1. **Principle**

Incubating whole blood with a low ionic sucrose solution enhances the uptake of complement components onto red cells. Trypsin will cleave C3b to expose the C3d and C4d sites. Anti-C3d is used in polyspecific anti-human globulin and monospecific anti-complement reagents to detect complement coating red cells *in vivo*.

1. **Scope and Related Policies**
   1. All reagents prepared in-house that contain a controlled substance must be labeled with a workplace label as per WHMIS legislation. 9.1
   2. Complement coated cells will be prepared on an as needed basis for the evaluation of AHG reagents.
   3. These cells may be frozen in LN2 for future use for up to 10 years.
   4. For procedures requiring control cells for Anti-C3d for example evaluation of antihuman globulin reagents or to confirm anti-C3 was added to the test. 9.2
2. **Specimen – N/A**
3. **Material**

**Equipment:** Centrifuge

Block for test tubes

Mechanical stirrer

Ice Bucket

Metal basin

**Supplies:** Test tubes – 10 x 75 mm

Serological pipettes

Erlenmeyer flask

Squeeze bottle

Ice Cubes

pH strips

**Reagents:** Phosphate Buffered Saline (PBS)

Anti-coagulated whole blood (CPD or ACD)

See Procedural Note 8.1

Solution A

Sucrose - 23.1g

Na2 EDTA 2H20 - 0.395g

NaH2 PO4 H20 - 0.173g

Distilled H20 - Made up to a final volume of 250 mL

Solution B

Sucrose - 23.1 g

Na2 EDTA 2H20 - 0.395 g

Na2H PO4 - 0.178 g

Distilled H20 - Made up to a final volume of 250 mL

Solution C

MgCl2 6H20 - 0.18 g in 10 mL Distilled H20

0.1% Trypsin (DIFCO Lab)

1. **Quality Control** 
   1. Serological pipettes should be maintained as per manufacturers recommendations including adequate volume delivery, reduction of carryover and absence of contamination. 9.3
   2. Centrifuge equipment shall be maintained as per manufacturer’s instruction including speed of rotation and timing device9.3
2. **Procedure**

Procedure 1: For Preparation of C3b coated RBC

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| * 1. Prepare the above solutions, chill "squeeze" bottle with 9% saline 0-1°C (in ice bucket). |
| * 1. To 250 mL of Solution A, add a sufficient amount of Solution B, (usually 15- 25 mL) to produce a mixture at pH 5.1. |
| * 1. Place 195 mL of the mixture in Erlenmeyer flask and add mechanical stirrer. |
| * 1. Place water and a few ice cubes in metal basin. |
| * 1. Put flask into basin. |
| * 1. Once mixture is chilled, add 1 mL of ACD or CPD anticoagulated blood. |
| * 1. Immediately add 0.1 mL of solution C. |
| * 1. Place on Magnetic Stirrer and stir for 1 hour maintaining temperature at 0-1°C with ice in basin. |
| * 1. Wash and suction x 3 with 0.9% cold saline kept in ice bucket. |
| * 1. Prepare appropriate cell suspension. |

Procedure 2: For Preparation of C3d (and C4d) coated RBC

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| * 1. Pack C3b coated RBCs prepared in Procedure # 1, suction off saline. |
| * 1. For each drop of packed C3b coated cells, add 1 drop of 0.1% Trypsin in PBS (pH 7.3) ratio 1:1. See Procedural Note 8.2. |
| * 1. Incubate at 37°C for 30 minutes. |
| * 1. Wash RBCs x 3 in 0.9% saline. |
| * 1. Make appropriate cell suspension. |
| * 1. These cells may be frozen in LN2 for future use for up to 10 years. |

1. **Reporting – N/A**
2. **Procedural Notes**
   1. EDTA inhibits the uptake of complement therefore EDTA anticoagulated blood must not be used for this procedure.
   2. 0.1% Trypsin is prepared from 1% stock solution frozen at minus 18°C.

1. **References**
   1. WHMIS [www.whmis.ca](http://www.whmis.ca)
   2. Judd WJ, Methods in immunohematology, 3rd ed. Montgomery Scientific Publications, 2008: 257-259.
   3. CSTM Standards for Hospital Transfusion Services, ver 3 February 2011: 3.4.3.1; 3.4.5.1.
   4. Hoffman et al Reproducible *in vitro* preparation of intermediate C3d coated red blood cells. Transfusion 1982; 22(3):180-184.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| December 1, 2014 | * Revised name of manual * Revised and renumbered sections 2.0, 5.0 & 6.0 * Revised list of *Equipment* and *Supplies* in section 4.0 * Updated list of references to include most recent editions |