1. **Principle**

To evaluate the results obtained with a panel of red cells and determine whether additional testing is required to confirm or exclude the presence of an antibody.

1. **Scope and Related Policies**

This procedure is used when positive results are obtained with any cell in the panel of cells while performing antibody identification.

1. **Specimens – N/A**
2. **Materials**

**Supplies:** Antigram with recorded reaction gradings.

1. **Quality Control – N/A**
2. **Procedure**

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| * 1. Review each negative IAT result obtained with the panel and screening cells as follows: | * + 1. Determine whether the antigen expressed is homozygous (double dose) or heterozygous (single dose) on the panel and/or screening cells. See the table in step 6.1.4. | * + - 1. Look at the first negative cell in the sample panel displayed below (cell number 1). An example of allellic genes are Jka and Jkb. Cell number 1 possesses the Jka antigen but not the Jkb antigen. In other words, this cell most likely has a “double dose” or homozygous expression of the Jka antigen. The s, k, c and E antigens are also demonstrated in homozygous expression. |
| 6.1.1.2 Cell number 1 has a “single dose” or heterozygous expression of the Fya, Fyb, M and N antigens. |
| * + 1. When the antigen expression is homozygous on the cell, “cross off the cell” by placing an “X” over the antigen excluded e.g. Jka, s, k, c and E. | |
| * + 1. When the antigen is expressed heterozygously on the cell, “cross off the cell” by placing a slash “/” over the antigens Fya, Fyb, M and N. | |
| * + 1. Repeat steps 6.1.2 and 6.1.3 until all negative cells have been marked on the antigram sheet. See the example below. An antibody can be excluded if at least 2 “X” appear in the column below the antigen (e.g. E) | |

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| Cell | E | e | C | c | Lea | Leb | K | k | M | N | S | s | Fya | Fyb | Jka | Jkb | **IAT** |
| 1 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | + | + | 0 | **0** |
| 2 | 0 | + | + | 0 | + | 0 | + | + | - | + | 0 | + | 0 | + | 0 | + | **2+** |
| 3 | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | + | + | **0** |
| 4 | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | + | + | 0 | 0 | + | **2+** |
| 5 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | + | **2+** |
| 6 | 0 | + | + | 0 | 0 | 0 | + | + | 0 | 0 | 0 | + | 0 | + | + | 0 | **2+** |
| 7 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | **2+** |
| 8 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | + | + | + | **2+** |
| Auto |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | **0** |

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| Cell | E | e | C | c | Lea | Leb | K | k | M | N | S | s | Fya | Fyb | Jka | Jkb | **IAT** |
| 1 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | + | + | 0 | **0** |
| 2 | 0 | + | + | 0 | + | 0 | + | + | - | + | 0 | + | 0 | + | 0 | + | **2+** |
| 3 | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | + | + | **0** |
| 4 | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | + | + | 0 | 0 | + | **2+** |
| 5 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | + | **2+** |
| 6 | 0 | + | + | 0 | 0 | 0 | + | + | 0 | 0 | 0 | + | 0 | + | + | 0 | **2+** |
| 7 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | **2+** |
| 8 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | + | + | + | **2+** |
| Auto |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | **0** |

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| * 1. Review the panel and screening cells that have reacted (positive results) with the plasma tested. Check to see if a specific antibody pattern is formed. Circle the antigen(s) at the top of each column that corresponds to the probable antibody(ies). | |
| * 1. List the following information on the antigram sheet: * The probable antibody(ies) identified * The antibody(ies) that requires more testing to be excluded (excluded with heterozygous cell only or only one example of a homozygous cell) * The antibody(ies) that have not been excluded (not excluded with any cell). | |
| * 1. If, for the suspected antibody(ies), there are less than three positive cells reacting and three negative cells not reacting, additional selected cells should be tested to confirm identification. See Procedural Notes 8.1. | |
| * 1. When it is not possible to find the right selected cells to exclude an antibody, antigen typing the patient cells may be helpful. Generally, if the patient cells possess the antigen, the corresponding antibody can be excluded. Phenotyping of patient cells is only valid if the patient has not been transfused in the past three months. | |
| * 1. If a specific antibody pattern cannot be identified, consider: | * + 1. Multiple antibodies (variable strength of reactions will often be apparent). Perform the following tests: * Phenotype the patient cells for the antigens to the antibody(ies) that cannot be excluded. See Procedural Notes 8.2 * Test additional selected panel cells to exclude other specificities. * Prewarm technique may be helpful if the presence of a cold reactive antibody is suspected. See NRT.001 – Prewarm Technique |
| * + 1. Antibodies to HLA class I antigens (Bg antibodies) can be expressed on red cells. If approximately 30% of the cells are positive, suspect HLA antibodies. Some characteristics of HLA antibodies are: * Found in patients who have been previously transfused or pregnant * Clinically insignificant 9.2 |
| * + 1. If more than 90% of the cells are positive and reaction strengths are similar by the IAT phase, suspect the following types of antibodies: * Antibody to a high incidence antigen (e.g. Anti-k, anti-Lub). Try to find a cell that is negative for the antigen to test. * Cold agglutinins (e.g. anti-I or anti-HI). Reactions should be stronger at room temperature and/or 37°C. Prewarm technique may be helpful. See NRT.001 – Prewarm Technique. |
| * + 1. High titre-low avidity (HTLA) antibody.   HTLA antibodies are not generally   considered to be clinically insignificant. |
| * + 1. If the autocontrol, direct antiglobulin test (DAT) and all panels cells are positive, an autoantibody may be present. Additional testing may be required to exclude the presence of underlying clinically significant alloantibodies. |

1. **Reporting – N/A**
2. **Procedural Notes**
   1. If a patient plasma gives positive results with a minimum of three cells (carrying antigen “X”) and negative results with a minimum of three cells (lacking the antigen “X”), one can conclude that the plasma contains an antibody directed against the “X” antigen.
      1. In this case, the probability level (p value) is 0.05 (95%). This is the accepted minimum statistical value.9.1
      2. The use of two reactive and two nonreactive red cells is also an acceptable approach for antibody confirmation.9.2
   2. It is not always possible to find examples of homozygous cells. For some antigens it is acceptable to use 2 heterozygous cells to exclude the presence of an antibody (e.g. K, D).
   3. Routine exclusion of antibodies to low frequency antigens such as Cw, V, Kpa, Jsa, Lua, Wra is not required unless a reactive cell increases suspicion of the possibility.
   4. If the patient has not been recently transfused and has a negative DAT, phenotyping the patient’s red cells can aid in exclusion of antibodies (i.e. if antigen positive the correlating antibodies may be excluded).
3. **References**
   1. Judd’s Methods in Immunohematology 3rd Edition; 2008:309-313
   2. Roback JD, ed. AABBTechnical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 470-471.
4. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised manual name * Revised wording in section 6.1.4 to include “an antibody can be excluded if at least 2 “X” appear in the column below the antigen * Renumbered and revised wording of section 8.0 * Updated list of references to include latest versions/editions |