1. **Principle**

If RBCs are spontaneously agglutinating because of RBC-bound IgM:

IgM molecules are susceptible to cleavage by thiol reagents such as Dithiothreitol (DTT), resulting in dispersement of spontaneously agglutinating RBC’s due to IgM antibodies. This results in RBCs suitable for ABO and Rh typing, and the detection of IgG and C3 by direct antiglobulin testing.

1. **Scope and Related Policies**

Treatment with chloroquine diphosphate may be more efficient than DTT for ABO and Rh typing, but cannot be used for detecting IgG as it removes IgG bound to RBC’s.

1. **Specimen**

50% suspension of washed RBCs in normal saline

1. **Material**

**Equipment:** Block for test tubes

Water bath/Heating block at 37°C

**Supplies:** Test tubes – 10 x 75 mm

Serological pipettes

**Reagents:** 0.9% Saline

Anti-IgG

IgG-coated cells

Bovine serum albumin (BSA) - 6% wt/vol. in PBS.

0.01 M DTT - 0.154 g DTT diluted to 100 ml in saline.

Alternatively: A 1 in 10 dilution in saline of 0.1 M DTT (0.154 g DTT in 10 ml PBS) can be made. Aliquots of 0.01 M DTT can be frozen in glass at -20°C for up to 6 months.

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

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| * 1. Mix equal volumes of .01 M DTT and a 50% suspension of RBCs. |
| * 1. Incubate at 37°C for 15 min. |
| * 1. Wash the RBCs 3-4 times with saline and resuspend to 3%. |
| * 1. Test the treated RBCs with 6% BSA. If agglutination is still present, repeat treatment. See Procedural Notes 8.2. |
| * 1. Perform ABO, Rh or DAT as necessary. |

1. **Reporting – N/A**
2. **Procedural Notes**
   1. Treatment of RBCs with DTT will not remove in vivo bound IgG or complement components from the RBC membrane.
   2. If RBCs are still reactive with 6% BSA, try treating a fresh aliquot with 0.05M DTT for 15-30 minutes at 37°C. Treating with 0.1 M DTT causes increased hemolysis.
3. **References**
   1. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: pg 890.
4. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Revised wording of sections 1.0 & 2.0 * Specified “0.9% saline” in section 4.0- *Reagents* * Updated list of references to include most recent editions |