STANDARD OPERATING PROCEDURE FOR ANTIGEN TYPING
1.0 Principle

1.1 The patient’s pre-transfusion red cells and donor red cells must be tested to determine if the corresponding antigen is present, once an alloantibody has been identified.

2.0 Scope and Related Policies

2.1 When antibodies are found in patient’s serum/plasma or documented in their history, antigen typing shall be performed on patient’s red blood cells to determine the presence/absence of the corresponding antibody.

2.2 Antigen typing may be performed for patients requiring long term transfusion therapy (example: Sickle cell disease.) to help obtain phenotypically compatible units.

2.3 Antigen typing may be performed on patients with clinically significant autoantibodies.

2.4 To confirm any agglutination is a result of antigen-antibody reactions, a Direct Antiglobulin Test (DAT) must be performed when antigen typing requires an Indirect Antiglobulin Test (IAT) method.

2.5 Antigen typing can be difficult to perform if patients have been transfused in the past three months, a pre-transfusion sample should be used if available.

2.6 All donor red blood cells must be tested and found negative for any corresponding clinically significant antibody in the patient’s plasma/serum and/or in the patient’s history.

2.7 Release of blood before completion of testing may occur only with documented approval of a physician and must be clearly labelled that testing has not been completed.

2.8 Related Standard Operating Procedures:
   2.8.1 NL2010-012 Determining Specimen Suitability
   2.8.2 NL2010.013 Patient History Check
   2.8.3 NL2012-033 Preparation of Red Cell Suspensions
   2.8.4 NL2012-042 Quality Control of Reagents and Antisera
   2.8.5 NL09-005 Performing the Direct Antiglobulin Test
3.0 Specimens

3.1 Patient samples must be collected in EDTA anticoagulated tube or clotted tube.
3.2 Samples collected in EDTA can be used for antigen typing up to 14 days.
3.3 Donor cells can be antigen typed until expiration of donor unit.

4.0 Materials

Reagents:
- Polyspecific AHG containing anti IgG, C3d
- Antisera (specific to the antigen in question)
- Coombs control cells
- Positive and negative control cells
- Patient or donor red cell suspension

Supplies:
- Transfer Pipettes
- Test tubes (10 x 75mm or 12x75mm)
- Timer
- Manufacturer’s instructions specific to the antisera being used
- Isotonic saline

Equipment:
- Serological centrifuge
- Refrigerator with temperature between 1-10°C
- Cell washer (if used at your facility)
- 37(±1) °C waterbath or incubator

5.0 Quality Control

5.1 Perform quality control on all antisera used with a positive and negative control. The positive control must be heterozygous for the antigen in question whenever possible. If a heterozygous positive control is not available, any cell positive for the antigen can be used.

5.2 Record lot number, panel cell number and expiry date of all cells chosen for positive and negative controls.

5.3 Reagents shall be used according to manufacturer’s instructions.

5.4 The negative control must lack the antigen in question.
5.5 Positive and negative controls must be performed each day of use.

5.6 Each antigen tested must have a set of positive and negative controls.

5.7 If more than one lot number of antisera is being used for the same antigen, each lot number must have a set of positive and negative controls.

5.8 IgG coated cells must be added to all negative indirect antiglobulin test results to confirm AHG is present and working.
6.0 Process Flowchart

6.1 Process Flow for Patient Antigen Typing

Check specimen suitability

Is specimen suitable?

Yes

Has patient been transfused in the last 3 months?

No

Retrieve pre-transfusion sample, complete antigen worksheet with patient identification

No

Record lot number, cell number and expiry date on worksheet

Select cells for positive and negative controls

Add IgG coated cells (Indirect antiglobulin method)

Agglutination of red blood cells

No

Repeat test

Yes

Record on worksheet and LIS (confirm controls are valid)

Negative

No

Positive

Record on worksheet and enter results into LIS (confirm controls are valid)

Read, grade and record all test results

If incubation is required record incubation time and temperature on worksheet

Add antisera and red blood cell suspension to labeled test tubes

Prepare red blood cell suspension

Review manufacturers instructions for test procedure

Collect new sample

Is specimen suitable?

Yes

Is pre-transfusion sample available?

No

Do not antigen type

Has patient been transfused in the last 3 months?
6.2 Process Flow for Donor Antigen Typing

Determine the number of donor units that need to be screened

Obtain donor unit(s), check expiry date(s) and remove segment for testing

Retrieve donor antigen typing worksheet and complete appropriate information

Label test tubes for donor red cell suspension and positive and negative controls.

Select cells for positive and negative controls

Record lot number, cell number and expiry date on worksheet

Prepare RBC suspension

Review manufacturers instructions for test procedure

Add antisera and RBC suspension to labeled test tubes.

Record incubation time and temperature on worksheet.

Immediately Read, grade and record test results

Negative

Positive

Add IgG coated cells (indirect antiglobulin method).

Agglutination of red blood cells.

Record information on worksheet and enter into LIS (confirm controls are valid)

Record results on worksheet and enter into and LIS (confirm controls are valid)

Record lot number, cell number and expiry date on worksheet
Standard Operating Procedure for Antigen Typing

7.0 Procedure

7.1 Patient Antigen Typing

7.1.1 Check suitability of specimen (i.e. age of specimen and recent transfusions).

7.1.2 Retrieve antigen typing worksheet for patient. Transcribe patient’s name and identification number from specimen onto worksheet. Record date of testing.

7.1.3 If performing antigen typing by an indirect antiglobulin test confirm DAT is negative. If DAT is positive with anti-C3d only, antigen typing may be performed using anti-IgG reagent. If DAT is positive with anti-IgG reagent do not antigen type or pretreat cells with chloroquine or EGA.

7.1.4 Label a test tube for the patient with patient identification and the antigen being tested. Label a test tube with identification for a positive control and a negative control.

