1. **Principle**

Determination of the titre of an antibody in a patient's blood sample is performed by straightforward serial dilutions of the plasma. Cold reactive autoantibodies, if reactive at very high titres, may suggest a pathologic cold agglutinin disease.

1. **Scope and Related Policies** 
   1. All tests will be set up using warm separated (37°C) plasma.
   2. The antibody test – cold autoagglutinins procedure must be positive to proceed with this procedure.
2. **Specimen**

EDTA anticoagulated whole blood preferably less than 72 hours old.

1. **Material**

**Equipment:** Serological centrifuge

Block for test tubes

Microscope

4°C refrigerator

37°C water bath/heating block

**Supplies:** Test tubes – 10 x 75 mm

Test tubes - 12 x 75 mm

Serological pipettes

**Reagents:** Normal saline

3% cell suspension of group O Adult (I) Cells (P1 neg screening cell). See Procedural Notes 8.5.

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. 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. Prepare a master titre of the test plasma. Label required number of 12 x 75 mm tubes numerically and with the first 3 letters of the patient's family name. See Procedural Notes 8.1. |
| * 1. Dispense 0.5 mL of normal saline into each tube except # 1. See Procedural Notes 8.2. |
| * 1. Dispense 0.5 mL of patient’s plasma into tubes #'s 1 and 2. |
| * 1. Mix the contents of tube # 2 ten times avoiding air bubbles. Using a clean pipette tip transfer 0.5 mL to tube #3 See Procedural Notes 8.3. |
| * 1. Repeat step 6.6 mixing the contents of tube #3 and transferring to tube #4 and continue until the last tube is reached. |
| * 1. Label the required number of 10 x 75 mm tubes numerically and with the first three letters of the patient’s family name. |
| * 1. Transfer 2 drops of each diluted plasma into the appropriately labelled tubes. |
| * 1. Dispense one vertical drop of OI P1 negative cells to each tube. |
| * 1. Mix and incubate at 4°C for 1-2 hours. Centrifuge at 3400 rpm for 10-15 seconds. |
| * 1. Examine each tube for agglutination macroscopically and record results. |

1. **Reporting** 
   1. Report the temperature and the reciprocal of the highest dilution giving a grade 1 reaction (e.g. titre 16 OI cells at 4°C).
   2. See Procedural Notes 8.4 for clinical significance.

1. **Procedural Notes**
   1. Titres are often set up to a final titre of 4096. This would require 13 tubes (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048, 1:4096).9.1
   2. Do not use less than 0.5 mL volumes for diluting, inaccuracies can occur with smaller volume.9.1
   3. It is critical that separate pipette tips are used when transferring the diluted plasma. This will minimize carryover of antibody.
   4. If titre is > 64, it is considered clinically significant, > 1000 it is likely to cause hemolysis, < 64 likely not clinically significant. 9.1, 9.2
   5. If the autoantibody specificity is not anti-I, cells that have the relevant antigen should be selected (instead of or in addition to the OI cell) to perform the titration e.g. anti-i.9.2
2. **References**
   1. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: pg 923-924.
   2. Judd WJ ed. Judd’s Methods in Immunohematology 3rd ed. Bethesda, MD; 2008:439.
3. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Revised wording in section 3.0 from “red cells” to “whole blood.” * Added “37ºC waterbath/heating block” to section 4.0 – *Equipment* * Revised wording of section 6.0 * Added section 7.2 * Revised and renumbered section 8.0 * Updated list of references to include most recent editions. |