1. **Principle**

To identify cold reactive allo-antibodies such as anti-M, anti-Lea or anti-P1 .

Plasma is tested against a panel of eight or more group O cells of known antigenic composition, in the phase by which the antibody was initially detected. Positive reactions are compared to the reaction pattern of the antigens present on the panel of cells. These reactions are evaluated to identify the antibody(ies) present.

This procedure is also used in the identification of cold reactive auto-antibodies. Many cold reactive autoantibodies possess anti-I or anti-IH specificity.9.2 Include group O adult and cord cells as well as group A1 and A2 cells (if the patient is group A) and B cells (if the patient is group B) when identifying cold reactive antibodies.9.2

1. **Scope and Related Policies**

A cold panel is usually done when a cold reactive antibody is suspected. Examples:

* During an investigation of an ABO discrepancy
* When positive result(s) obtained in an immediate spin crossmatch and antibody screen was negative
* When the results of the panel for warm antibodies are inconclusive (especially when the 37°C results are significantly stronger than the IAT results)

1. **Specimens**

EDTA anticoagulated whole blood

1. **Materials**

**Equipment:** Serological centrifuge

Block for test tubes

Refrigerator

Microscope

**Supplies:** Test tubes – 10 x 75 mm

Serologic pipettes

**Reagents:** Panel of cells with corresponding antigram sheet

Normal saline

1. **Quality Control**
   1. An autocontrol should be tested in conjunction with the panel to help differentiate whether antibody(ies) detected are alloantibodies or autoantibodies.
   2. See QCA.001 – Quality Control of Reagent Red Cells and Antisera.
2. **Procedure**

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| * 1. Check the suitability of the specimen(s) | * 1. See PA.002 – Determining Specimen Suitability. |
| * 1. Record the following information on the antigram sheet | * + 1. Transcribe the following information from the specimen label: * Patient family and given names * Patient Identification number * Date of collection (time, optional) * Or, affix a computer label |
| * + 1. Date the test is performed. |
| * + 1. The method used for testing. This information is usually written above the column where the test results will be recorded. |
| * + 1. Record the identification of all cells to be tested e.g. cord cells, A1, A2 or B cells on the antigram sheet as required. |
| * + 1. Ensure that the antigram sheet corresponds to the panel of cells by comparing the lot number on the antigram sheet and on the vials of panel cells. |
| * 1. Centrifuge | 1. Centrifuge specimen(s) for 5 minutes at 3500 rpm or equivalent. |
| 1. Check the patient’s specimen(s) for abnormal appearance. See PA.002 – Determining Specimen Suitability step 6.5. |
| 1. Compare the patient name and identification number on all specimens with the corresponding information on the request form or computer screen. |
| * 1. Prepare a 3% cell suspensions (patient and cord, group A or B as required). | * + 1. Label a test tube with the patient’s family name; transcribe the family name from the specimen tube not from the request form. A preprinted 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 commercial cell suspension and adjust the strength of the suspension if necessary. |
| * + 1. Prepare 3% suspensions of group O cord cells, group A1, group A2 and B cells as required. |
| * 1. Label tubes | * + 1. Label the required number of tubes to be set up with family name and the panel cell /donor unit number or cord cell identification. The family name may be abbreviated to the first three letters. Place the tubes, in numerical order, in the block. |
| * + 1. Label one tube with patient’s family name and “auto.” Place the tube in the block. |
| * 1. Add specimen and cells to the labeled tubes as follows: | * + 1. Retrieve the specimen and compare the name and identification number on the specimen with the corresponding information on the antigram. | |
| * + 1. 2-3 9.2 drops of plasma into each tube. | |
| * + 1. 1 drop of the patient 3% red cell suspension into the tube labeled “auto.” | |
| * + 1. 1 drop of the appropriate panel cell into the corresponding labeled tubes. | |
| * + 1. Mix the contents of each tube. Compare each tube for appearance and volume. | |
| * 1. Incubate and Centrifuge | * + 1. Incubate at room temperature (RT), approximately 20-22°C, for 30 minutes. | |
| * + 1. Centrifuge tubes in a serologic centrifuge at 3400 rpm for 15 seconds. | |
| * + 1. Examine for hemolysis, resuspend and read macroscopically. | |
| * + 1. Grade and record results on the antigram sheet. See RT. 001 –Reading and Recording Hemagglutination Reactions. | |
| * + 1. If all panel cells are negative, report as 7.0 – Reporting. | |
| * 1. Interpret Results | |  |  | | --- | --- | | ***If*** | ***then*** | | panel cells are reactive but the autocontrol is negative | perform the antibody exclusion. See NRT.008 – Exclusion of Antibodies.  When there is no discernible specificity, consider reasons such as antigen variability and dosage. Some antigens (e.g., I, P1, Lea, Sda) are expressed in varying degrees on red cells from different adult donors. Red cells from individuals heterozygous for the gene that determines the antigen may express less antigen and may react weakly or be non-reactive. Consider the possibility of an antibody to a low incidence antigen | | all group O cells including the autocontrol are positive | determine specificity if possible with the use of cord cells and/or A1, A2 or B cells if applicable.9.1 See Procedural Notes 8.2. | | |
| * 1. Review history | * + 1. Review transfusion and pregnancy history (and drug therapy if auto control or DAT is positive). Record the history on the antigram sheet.  |  |  | | --- | --- | | ***If*** | ***then*** | | the patient has not been transfused in the last three months | confirm the antibody identified by phenotyping the patient cells for the corresponding antigen. The patient’s cells should type negative for the antigen to which the patient has the corresponding alloantibody.  See Procedural Notes 8.1. | | the patient has been transfused in the last three months | do not perform the phenotype testing on the current specimen. Phenotype testing should only be done if a pre-transfusion specimen is available. | | the specificity of the antibody is not considered clinically significant | antigen typing of the patient’s cells or donor units is not required. | | |
| * 1. Report Results | * + 1. Report the result of the antibody identification. See 7.0 – Reporting. | |
| * 1. Cross   Matching | 1. Donor units should be crossmatched using an antiglobulin test; donor units that are compatible may be transfused. A prewarm technique may be useful to obtain compatible donor units. | |
| 1. If crossmatch compatible donor units cannot be found by prewarm technique, antigen negative blood should be crossmatched. | |
| 1. Example of clinically insignificant cold/reactive antibodies: anti-HI, anti-P1, anti-Lea, anti-M, anti-N, anti-Lua, anti-Bg, anti-Sda, most examples of anti-Leb and anti-A1. | |
| * 1. Perform a clerical check. | * + 1. Complete the following checklist to ensure all areas have been completed: See Form NRT.006F. | |
| * + 1. Prepare an “antibody file” or card (for internal files) if it is considered clinically significant. | |
| * + 1. Keep all worksheets (i.e. antigram, phenotype sheets, etc.) as required by provincial regulations. | |
| * + 1. Report the antibody on the request form or in the computer. | |

