1. **Principle**

Red Cells are tested with specific antisera to determine the presence or absence of blood group antigen(s).

In direct antigen typing, the specific antisera will agglutinate red cells that have the corresponding antigen. Agglutination may occur at room temperature, at 4°C or at 37°C depending on the antisera used.

In indirect antigen typing, the specific antisera will sensitize red cells that have the corresponding antigen. Agglutination will occur by the indirect antiglobulin test (IAT).

Bone Marrow Transplant

When performing antigen typing for Bone Marrow Transplant (BMT) patients, the patient history should always be considered. Post BMT patients may exhibit chimerism due to the presence of donor and recipient red cells.

1. **Scope and Related Policies**
   1. An autocontrol or a direct antiglobulin test shall be performed in conjunction with red cell phenotyping that requires an indirect antiglobulin test.9.1
   2. Antigen typing (phenotype testing) of patient cells is done on a specimen from a patient who has not been transfused in the past three months.
      1. It should be performed where possible before assigning specificity to a newly detected antibody.
      2. It may be performed to aid in exclusion of an antibody(ies) during an antibody identification.
      3. It is recommended for patients who will require long term transfusion therapy [e.g., patients with chronic anemia (sickle cell, thalassemia)] or for patients with a warm reactive autoantibody. Consider typing with antisera such as anti-C, -c, -E, -e, -K, -Jka, -Jkb, -Fya, -Fyb, -S and –s to provide baseline information in case of future antibody production.
   3. Antigen typing of donor units is required when selecting donor units for a patient who has, or has a history of, clinically significant antibody(ies).
      1. Antigen typing is not necessary when a patient has a clinically insignificant antibody(ies) and compatible donor units can be found using serological testing.
2. **Specimens**
   1. Antigen typing must be done on a pre-transfusion specimen. (EDTA anticoagulated whole blood.) See Procedural Notes 8.2.

* Preferably specimens should be tested within two days of collection
* Specimens that cannot be tested immediately should be stored at 4°C.
  1. Red cells from donor unit segments may be tested up to the expiration date of the unit if stored at 1-6oC.

1. **Materials**

**Equipment:** Serological centrifuge

Cell washer

Block for test tubes

Water bath/Heating block at 37° C

Microscope

**Supplies:** Test tubes – 10 x 75 mm

Serological pipettes

Segment device

**Reagents:** 3-5 % Panel or screening cells (for controls)

Commercial antisera (with manufacturer’s insert)

Anti-IgG

IgG-coated cells

Normal saline

**Worksheets:** Patient Antigen Typing Worksheet – NRT.009F

1. **Quality Control**
   1. Commercial antisera must be tested with the appropriate positive and negative control cells each time of use.9.1
   2. The positive control cell must have the weakest expression (i.e., single dose, or heterozygous expression of the antigen whenever applicable) of the corresponding antigen.9.1 The negative control cell must lack the corresponding antigen.
2. **Procedures**

