1. **Principle**

To confirm the presence of passively acquired clinically significant immune anti -A and/or -B antibodies.

The antibody screen consists of testing the patient plasma against group A and B red cells.

The immune antibodies may cause direct agglutination of red cells, hemolysis at 37° C or coat the red cells with globulin (i.e., IgG). The selected cells are incubated with patient plasma at 37° C. After incubation, the cells are observed for direct agglutination and/or hemolysis, washed to remove unbound globulin and tested with antihuman globulin (AHG).

Direct agglutination, hemolysis or agglutination with AHG (indicating that the screening cells have been coated with globulin) confirms the presence of Immune anti-A and/or -B.

1. **Scope and Related Policies**
	1. Antibody screen shall be done at 37° C and include an indirect antiglobulin procedure which has been shown to have good sensitivity. Alternative test methods may be used provided there is appropriate documentation of sensitivity and the supplier’s instructions are followed. The use of an antiglobulin reagent that contains only anti-IgG is acceptable when performing an antibody screen.9.1
		1. A control system using red cells sensitized with IgG shall be applied to each antiglobulin test interpreted as negative.9.1
	2. Reverse grouping (A and B) cells may be used, or known donor cells. Group O cells are included as a control.
	3. Antibody screen for Immune anti-A and/or -B:
		1. A venous, capillary or cord blood specimen may be used for testing.
		2. Tests for antibody resulting from an ABO discrepancy between mother and neonate.
		3. If the neonate is receiving non-group O red cells, test for passively acquired maternal anti-A and/or anti-B must be performed by indirect antiglobulin test. Red cells selected for transfusion shall lack the corresponding antigen. 9.1

2.3.4 Tests for the presence of passive anti-A and/or B acquired
 through recent transfusion of non-ABO identical red cells,
 platelets, plasma or Intravenous Immune Globulin (IVIG).

1. **Specimens**

EDTA anticoagulated whole blood

Cord Blood

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

**Reagents:** Set of Reverse grouping cells or 3% saline suspensions from known group A and B donors

3% saline suspension known group O cells

 Anti-IgG

 IgG-coated control cells

 Normal Saline

1. **Quality Control**

See QCA.001 – Quality Control of Reagent Red Cells and Antisera

1. **Procedure**

|  |  |
| --- | --- |
| **STEP** | **ACTION** |
| 1. Check the suitability of the specimen(s)
 | 1. Ensure that the specimen information matches the request form. See PA.002 – Determining Specimen Suitability steps 6.1 – 6.4.
2. Centrifuge specimen for 5 minutes at 3500 rpm or equivalent, if required.
3. After centrifugation, check the patient’s specimen(s) for abnormal appearance. See PA.002 – Determining Specimen Suitability step 6.5.
 |
| 1. Perform a patient history check.
 | 1. See PA.003 – Patient History Check
 |
| 1. Label tubes
 | 1. Compare the patient name and identification number on all specimens with the information on the request form or computer screen
2. Label appropriate tubes: one with the patient's name, one with Imm A, and/or Imm B and one with O control. See Procedural Notes 8.1
 |
| 1. Add Patient Plasma/Serum
 | 1. Dispense 2 drops of patient plasma/serum to the tubes. See procedural note 8.4
 |
| 1. Add the appropriate 3% red cell
 | 1. 1 drop of A cell to the appropriate tube and/or 1 drop of B cell to the appropriate tube
2. 1 drop of O cell to the appropriate tube

***Note: Hold the pipette or dropper vertically when dispensing the cells***1. Mix all tubes. Examine all tubes for appearance and volume. If the volume or appearance is not consistent, test tubes should be discarded and all of the tests repeated.
 |
| 1. Incubate at 37° C.
 | 1. Incubate the tubes for 30 minutes at 37° C
2. Check and record the temperature of the water bath or heating block on QCA.006F
3. Remove the tubes from the water bath after incubation
4. Centrifuge tubes at 3400 rpm for 10-15 seconds
5. Observe for hemolysis. Record hemolysis, if present. See RT.001– Reading and Recording Hemagglutination Reactions
6. Resuspend and read macroscopically
7. Grade and record the 37° C results as per established procedure. See RT.001– Reading and Recording Hemagglutination Reactions
 |
| 1. Perform an antiglobulin test
 | 1. Wash the tubes 4 times. See RT.002– Cell Washing Automated and Manual
2. Add 2 drops of anti-IgG
3. Mix the tubes immediately and centrifuge tubes at 3400 rpm for 10-15 seconds
4. Immediately after centrifugation resuspend the cells and read macroscopically. If negative, read microscopically. See Procedural Notes 8.3
5. Grade and record results. See RT.001– Reading and Recording Hemagglutination Reaction
6. Add 1 drop of IgG-coated control cells to the tube(s) with negative results. Centrifuge tubes at 3400 rpm for 10-15 seconds, resuspend cells, read macroscopically and record results. Agglutination of grade 2 must be present or the test(s) must be repeated
 |
| 1. Interpret Results
 | 1. Interpret antibody screen results. See 7.0 – Reporting
 |
| 1. Perform a clerical check
 | 1. For each patient, check that:
* The name and identification number(s) are identical on all specimens and on the request form
* The donor unit numbers are identical on the test tubes and on the request form
* All test results have been recorded
1. The test results have been reported and interpreted correctly
 |
| 1. Complete paperwork
 | 1. Initial or sign and record the completion time and date on the request form or verify in the computer
2. Verification of results must be recorded. See 7.0 Reporting
 |

1. **Reporting**
	1. No agglutination or hemolysis of red cells indicates that antibodies were not present or were undetected. Report the antibody screen as negative.
	2. Agglutination or hemolysis indicates the presence of immune antibodies. Report the antibody screen as: Immune \_\_\_\_\_\_ (anti -A and/or -B) present.
	3. If the Group O cell is positive initiate an antibody investigation. See NRT.007 - Antibody Identification of Warm Reactive Antibodies.
2. **Procedural Notes**
	1. Label a test tube with the first three letters of the patient’s family name; transcribe this from the specimen tube, not from the request form. A pre-printed label may be used.
	2. Hold the pipette or dropper vertically when dispensing the plasma or reagents.
	3. Tests should be read immediately after centrifugation. Delay may cause bound IgG to dissociate from red cells and either leave too little IgG to detect or neutralize AHG reagent causing false negative results.9.2
	4. The incubation times and the volume and concentration of red cells indicated are those given in the literature. Individual laboratories may choose to standardize with somewhat different values. Sensitivity can be increased by increasing ratio, decreasing the red cell concentration from 5% to between 2% and 3% or by adding 4 drops serum/plasma to 1 drop of a standard red cell suspension.9.2
3. **References**
	1. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine, 5.3.5.3, 5.3.5.4, 5.9.2.4.
	2. Roback JD ed AABB Technical Manual 17th ed. Bethesda MD; AABB; 2011: 443-446.
4. **Revision History**

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| --- | --- |
| **Revision Date** | **Summary of Revision** |
| January 31, 2014 | * Revised name of manual
* Changed document number from RT 008 to RT 014
* Revised wording in principle to specify “the presence of passively acquired” antibodies
* Added section 2.3.4 regarding tests for the presence of passive anti-A and/or anti-B
* Added reference to “*Procedural Notes* 8.4” in section 6.7
* Changed reference in 6.12.3, 6.12.5, 6.12.6.5 from PA 006 to RT 001.
* Changed reference in 6.12.6.1 from PA 005 to RT 002
* Renumbered 8.0 *Procedural Notes*
* Updated references to include the most recent editions/versions
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