1. **Reporting**
   1. For all antibody(ies) report the name of the antibody(ies) identified and indicate if it is or is not considered clinically significant.

* 1. **Procedural Notes**
  2. It is not necessary to antigen type the patient if the antibody is not considered to be clinically significant.
     1. Some facilities choose to phenotype for the corresponding and antithetical antigens if the antibody is considered clinically significant. Although desirable in some circumstances (patient will be chronically transfused), it is not necessary for antibody identification. See NRT.009 – Antigen Typing – Direct and Indirect Agglutination.
     2. Phenotyping for Lea and Leb antigens may be performed if either an anti-Lea or anti-Leb have been identified. Patients with one Lewis system antibody will type as Le (a-b-) except when the patient is pregnant. Lewis antigen typing is not reported during pregnancy due to weakened or missing expression.
  3. If all cells are reactive (equal strength), including the auto control, the specificity could be anti-Pr. Enzyme treated cells should be non-reactive if anti-Pr is present. 9.1

1. **References**
   1. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 923.
   2. Judd WJ. Methods in Immunohematology, 3rd ed., 1998: 68-70.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised name of manual * Revised name of procedure * Revised wording in section 1.0 to include “many cold reactive autoantibodies possess anti-I or anti-IH specificity” and list examples of cold reactive allo-antibodies. * Renumbered sections 6.0 and 8.0 and made minor revisions to wording * Added sections 6.2.4 and 6.4.5 * In section 6.6, added donor unit and cord cell and specified 2-3 drops of plasma according to the reference cited * Added “if antibody is not clinically significant then antigen typing is not required” in section 6.9 * Added “or is not considered to be clinically significant” in section 7.1 * Added “it is not necessary to antigen type if the antibody is not considered to be clinically significant” in section 8.1 * Updated list of references to include latest versions/editions |