1. **Principle**

To identify unexpected antibodies detected in the antibody screen.

Plasma is usually 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 reaction patterns of the antigen present on the red cells. The reactions are evaluated to identify the antibody(ies) present.

1. **Scope and Related Policies**
	1. A panel of cells is tested when the initial antibody screen is positive.
	2. When a patient has a clinically significant antibody or a previous history of clinically significant antibodies, red cells lacking the corresponding antigen(s) should be crossmatched using an antiglobulin or comparable technique.9.1
	3. “Selected cells” from a panel of cells are tested to exclude the presence of other clinically significant antibody(ies) when:
* An antibody, previously identified, reacts with the screening cells
* Additional cells are required to exclude an antibody (i.e., after exclusion procedure has been performed on an initial antibody panel).
1. **Specimens**

EDTA anticoagulated whole blood

1. **Materials**

**Equipment:**  Serologic centrifuge

 Cell washer

 Block for test tubes

 Water bath/Heating block at 37° C

 Microscope

**Supplies:** Test tubes – 10 x 75 mm

 Serological pipettes

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

 Anti-IgG

 IgG-coated cells

 Normal saline

 Potentiating reagents if used:

* Polyethylene glycol (PEG)
* Low Ionic Strength Solution (LISS)
1. **Quality Control**
	1. An autocontrol should be tested in conjunction with the panel to help differentiate whether antibody(ies) detected are allo or autoantibodies.9.2
	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). 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 computer label.
 |
| * + 1. Date the test is performed.
 |
| * + 1. The method used for testing (e.g., SIAT). This information is usually written above the column where the test results will be recorded.
 |
| * 1. Centrifuge the specimen(s) for 5 minutes @ 3500 rpm or equivalent.
 |
| * 1. Ensure that the antigram sheet corresponds to the panel of cells by comparing the lot number on the antigram sheet to the lot number on the vial of panel cells. Ensure the cells are not past the expiry date.
 |
| * 1. Prepare a 3% patient red cell suspension.
 | * + 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 pre-printed label may be used (ensure that the information on the label coincides exactly with the information on 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 red cell suspension and adjust the strength of the suspension if necessary.
 |
| * 1. Label the required number of tubes to be set up with patient’s family name and the panel cell number. 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. Compare the name and identification number on the specimen with the one on the antigram sheet.
 |
| * 1. Pipette 3 9.3 drops of plasma in each labeled tube. If a potentiating solution is being used add 2 drops of plasma.
 |
| * 1. Add 1 drop of patient 3% red cell suspension to the tube labeled “auto”.
 |
| * 1. Add 1 drop of the appropriate panel cell to each of the corresponding tubes.
 |
| * 1. If using, add 2 drops of potentiating solution to each tube.
 |
| * 1. Mix the contents of each tube. Compare each tube to ensure consistent volume.
 |
| * 1. Incubate at 37°C for 30-60 minutes. (Incubate at 37°C for 15 minutes if potentiating solution is being used.)
 |
| * 1. Check and record the temperature of the water bath or heating block on form QCA.006F.
 |
| * 1. After Incubation:
 | * + 1. Remove the tubes from the water bath observe the tubes for hemolysis and record if present.
 |
| * + 1. Mix tubes gently and observe for agglutination, record if present. (If PEG has been used, omit steps 6.16.3 and 6.16.4.)
 |
| * + 1. Centrifuge tubes at 3400 rpm for 10-15  seconds.
 |
| * + 1. Resuspend and read macroscopically only.  Grade and record the 37°C results on the  antigram sheet.
 |
| * + 1. Perform an antiglobulin test on all tubes.
* Wash the tubes 4 times if using a cell washer.
* Add 2 drops of anti-IgG.
* Mix the tubes immediately and centrifuge at 3400 rpm for 10-15 seconds.
* Immediately after centrifugation resuspend the cells and read macroscopically. If negative, read microscopically. See Procedural Notes 8.1.
* Grade and record results. See RT.001 – Reading and Recording Hemagglutination Reactions.
* Add 1 drop of IgG-coated red cells to any tube(s) with negative results. Centrifuge, resuspend cells, read macroscopically and record results.
 |
| * 1. Perform the antibody exclusion. See NRT.008– Exclusion of Antibodies.
 | * + 1. When there is no discernable specificity  consider reasons such as antigen dosage.  Red cells from individuals heterozygous for  the gene that determines the antigen may  express less antigen and may react weakly  or be non-reactive.
 |
| * + 1. When the results obtained with the panel  are **inconclusive**, antibody identification for  cold reactive antibodies may be helpful to  determine antibody specificity (especially if  the reactions at 37ºC are significantly  stronger than reactions in IAT). See  NRT.006 – Antibody Identification of Cold  Reactive Antibodies.
 |
| * + 1. If a passive anti-D is suspected, see  Procedural Notes 8.2.
 |
| * + 1. If testing required to complete the antibody  identification is not performed in your  laboratory, send the specimen and copy of  the worksheet (request form, antigram  sheets, antigen typing, etc.) to a reference  laboratory.
 |
| * + 1. If the panel is unexpectedly negative, repeat  the antibody screen. If the antibody screen  is negative, check for transcription error.
 |
| * 1. Review the diagnosis, transfusion and pregnancy history (and drug therapy if auto control or DAT is positive). Record the history on the antigram sheet.