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| * 1. Check the suitability of the specimen(s). See PA.002- Determining Specimen Suitability. | |
| * 1. Complete a Patient Antigen Typing Worksheet – NRT.009F. Transcribe the name and identification number from the specimen onto the worksheet, or use computer worksheet.  |  |  | | --- | --- | | *If* | Then | | Typing donor units | Complete Antigen Typing Worksheet – NRT.009F. To estimate the number of donor units to test, see Procedural Notes 8.1 | | Computerized | Proceed with computer result entry. | | |
| * 1. Confirm that the patient has not been transfused in the past three months. Record this information on the worksheet.  |  |  | | --- | --- | | *If* | *Then* | | The patient has been transfused in the last three months | Retrieve and use a pre-transfusion specimen, if available.  See Procedural Notes 8.2 | | |
| * 1. If antigen typing by indirect antiglobulin test, confirm that an autocontrol by IAT or the direct antiglobulin test (DAT) on the cells is negative.   Record autocontrol by IAT or DAT results on the worksheet.   |  |  | | --- | --- | | *If* | *Then* | | The IAT autocontrol or DAT result is positive with anti-C3 only | Antigen typing using indirect antiglobulin test, may be performed providing anti-IgG reagent is used | | The IAT autocontrol or DAT is positive with anti-IgG | Do not perform antigen typing using indirect antiglobulin test. Use monoclonal antisera for testing or pretreat the cells with chloroquine or EGA. See Special Procedures SP.011 or 012. | | |
| * 1. Prepare a 3% patient red cell suspension: | * + 1. Label a test tube with the patient full family name; transcribe the family name from the specimen tube, not from the request form. A pre-printed label may be used (ensure the information coincides exactly to the specimen label). |
| * + 1. Dispense 2 drops of whole blood (or equivalent: 1 drop of packed cells) to the labeled tube. |
| * + 1. Add 0.5 to 1.0 mL of normal saline and mix to resuspend to 3%. |
| * + 1. Compare with a 3% commercial red cell suspension and adjust the strength of the suspension if necessary. |
| * 1. Preparing a 3% donor unit cell suspension: | * + 1. Using a segment device squeeze one drop of blood into a test tube labeled with the full donor number. |
| * + 1. Add 0.5 – 1.0 mL of normal saline to resuspend to 3%. |
| * + 1. Compare with a 3% commercial red cell suspension and adjust the strength of the suspension if necessary. |
| * 1. Review the current manufacturer insert for antisera used (specific instructions for number of drops, method, temperature and time of incubation). | |
| * 1. Select the appropriate cells for positive and negative controls from a panel of cells or from the screening cells. * The negative control cell must not carry the antigen * The positive control cell must have the weakest expression of the antigen [i.e., whenever applicable, the positive control must have a single dose (heterozygous) of the antigen]. | |
| * 1. Label tubes as follows: | * + 1. One tube with the first three letters of the patient’s family name and the required number of tubes for the donor units being tested, each labeled with the last four numbers of the appropriate donor unit and the name of the antigen: e.g., “JON Fya”, (or 4267 Fya). |
| * + 1. One tube for positive control: e.g., “Fya +”. |
| * + 1. One tube for negative control: e.g., “Fya -”. |
| * + 1. Record the panel number and cell number of the cells used for positive and negative controls. |
| * 1. Add the appropriate number of drops (as per manufacturer’s insert) of antisera into each tube and record the antisera lot number and expiry date on the worksheet or in the computer. | |
| * 1. Add 1 drop of the patient, donor unit and appropriate control cell suspension to the appropriate tubes. | |
| * 1. Mix all tubes. | |
| * 1. Follow manufacturer insert instructions, perform the steps as directed | |
| * 1. Add IgG-coated cells to all negative IAT results; centrifuge, resuspend and read macroscopically. Record results. Macroscopic agglutination must be present or the test and controls must be repeated. Refer to manufacturer instructions. | |
| * 1. Sign or initial and record the completion time and date on the worksheet or in the computer. | |
| * 1. Record the result of the antigen typing. See 7.0 – Reporting. | |

1. **Reporting**
   1. Macroscopic agglutination with specific antiserum indicates the presence of the corresponding antigen on the cells and no agglutination indicates the absence. Refer to manufacturer instructions for interpretation for each specific antisera in use.
2. **Procedural Notes**
   1. To obtain a certain number of donor units negative for a given antigen, consider the frequency of the antigen in a population.

Example:  
Frequency of E antigen = 30%  
or % of E negative donor units = 70%

If there are 70 E negative donors in a population of 100 donors, there   
should be approximately 2 E negative donors in a population of 3 donors.

Therefore, to obtain 2 donor units negative for the E antigen, typing 3   
 donor units will likely be required.

* 1. If the patient has been transfused within the last three months, separation of the patient’s own cells9.2 can be attempted or refer the sample out for genotype testing. Genotype testing can be performed in house if available.

1. **References**
   1. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine,5.3.4
   2. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 472,892.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised manual name * Renumbered and made minor wording revisions to section 2.0, 6.0 and 7.0 * In section 3.2, clarified “if stored at 1-6ºC * Revised wording to include “3-5% panel or screening cells” in section 4.0 * Revised wording of section 6.14 to include “Macroscopic agglutination” and “refer to manufacturer instructions” * Revised wording to include “genotype testing can be performed in house if available” in section 8.2 * Updated list of references to include latest versions/editions |