1. **Principle**

To detect unexpected clinically significant antibodies.

The antibody screen consists of testing the patient plasma against a set of screening cells of known antigen composition.

Red cell antibodies may cause direct agglutination or lysis of red cells, or may coat the red cells with globulin (i.e., IgG). Screening cells are incubated with patient plasma at 37° C. After incubation, the cells are observed for direct agglutination or hemolysis, washed to remove unbound globulin and antihuman globulin (AHG) is added. After centrifugation, reactions are read macroscopically and microscopically. The addition of a potentiating reagent that accelerates antibody binding to the red cells may be used. (Low ionic strength solution – LISS or polyethylene glycol – PEG).

Direct agglutination or hemolysis usually indicates the presence of IgM antibodies (e.g., cold antibody). Agglutination with AHG indicates that the screening cells have been coated with globulin (IgG).

Some of the clinically significant antibodies usually detected by AHG phase are antibodies of the Rh, Kell, Duffy, Kidd, and MNS systems.

1. **Scope and Related Policies**

Note: ABO grouping, Rh typing and antibody screen together make up a type and screen procedure.

* 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. A minimum of two reagent cells that express a wide variety of blood group antigens shall be used for antibody screening. Red cells with a double expression of antigens should be used.9.1
	3. Reagent red cells for prenatal and pre-transfusion antibody screening shall not be pooled.9.1
	4. Antibody screen on neonatal patients:
		1. A venous or capillary blood specimen shall be used for all pre-transfusion testing. Cord blood must not be used for pre-transfusion testing.9.1
			1. Compatibility testing should be performed with maternal plasma. A venous or capillary blood specimen collected from the neonate may be used if maternal plasma is not available.9.1
		2. The initial pre-transfusion blood specimen shall be tested for ABO and Rh antigens and for clinically significant antibodies.9.1
			1. If the initial antibody screen is negative, further compatibility testing during the current hospital admission in the first four months of life is not required.9.1

If the initial pre-transfusion antibody screen demonstrates clinically significant red cell antibodies, the donor red cells shall have compatibility testing performed using an antigloblin or comparable technique and shall lack the corresponding antigens, unitl the antibody is no longer demonstrated in the neonates’s plasma/serum. then all red cells required for transfusion shall have compatibility testing performed and must be phenotypically negative for the corresponding antigens.9.1

* 1. Antibody screen on patients who have received RhIg:
		1. Select cells may be used for patients who have received RhIg within the previous three months. These should include one R2 R2 cell andr', r'' and r cells, in combination to exclude the presence of clinically significant antibodies that may have developed.
1. **Specimens**

EDTA anticoagulated whole 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 screening cells (2 or 3 vials, not pooled)

 Anti-IgG

 IgG-coated control cells

 Normal saline

 LISS or PEG (if used)

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 label information matches the request form. See PA.002 – Determining Specimen Suitability steps 6.1 – 6.4.
 |
| 1. Perform a patient history check.
 | 1. See PA.003 – Patient History Check.
 |
| 1. Prepare specimen
 | 1. Centrifuge specimen for 5 minutes at 3500 rpm or equivalent.
2. Retrieve the patient specimen(s) from the centrifuge and check the specimens for abnormal appearance. See PA.002 – Determining Specimen Suitability step 6.5.
3. Compare the patient name and identification number on all specimens with the information on the request form or computer screen.
 |
| 1. Label tubes
 | 1. Label tubes as per established procedure. See PA.004 – Labeling of Test Tubes and Block Set Up for Compatibility Testing. An autocontrol test is not required. See Procedural Notes 8.1.
 |
| 1. Add plasma and 3% reagent red cell suspensions
 | 1. Dispense 3 drops of patient plasma to the tubes9.2 (see procedural notes 8.3). Hold the pipette or dropper vertically when dispensing the plasma or reagents. If a potentiating solution is used, add 2 drops of patient plasma to the tubes.
2. 1 drop of screening cell 1 to the appropriate tube.
3. 1 drop of screening cell 2 to the appropriate tube.
4. 1 drop of screening cell 3 to the appropriate tube. See Scope and Related Policies 2.2
5. 1 drop of patient 3% red cell suspension to tube labeled “auto” (optional).
6. If using, add 2 drops of potentiating solution to each tube (or follow reagent manufacturer’s instructions)
7. Mix all tubes. Examine all tubes for appearance and volume.

