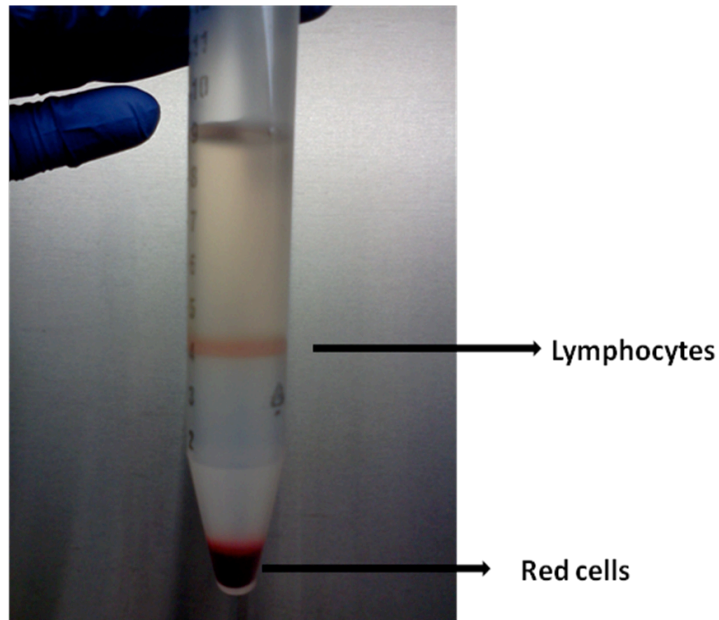


Isolation of Leukocytes from *X. laevis* adult frog's blood (7/8/2015):

1. Mix 50 μ L of Heparin (Alfa Aesar Cat. No. A16198) with 5 mL Sterile APBS.
2. Collect blood from the adult frog (usually is collected from the leg).
3. In 15 mL centrifuge tube add \sim 4 mL 51% Percoll (colloidal silica with a specific diameter used to separate cells by density).
4. Then add the blood in heparin carefully on the top of the Percoll.
5. Centrifuge for 25 minutes at 4°C and 1500 RPM.
6. Collect middle part of the solution that contains the lymphocytes.



7. You can repeat step 5 and 6 to separate completely the red cells from the lymphocytes (optional).
8. Wash lymphocytes with 10 mL sterile APBS.
9. Centrifuge for 10 minutes at 4°C and 1000 RPM.
10. Resuspend pellet in 500 μ L sterile APBS.
11. Count lymphocytes cells using a Hemocytometer.
12. Centrifuge cells at for 1 minute at RT and 14000 RPM.
13. Take out the supernatant.
14. Add 1 mL Trizol for RNA/DNA isolation.