms to ensure compatibility. Use only for recipients with a negative antibody screen and independently confirmed blood group.
- If crossmatch-compatible blood products cannot be found, incompatible units may be used at the physician’s discretion if the need for transfusion outweighs the risk of transfusing incompatible blood.

Select compatible units for transfusion
- If transfusion is needed so urgently that pre-transfusion testing cannot be completed, unmatched blood products may be considered (Type O-positive or, for women with childbearing potential, O-negative). If ABO and Rh testing has been completed, group-specific non-crossmatched red blood cell products may be transfused.

Figure 2. Summary of the pre-transfusion testing process.

EMERGENCY BLOOD RELEASE

When the urgency of the transfusion requirement prevents the initiation or completion of pre-transfusion testing, emergency release of unmatched blood products may be considered. If ABO and Rh (D) testing have been completed, group-specific non-crossmatched red blood cell products may be provided. If no testing has been initiated when blood is required, Type O-positive or O-negative unmatched red blood cell units could be released. Type O-negative red blood cells should be conserved in emergencies for woman of childbearing potential to prevent overuse of the limited supply of Type O-negative red blood cell units. A sample for

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compatibility testing should be obtained as soon as clinical circumstances permit. This allows appropriate switching to group-specific crossmatched blood as soon as possible.

CONTINUING PROFESSIONAL DEVELOPMENT CREDITS

Fellows and health-care professionals who participate in the Canadian Royal College’s Maintenance of Certification (MOC) Program can claim the reading of the Clinical Guide to Transfusion as a continuing professional development (CPD) activity under Section 2: Self-learning credit. The reading of one chapter is equivalent to **two credits**.

ACKNOWLEDGEMENTS

The author, Debra Lane, acknowledges Kate Chipperfield, MD FRCP, and Darlene Mueller, MA, ART, for their review of this chapter.

We’re here to answer your questions about the Clinical Guide to Transfusion. We’d also appreciate your ideas on how to improve the Guide. Please contact us through the [Clinical Guide feedback form](https://professionaleducation.blood.ca/en/transfusion/guide-clinique/pre-transfusion-testing).

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