|  |  |
| --- | --- |
| ***If*** | ***Then*** |
| volume or appearance is not consistent, | test tubes should be discarded and repeat steps beginning at 6.4. |
| Volume or appearance is consistent | Proceed to 6.6 |

 |
| 1. Incubate at 37° C
 | 1. Incubate the tubes 30-60 minutes at 37° C (incubate at 37°C for 15 minutes if potentiating solution is used).
2. Check and record the temperature of the water bath or heating block on QCA.006F.
3. Remove the tubes from the water bath. *If PEG solution is in use, go directly to 6.7*
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 Antiglobulin test
 | 1. Wash the tubes 4 times. See RT.002 – Cell Washing Automated and Manual.
2. Add 2 drops of anti-IgG to each tube.
3. Mix the tubes immediately and centrifuge at 3400 rpm for 10-15 seconds.
4. Immediately after centrifugation re-suspend the cells and read macroscopically. If negative, read microscopically. See Procedural Notes 8.4.
5. Grade and record results as per established procedure. See RT.001–Reading and Recording Hemagglutination Reactions.
6. Add 1 drop of IgG-coated 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 2 or greater must be present or the test(s) must be repeated.*
7. Interpret antibody screen results. See 7.0 – Reporting.
 |
| 1. Perform a clerical check.
 | For each antibody screen check that:* The patient name and identification number are identical on all specimens and on the request form
* The patient name is the same on all test tubes and on the request form
* The test results have been recorded, including the results of the IgG-coated control cells
 |
| 1. Complete paper work
 | * + 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 unexpected antibodies were not present or were undetected. Report the antibody screen as negative.
	2. Agglutination or hemolysis may indicate the presence of unexpected antibodies. Report the antibody screen as positive and investigate.
2. **Procedural Notes**
	1. An autocontrol is optional and would not usually be required on a routine basis.
		1. If an autocontrol is set up, mix the specimen and prepare a 3% red cell suspension of the patient’s cells. See RT.003 Preparation of a 3% Red Cell Suspension.
	2. Hold the pipette or dropper vertically when dispensing the plasma or reagents.
	3. 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 of plasma to cells (i.e. by adding 4 drops of plasma to 1 drop of a standard red cell suspension or by decreasing the red cell concentration from 5% to between 2% and 3%.9.3
	4. 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.9.3
3. **References**
	1. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine, 5.3.5 5.9.2.
	2. Judd WJ. Methods in Immunohematology, 3rd ed. Bethesda, MD: American Association of Blood Banks, 2008; 71-74.
	3. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda,MD: American Association of Blood Banks, 2011; 446; 443
4. **Revision History**

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| --- | --- |
| **Revision Date** | **Summary of Revision** |
| January 31, 2014 | * Revised name of manual
* Changed document number from RT 005 to RT 008
* Revised wording of section 1.0 with reference to after centrifugation.
* Renumbered section 2.4.2 and revised wording of section 2.4.2.1 to clarify
* Added “LISS or PEG (if used) to section 4.0 under Reagents
* Added “if potentiating solution is used, add 2 drops of patient plasma to the tubes” to section 6.7
* Added section 6.8.5
* Added “Note: if PEG solution is in use, go directly to 6.12.6” to section 6.12
* Changed reference made in 6.12.3 from PA 006 to RT 001
* Changed reference made in 6.12.5 from PA 006 to RT 001
* Changed reference made in 6.12.6.1 from PA 005 to RT 002
* Changed reference made in 6.12.6.5 from PA 006 to RT 001
* Renumbered section 7 and 8.
* Changed reference made in 8.1.1 from RT 014 to RT 003
* Revised wording of section 8.3 referring to incubation times
* Updated list of references to include the most updated versions/editions
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