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| *If* | *Then* |
| The patient has not been transfused in the last three months | Antigen type the patient cells for the corresponding antigen(s). The patient’s cells should be negative for the antigen corresponding to the alloantibody identified. See Procedural Notes 8.3 and NRT.009 – Antigen Typing – Direct and Indirect Agglutination. |
| The patient has been transfused in the last three months | Do not perform the antigen typing on the current specimen. Perform the antigen typing on a pre-transfusion specimen, if available. See Procedural Notes 8.4. |

 |
| * 1. Complete the following checklist to ensure that all steps have been completed: See Form NRT.007F
 |
| * 1. Sign or initial the antigram and/or worksheet(s).
 |
| * 1. Report the result of the antibody identification. See 7.0 – Reporting.
 |

1. **Reporting**
	1. Report the name of the antibody(ies) identified. A comment may be added indicating whether the antibody is considered to be clinically significant. Examples of most commonly encountered clinically significant warm reactive antibodies are: anti-D, anti-C, anti-E, anti-c, anti-e, anti-K, anti-Fya, anti-Fyb, anti-Jka, anti-Jkb, anti-S, anti-s.9.3
		1. If the required commercial antisera is not available, antigen negative donors must be obtained from the blood supplier. Refer to Procedural Notes 8.3.2
		2. Prepare the following documentation:
* An “antibody file” card or file for internal file
* A patient antibody card (wallet card)
* A report for the attending physician and copy to the medical record.
	+ 1. Keep all worksheets (i.e., antigram, phenotype worksheets, etc.) as required by provincial regulation.
	1. If a passive anti-D has been identified in an Rh negative woman who has received Rh Immune Globulin (RhIG) within the last 12 weeks, see Procedural Notes 8.2. Report: “Passive anti-D probably due to the injection of RhIG on \_\_\_\_(date of injection).”

7.3 If a clinically insignificant antibody is suspected, report: “Anti-\_\_\_\_\_ clinically insignificant.” Donor units should be crossmatched using an antiglobulin or comparable test; donor units that are compatible may be transfused. A prewarm technique may be useful to obtain compatible donor units. If crossmatch compatible donor units cannot be found by prewarm technique, phenotyped blood may be considered.

Examples of clinically insignificant antibodies are: anti-HI, anti-P1, anti-Leb, anti-M, anti-N, anti-Lua, anti-Bg, anti-Sda, most examples of anti-Lea and anti-A1.

1. **Procedural Notes**
	1. Tests should be read immediately after centrifugation. Interruption in testing may cause bound IgG to dissociate from red cells and either leave too little IgG to detect or may neutralize AHG reagent causing false negative results.
	2. Usually the reaction of a passive anti-D with D positive cells is weaker than grade 2 using the indirect antiglobulin test however, this is dependent on the date RhIG was administered, 3-5 days post administration the reaction strength may be significantly stronger. Patient history should be checked to confirm a recent injection of RhIG.
	3. Some facilities choose to phenotype for the corresponding antithetical antigens (e.g. when phenotyping for Fya antigen, test for Fyb as well). 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.
		1. 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.
		2. The specimen may have to be sent to a reference laboratory for phenotype testing when antibodies to low or high incidence antigens are identified:

Examples: anti-k, anti-Kpa, anti-Cw, anti-Lua, anti-Lub.

* + 1. Do not delay transfusion awaiting typing results if the suspected antibody is directed against a low prevalence antigen. Refer to facility policy to determine if confirmation of the patient’s antigen type is required.
		2. When anti-D is identified it is rarely possible to find the right panel cells (homozygous for C or E) to exclude anti-C or anti-E. In this case consider crossmatching CDE negative donor units.
		3. When red cells are coated with IgG autoantibodies it will be necessary to remove the IgG antibodies from the red cells before typing with antisera that requires an IAT methodology. Chemical modification of red cells can be done by using e.g.chloroquine, EGA. Alternatively, if available, monoclonal antisera may be used.
	1. If the patient has been transfused within the last three months, refer the sample out for genotype testing or perform in house if available.
1. **References**
	1. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine, 5.3.4.3, 5.3.7.2.3.
	2. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 469,475.
	3. Judd WJ. Methods in immunohematology, 3 rded. 1998:72-73, 290-293.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| March 1, 2014  | * Revised name of manual
* Revised wording of section 6.3 to include “Centrifuge the specimen(s) for 5 minutes @ 3500 rpm or equivalent”
* Changed wording in section 6.9 to “pipette 3 drops” in accordance with reference 9.3.
* Section 6.16.5- changed PA. 006 to RT. 001
* Renumbered section 7.0 and made minor grammatical changes
* Added “Patient history should be checked to confirm a recent injection of RhIG” to section 8.2
* Revised wording of section 8.3.5 and 8.4
* Updated list of references to include latest editions/versions.
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