**PURPOSE**

This procedure provides instructions for testing the red cell antigen(s) of a patient in antibody identification or testing of donor red cell antigen(s) when selecting antigen negative units for a patient with history of clinically significant antibodies. Patient makes alloantibody to the antigen they are lacking. Antigen typing of the patient is to confirm the antibody identification or to rule out alloantibody when the patient tests positive for the corresponding antigen.

**REAGENTS**

A. Anti-sera

B. Anti-IgG anti human globulin

C. IgG coated Coombs control cells

**EQUIPMENT**

A. 12 x 75 mm Tubes

B. Transfer pipettes

C. Heat block

D. Centrifuge

E. Timer

F. Cellwasher

**SPECIMEN**

A. Patient specimen in EDTA anticoagulant

B. Donor red cells

**SCOPE**

Transfusion Service CLS trained in this procedure.

**CONTROLS**

A. Positive and negative control performed each day of use.

B. Antigen typing procedures must follow manufacturer's insert.

C. Antigen typing quality control is reviewed by supervisor or designee the next day.

**PROCEDURE:**

A. **Perform quality control of the antiserum** **once a day** according to the current manufacturer's insert.

1. Verify that the antiserum is within its expiration date before testing. Do not use expired antiserum. Remove expired antiserum and place on the Expired Reagents shelf in Fridge#2.

2. Find the lot# of the vial on the Reagent Supply Order & Receipt Log and follow the manufacturer's instructions that has the corresponding insert code.

3. Positive control cell is selected from screening cells or panel cells showing **heterozygous** or weaker expression of the antigen to be tested.

4. Negative control cell is selected from screening cells or panel cells lacking the antigen to be tested.

5. Record antisera QC results in the Anti-sera Package Inserts binder.

6. Verify results of controls for acceptability according to manufacturer's insert before reporting patient results.

B. **Patient red cell antigen typing - follow manufacturer's insert**

1. **Complete Phenotype** **or Genotype**

1. It is recommended to obtain a full phenotype of **all clinically significant antigens** (ABO, Rh, Kell, Duffy, Kidd, and Ss), on patients who are diagnosed with the following diseases:
2. sickle cell anemia
3. thalassemia major (e.g. Cooley's)
4. severe congenital anemias (e.g. Fanconi, Diamond Blackfan)
5. autoimmune hemolytic anemia (e.g. WAIHA, Cold Agglutinin Disease, Paroxysmal Cold Hemoglobinuria)
6. or any disease that may require chronic red cell transfusion

1. This will enable the Transfusion Service to provide phenotypically matched red cells in the future, if necessary.
2. A **full genotype** is recommended for patients diagnosed with Sickle Cell anemia and for recently transfused patients who need chronic transfusions.
3. Request a second sample for send-out to Reference Lab.
4. Perform serological phenotype if transfusion cannot wait till after completion of genotype testing.

2. **Transfusion History**

a. Antigen typing of patient's red cell is accurate if the patient has not been transfused in the last 3 months. Verify transfusion history with the patient's physician or nurse.

b. If the patient has been transfused within the last 3 months send specimen to Reference Lab for phenotyping using reticulocytes or molecular genotyping.

3. **Patient has positive DAT**

* + 1. Antigen typing by IAT method should not be performed.
		2. Saline or monoclonal reagents by Direct antiglobulin method can be used for phenotyping.
		3. Send sample to Reference Lab if necessary for complete phenotyping/genotyping.

4. **RBCs Selection for Sickle Cell Disease/Thalassemia Major/Congenital Anemias**

a. Match donor RBC antigens with the patient's complete Rh and K phenotypes if the patient has no history of antibody.

b. Match donor RBC antigens with the patient's complete Rh, Kell, and the patient’s clinically significant antibody e.g. if patient has anti-Jka, order donor pRBC pheno-matched for Rh, K and Jka.

c. Give c antigen negative RBC when patient is C+c+E+e+ due to the possibility of making anti-f.

d. Donor RBCs should also be Hgb S negative for Sickle Cell patients.

