1. **Principle**

To determine the thermal range of cold autoantibody by performing a screen test at 37°C, 32°C, 28°C and 22°C.

1. **Scope and Related Policies** 
   1. The Antibody Test - Cold Autoagglutinins procedure must be positive to proceed with this procedure.
   2. All tests will be set up using warm separated (37°C) plasma.
2. **Specimen**

EDTA anticoagulated whole blood

1. **Materials**

**Equipment:** Serological centrifuge

Block for test tubes

Water bath/Heating block at 37°C, 32°C, 28°C and

22°C

**Supplies:** Test tubes - 10 x 75 mm

Serological pipettes

**Reagents:** 3% saline suspension of Group O P1 negative adult (I) cell (screening cell)

3% saline suspension of patient’s red cells

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

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| * 1. Check the suitability of the specimen to ensure that the specimen label information matches the request form. See PA.002 - Determining Specimen Suitability. |
| * 1. Perform a patient history check. See PA.003 - Patient History Check. |
| * 1. Warm separate patient specimen by setting in 37°C water bath/heating block for a minimum of 15 minutes (with periodic mixing). Spin specimen approximately 2-5 minutes at 3000 rpm and place in 37°C water bath immediately after spinning. With a pipette warmed to 37°C remove plasma into a clean, labeled tube avoiding any cells. Re-spin plasma sample to clear. |
| * 1. Label 2 - 10 x 75 mm tubes with the first 3 letters of the patient family name. On one of the tubes write adult and on the 2nd tube write auto. |
| * 1. Warm RBCs, plasma and the 10 x 75 mm tubes to 37°C. |
| * 1. Dispense 3 drops of patient plasma into each of the 2 labeled tubes. |
| * 1. Dispense 1 drop of the 3% O adult suspension into the tube labeled adult. |
| * 1. Dispense 1 drop of the 3% patient suspension into the tube labeled auto. |
| * 1. Place tubes in a 37°C water bath/heating block and incubate for 1 hour. |
| * 1. Centrifuge at 3400 rpm for 10-15 seconds. Resuspend tubes and examine macroscopically for agglutination. Grade and record the results as per established procedure. See RT.001 - Reading and Recording Hemagglutination Reactions. |
| * 1. Place tubes in a 32°C water bath/heating block and incubate for 1 hour. |
| * 1. Centrifuge at 3400 rpm for 10-15 seconds. Resuspend tubes and examine macroscopically for agglutination. Grade and record the results as per established procedure. |
| * 1. Place tubes in a 28°C water bath/heating block and incubate for 1 hour. |
| * 1. Centrifuge at 3400 rpm for 10-15 seconds. Resuspend tubes and examine macroscopically for agglutination. Grade and record the results as per established procedure. |
| * 1. Place tubes in a 22°C water bath/heating block and incubate for 1 hour. |
| * 1. Centrifuge at 3400 rpm for 10-15 seconds. Resuspend tubes and examine macroscopically for agglutination. Grade and record the results as per established procedure. |
| * 1. Interpret the thermal range results. See 7.0 Reporting. |
| * 1. Initial or sign and record the completion time and date on the request form or verify in the computer. |
| * 1. Perform a clerical check. Check that the patient name and identification number are identical on all specimens and on the request form. Check that the patient name is the same on all test tubes and on request form. Check that the test results have been recorded. Check that the test results have been interpreted correctly. |
| * 1. Report the result of the thermal range. See 7.0 Reporting. |

1. **Reporting**
   1. The thermal range is reported as the highest temperature where autologous cell reactivity occurs. For example you may have no reaction at 37°C but reactivity at 32°C, so the thermal range would be reported as 32°C.
2. **Procedural Notes**
   1. Cold agglutinin titrations and assays to determine thermal range are not routinely performed. These assays are performed only upon physician request.
3. **References**
   1. Judd, WJ ed. Judd’s Methods in Immunohematology, 3rd ed., Bethesda, MD: pg. 435-436.
4. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Revised section 4.0 * Revised and renumbered section 6.0; added section 6.2 * Changed PA.006 to RT.001 in section 6.10 * Revised list of references |