Protocol for obtaining a lymph node aspirate for the purposes of PCR:

**Note:** We would prefer that two lymph nodes be aspirated and tested for each patient. The following recommended procedure for obtaining a lymph node aspirate for PCR is similar to the aspiration procedure routinely used for flow cytometry at the NCSU-Veterinary Teaching Hospital, except that sterile saline is used for sample transport rather than RPMI medium.

1. Before obtaining the aspirate, inject 1 mL of sterile saline into a sterile, unopened red-topped tube. Do **NOT** use a serum separating tube (with gel plug). **Label the tube with the animal’s name, date of sample collection, lymph node aspirate, and which node was aspirated.**

2. Aspirate lymph node using an 18 to 20 gauge needle and a 12cc syringe for adequate suction.

3. Using the same needle and syringe, uptake sterile saline from the sterile red-topped tube into the syringe containing the aspirate. Rinse 3 to 5 times to wash the lymphoid cells from the needle into the collection tube. Do not remove the needle from the tube until the washing is complete.

4. After washing, leave the aspirate and saline in the red-topped tube and ship to the VBDDL for further analysis.

5. Repeat the aspiration process as needed using a new tube for each additional lymph node.