5. **RBCs Selection for Autoimmune Hemolytic Anemia**

a. When unable to rule out underlying alloantibodies, provide phenotypically match RBC for transfusion.

6. **Special Requirements for Transfusion in LIS**

a. Enter special requirements e.g. IRR, HgbS negative, antigen negative requirements in the LIS.

C. **Donor red cell antigen typing**

1. When a patient has **clinically significant alloantibody**, RBC that lacks the corresponding antigen should be selected for transfusion unless antigen negative units are not available due to rarity or at the recommendation of Blood Supplier’s Medical Director.

a. Antigens to clinically significant antibodies are ABO, Rh (D, C, E, c, e), K, k, Fya, Fyb, Jka, Jkb, S and s.

b. Even if the antibody is no longer detectable, RBCs for all subsequent transfusions should lack the antigen, to prevent a secondary immune response.

c. IAT crossmatch of the antigen negative donor RBC is required using the same technique (in general) that identified the antibody.

d. **Low Frequency Antigens**

i) Check the Antigen QC binder for available **low frequency** antisera i.e. anti-Cw, Kpa, etc. If not available in house, check with Blood Supplier if they provide confirmed antigen negative RBC for the specific low frequency antigen that the patient requires.

ii) If there is no commercial antisera to phenotype low frequency antigens, IAT crossmatch is required using the same technique (in general) that the antibody is identified.

e. Expired rare antisera can be used for antigen typing as long as the positive and negative QC results are acceptable.

2. **Clinically insignificant alloantibody not reacting at 370C** ( Lea, Leb, M, N, P1, Lua, A1, Sda, HTLA)

a. Donor antigen typing is not necessary but IAT crossmatch is required using the same technique (in general) that identified the antibody.

3. **Screening donor units with patient's plasma**

a. In order to save expensive or rare antiserum, **if** **patient's antibody gives** **2+ or stronger reaction**, the patient's plasma can be used to find crossmatched compatible units. Result incompatible units (but do not set the units to 'crossmatched' status) in the computer to capture workload.

b. Nonreactive units must then be confirmed with the commercial antisera reagent.

4. **Frequency of compatible donor units**

a. When a patient has **multiple antibodies**, it can be helpful to determine the frequency of compatible donor units.

b. **Calculations**

i) For example, if a serum contains anti-c, Fya and S, while antigen frequencies among random donor population are 18 % c negative, 34 % Fy(a-), and 45 % S negative, then the frequency of compatible donor units would be 0.18 x 0.34 x 0.45 = 0.028. In other words, if you screen a hundred units you will find 2 to 3 units that is c-, Fy(a-) and S- (or 1/0.028 = screen 36 units to find one unit).

ii) If the calculated frequency is less than 5%, order historically antigen negative donor units from Blood Supplier. Otherwise, if workload permits, crossmatch with patient's plasma or antisera using analyzer. Confirm the compatible donor units using commercial antiserum following manufacturer's insert.

5. **Historically antigen negative units** must be reconfirmed with commercial antiserum, label with antigen typing label and result in the computer.

6. **Antigen typing Confirmed by Blood Supplier**

a. If Blood Supplier has confirmed the antigens to be negative, it is not necessary to reconfirm the antigen typing of the units.

b. Enter the antigens as Special Testing when the unit is received into inventory.

D. **Interpretation of Result**

1. Agglutination of the red cells in the presence of reagent is a positive test result and indicates the presence of the antigen.

2. Investigate any **mixed field** positive reaction or reaction **less than 2+.**  Refer to the manufacturer's insert for acceptable reaction strength**.**

3. No agglutination is a negative result and indicates the absence of the antigen.

4. **Label** the unit with antigen typing sticker and result the antigen typing in the computer.

E. **Resulting in computer**

Order the appropriate test mnemonics and enter result as the test is read in the LIS. See computer SOP.

**REFERENCE**

A. AABB, Standards for Blood Banks and Transfusion Services, current edition, Bethesda, MD.