1. **Principle**

To prevent the reactivity of cold reacting clinically insignificant antibodies while detecting warm reacting clinically significant antibodies.

Immediately before testing, the patient’s plasma and the test red cells are warmed separately to 37°C. The plasma and cells are then mixed together, incubated at 37°C, washed in warm saline to remove the unbound cold antibody and then carried to the antiglobulin test using anti-IgG. By maintaining a 37°C temperature, only clinically significant antibodies capable of reacting at body temperature should be detected. If a potentiating solution is used this must also be maintained at 37°C.

1. **Scope and Related Policies**
   1. A prewarm technique is usually done when a cold reactive, clinically insignificant antibody is present or suspected in a patient’s specimen.
   2. The prewarm technique should be performed by tube method.
2. **Specimens**

EDTA anticoagulated whole blood

1. **Materials**

**Equipment:** Serological centrifuge

Block for test tubes

Water bath/Heating block at 37°C

Microscope

**Supplies:** Test tubes – 10 x 75 mm

Serological pipettes

**Reagents:** Screening, donor and/or panel cells

Anti-IgG

IgG-coated cells

Normal saline

Potentiating reagents, if used:

* Polyethylene Glycol (PEG) **or**
* Low Ionic Strength Solution (LISS)

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

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| * 1. Warm normal saline | 1. Warm normal saline to 37°C to use for washing cells in the indirect antiglobulin technique in step 6.7 (minimum 10 minutes). |
| * 1. Warm the patient plasma.   *(See Procedural   Notes 8.1.)* | * + 1. Label a tube with the patient’s full name and identification number; * Transcribe the given name from the specimen tube not from the request form * A pre-printed label may be used (ensure the information coincides exactly with the information on the request form and specimen tube). |
| * + 1. Pipette sufficient amount of plasma for testing into the labeled tube. The amount of plasma may vary depending on the test method and numbers of tubes required in testing. |
| * + 1. Leave the pipette in the tube. |
| * + 1. Incubate at 37°C for 10 minutes. |
| * 1. Prewarm the potentiating solution if using.   (*See Procedural   Notes 8.1.)* | * + 1. Label a tube with the name of the potentiating solution (PEG or LISS). |
| * + 1. Pipette sufficient volume of the potentiating solution for tests being performed into the labeled tube. |
| * + 1. Leave the pipette in the tube. |
| * + 1. Incubate at 37°C for 10 minutes. |
| * 1. Set up Auto-control   *(if required)* | * + 1. Label a test tube with the patient full given name; * Transcribe the given name from the specimen tube not from the request form. * A pre-printed label may be used (ensure the information coincides exactly with the information on the request form and specimen tube). |
| * + 1. Dispense 2 drops of whole blood (or equivalent: 1 drop of packed cells) into the labeled tube. |
| * + 1. Add 0.5 mL of normal saline and mix to resuspend to 3%. |
| * + 1. Compare with a 3% commercial red cell suspension and adjust the strength of the suspension if necessary. |
| * + 1. Label a tube with the patient’s name and “auto”. |
| * + 1. Pipette 1 drop of the patient 3% red cell suspension into the labeled tube. |
| * + 1. Incubate at 37°C for 5 minutes. |
| * 1. Warm the cells to be tested. | * + 1. Label the appropriate number of tubes with the patient name and cell number (e.g. screening cell 1, panel cell 2, etc.) |
| * + 1. Pipette 1 drop of the appropriate well-mixed test cell suspension into each labeled tube. |
| * + 1. Incubate at 37ºC for 5 to 10 minutes. |
| * 1. Add patient plasma to labelled tubes and Incubate. | After incubation, using the pipette(s) left in the tube(s):   * + 1. Transfer 3 9.2 drops of warmed patient plasma to each tube containing cells.  |  |  | | --- | --- | | ***If*** | ***then*** | | PEG or LISS is used | add 2 drops of patient plasma then add 2 drops of warmed potentiating solution to each tube. | |
| * + 1. Mix each tube, keeping them at 37°C. |
| * + 1. Incubate the mixed tubes at 37°C for 30-60 minutes; if potentiating solution was added incubate tubes at 37°C for 15 minutes. |
| * + 1. Check and record the temperature of the water bath or heating block on form QCA.006F. |
| * 1. Antiglobulin Phase testing | * + 1. Proceed to the antiglobulin phase. See RT.002 – Cell Washing Automated and Manual. * *Manually wash all tubes times 4 with warm normal saline from step 6.1* |
| 1. Interpret the result | * 1. Refer to section 7.0 – Reporting for interpretation. |
| * 1. Perform a clerical check. | * + 1. Ensure that the specimen label information   for each specimen tested coincides with the   information on the corresponding test tubes   and request form. |
| * 1. Initial | 1. Initial or sign and record the completion time and date on the request form or in the computer. |
| 1. Verification of results must be recorded. See 7.0 Reporting. |

1. **Reporting**
   1. No agglutination or hemolysis of red cells, by prewarm technique, indicates that unexpected clinically significant antibodies were not

present or were undetected.

* 1. Agglutination or hemolysis of red cells by prewarm technique indicates the presence of unexpected clinically significant antibodies.

1. **Procedural Notes**
   1. One mL of patient plasma (or potentiating solution if used) will be sufficient to perform an antibody screen. If more red cells are to be tested, increase the volume of patient plasma/potentiating solution accordingly. One mL = 20 drops (approximately).
   2. For weak, cold reactive antibodies, warming up the patient plasma before adding it to the reagent red cells (and potentiating solution if used) may be sufficient to prevent interference by the cold reactive antibody.
   3. Test 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.
   4. The prewarm procedure will not detect alloantibodies that agglutinate at 37°C or lower and are not reactive in the antiglobulin phase. To demonstrate these antibodies, testing (including centrifugation) may have to be done at 37°C.
2. **References**
   1. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 900-901.
   2. Judd WJ, Methods in Immunohematology 3rd ed.; 2008:27
3. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised name of manual * Revised wording of section 2.2 * Restructured and renumbered section 6.0 * Revised wording of section 6.4.1 to include detailed instructions on labeling the test tubes * Added “See RT. 002- Cell Washing Automated & Manual” to section 6.7.1 * Updated reference list to include most recent editions/versions |