

## **Spleen and Cecal Tonsil Flow Prep Protocol**

### **Spleen**

1. Collect spleens from birds in incomplete RPMI
2. Press spleen through a cell strainer (40u) using a syringe plunger and 4ml of RPMI
  - a. Be careful to leave cells on ice at all times when possible
  - b. Be sure to keep the tissue wet at all times to reduce cell death
3. Collect filtered contents in a 15ml tube and fill to 5ml mark
4. In another 15ml tube, place 5ml of histopaque (1.077 g/ml)
5. Overlay the cell suspension onto histopaque without mixing
6. Spin cells at 1,200 xg for 10 minutes at 10C with no brake
7. Collect cell layer
8. Wash with 5ml complete RPMI by spinning at 300 x g for 10 minutes
9. Discard Supernatant and resuspend in 5ml complete RPMI

### **Cecal Tonsils**

1. Collect Ct from birds in incomplete RPMI
2. Press through a cell strainer (40u) using a syringe plunger and 4ml of RPMI
  - a. Be careful to leave cells on ice at all times when possible
  - b. Be sure to keep the tissue wet at all times to reduce cell death
3. Collect filtered contents in a 15ml tube and fill to 5ml mark
4. Spin at 300 x g for 10 minutes
5. Discard supernatant
6. Wash with 5ml complete RPMI by spinning at 300 x g for 10 minutes
7. Discard Supernatant and resuspend in 5ml complete RPMI