<table>
<thead>
<tr>
<th>Test tube number</th>
<th>Test tube label</th>
<th>Test tube contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>P1</td>
<td>Patient appropriate red cell suspension</td>
</tr>
<tr>
<td>#2</td>
<td>P1 Jkª</td>
<td>Patient red cell suspension + antisera</td>
</tr>
<tr>
<td>#3</td>
<td>Jkª positive control</td>
<td>Positive control cell suspension + antisera</td>
</tr>
<tr>
<td>#4</td>
<td>Jkª negative control</td>
<td>Negative control cell suspension + antisera</td>
</tr>
</tbody>
</table>

Example of labelling with Patient "P1" and antigen Jkª

7.1.5 Select appropriate red cells for a positive and negative control from antibody screening cells or identification panel cells.

7.1.6 Record lot number, cell number and expiry date on worksheet.

7.1.7 Prepare an appropriate patient red blood cell suspension according to the manufacturer’s directions for the antisera being used.

7.1.8 Review manufacturer’s instructions for method, temperature and time of incubation.

7.1.9 Add antisera to the appropriate tubes according to manufacturer’s instructions.
7.1.10 Add patient red cell suspension to appropriate tube according to manufacturer’s instructions for correct amount.

7.1.11 If manufacturer’s instructions includes an incubation period, record time of incubation and temperature in the appropriate place on the worksheet.

7.1.12 Immediately read all tubes following the manufacturer’s instructions, grade and record test result on worksheet.

7.1.13 Confirm all negative results performed by the indirect antiglobulin test by the addition of coombs control cells; centrifuge, grade and record results.

7.2 Donor Antigen typing

7.2.1 Determine the number of donor units that need to be screened. To do this use the following formula:

\[
\text{Number of antigen negative units required} = \frac{\text{number of screened units}}{\text{\% of antigen negative donors, expressed in decimal}}
\]

If you need 3 units of JKa antigen negative blood, the percent of the population that is negative for JKa is 23%.

3 ÷ 0.23 = 13. Thirteen units of blood will need to be screened to obtain 3 units of compatible blood.

If screening for multiple antigens is required, multiply the percent of antigen negative donors together. (Example: if you need K, S and JKa antigen negative blood, the percent negative for K in the population is 91%, the percent negative for S is 48% and JKa is 23%, then multiply those together 0.91 x 0.48 x 0.23 = 0.100. You need 4 units, therefore divide, 4 ÷ 0.100 = 40. Forty units will need to be screened to obtain 4 units negative for all 3 antigens).

7.2.2 Obtain donor units, check expiry date and remove segments for testing.

7.2.3 Retrieve donor typing worksheet and transcribe donor identification, expiry date and blood group
7.2.4 Label a test tube for the donor with donor identification and the antigen being tested. Label a test tube with identification for a positive control and a negative control.

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<td>D1</td>
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<tr>
<td>#2</td>
<td>D1 Jkª</td>
<td>Donor red cell suspension+ antisera</td>
</tr>
<tr>
<td>#3</td>
<td>Jkª positive control</td>
<td>Positive control cell suspension+ antisera</td>
</tr>
<tr>
<td>#4</td>
<td>Jkª negative control</td>
<td>Negative control suspension+ antisera</td>
</tr>
</tbody>
</table>

7.2.5 Select appropriate red cells for a positive and negative control from antibody screening cells or identification panel cell.

7.2.6 Prepare an appropriate donor red blood cell suspension according to the manufactures directions for the antisera being used.

7.2.7 Review manufacturer’s instructions for method, temperature and time of incubation.

7.2.8 Add antisera to the appropriate tubes according to manufacturer’s instructions for correct amount.

7.2.9 Add donor red cell suspension to appropriate tube according to manufacturer’s instructions for correct amount.

7.2.10 Record time of incubation and temperature in the appropriate place on the worksheet.

7.2.11 Immediately read all tubes following the manufacturer’s instructions, grade and record test result.

7.2.12 Confirm all negative results performed by the indirect antiglobulin test by the addition of coombs control cells and centrifuge; grade and record results.

8.0 Reporting

8.1 All reactions must be consistent with manufacturer’s instructions for grading and reporting.

8.2 Quality control performed must be ‘acceptable’.
8.3 Transcribe all information from worksheet(s) to Laboratory Information System (LIS), if applicable

9.0 Procedural Notes

9.1 False test results can occur when:

9.1.1 Specimen or reagents are contaminated

9.1.2 Specimen is not fresh

9.1.3 Red cell suspension is too strong/weak (always follow manufacturer’s instructions for red cell suspension when antigen typing)

9.1.4 Positive DAT when using Indirect Antiglobulin method

9.1.5 Improper incubation time or temperature

9.1.6 Improper centrifugation

9.1.7 Vigorous shaking when reading test results

9.1.8 Interruption of the wash procedure during an Indirect Antiglobulin Test method.

9.1.9 Any deviation from manufacturer’s instructions

10.0 Records Management

10.1 The recipient transfusion data file in the transfusion medicine laboratory shall be retained indefinitely.

10.2 All transfusion records in the recipient’s medical chart, including pre-transfusion serological tests results and worksheets for identification of atypical antibodies shall be retained in accordance with health care facility’s retention policy for medical records.

10.3 Quality control of blood components, blood products, reagents and equipment shall be retained for 5 years.

10.4 Date and time of specimen collection and phlebotomist’s identification shall be retained for 1 year.

10.5 Request form for serologic tests shall be retained for one month.
10.6 Documentation of staff training and competency must be kept for a minimum of ten years.

10.7 Temperature monitoring records for blood products must be kept a minimum of five years.

10.8 Records of blood components inspection prior to release must be kept for a minimum of five years.
References


