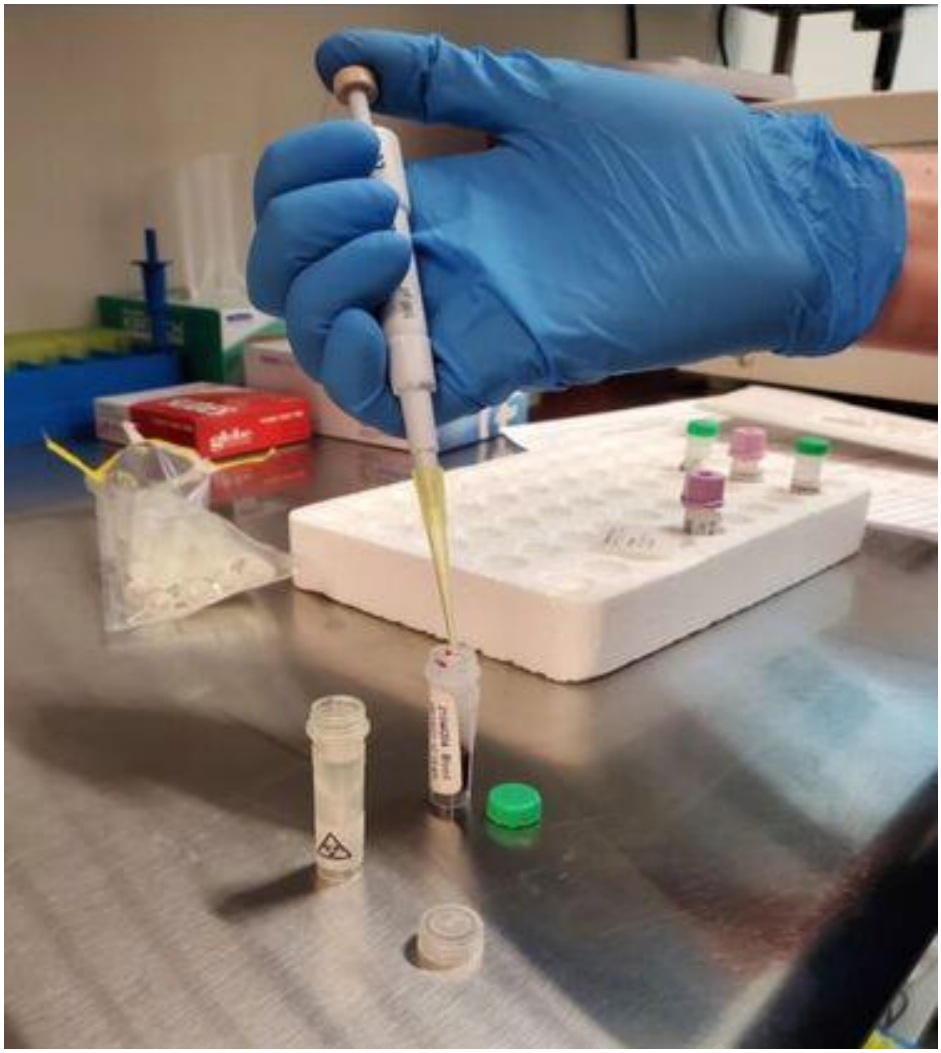
Sample Preparation

Preparing Non-Mammalian Blood for Cell Counts: Formalin-Fixation

Whole blood preserved 1:5 in 10% neutral buffered formalin at the time of collection, extends the sample stability period to days/weeks/months vs. requiring the cell count to be performed within hours. The formalin preserves morphology for blood cells, just as it does for tissue cells. Total leukocyte and erythrocyte counts are then determined from a dilution of that preserved whole blood in Natt-Herrick diluent, following standard methods. The formalin may lessen the intensity of the dye (cells appear less purple) but the cellular components are often more clearly visible (nucleus, granules, etc).

Important: Use unfixed whole blood for PCV, blood smears, hemoglobin, e.g. all CBC tests other than the hemocytometer counts. *The formalin-fixed cells are ONLY for the hemocytometer count.*



Procedure:

1. Prior to blood collection, prefill a small tube or vial with 400 µL of 10% neutral buffered formalin (NBF).
2. Collect blood per established protocols and transfer to anticoagulant tube (anticoagulant selection based on species requirements). Mix well.
3. Immediately transfer 100 µL of the whole blood to the tube containing pre-measured NBF to achieve a 1:5 dilution. Mix well by pipetting gently up & down.
4. Label tubes with ID & date.

The fixed specimen can be stored ambient or in refrigerator and NBF cells are stable for at least one month, or longer. Please note it is important to aliquot blood into NBF immediately after collection; delaying will negatively impact the quality of the sample. Thrombocytes will begin to aggregate in heparin samples quickly and other cell morphology distortions may occur.

For elasmobranch CBC and chemistry, the simplest option is to use lithium heparin. Draw approximately 1-2 mL blood and transfer to heparin tube(s) and follow the instructions below. Or, if preferred, the blood can be transferred to a serum (red top) tube (at least 1 mL) and into your anticoagulant of choice for the CBC.

Steps tank-side, at the time of collection:

1. Draw the blood per your usual SOP and transfer to anticoagulant of choice (heparin, EDTA, citrate, etc.)
2. Mix the anticoagulant tube and transfer 1-part whole blood into a tube containing 4 parts 10% formalin (e.g., 100uL blood + 400uL NBF). *NOTE: This formalin tube is ONLY for the hemacytometer count; do not use it to make smears or run any of the other CBC tests.*
3. Make 2 blood smears from the heparin (or EDTA, citrate) tube and air dry.

In the lab:

4. If you have a hematocrit centrifuge and refractometer, run the PCV and total dissolved solids. If you include the results on the submission form, we'll add them to the CBC report and note that these were read at the time of collection. If you don't have these items, or prefer us to run the PCV/TS, please include a small tube of the anticoagulant blood sample.
5. Spin the heparin (or serum) tubes, and aliquot 0.5-1.0 mL of plasma or serum for the chemistry tests.

Shipping:

6. The vial of formalin fixed cells (for WBC count on hemocytometer)
7. Blood smears in a hard case, packed in a separate zip-top bag from the formalin tube.
8. The vial of plasma/serum
9. If we are running the PCV/TS, include the heparin/EDTA vial.
10. Package these items and ship overnight with a cold pack.

For a short video demonstrating the sample collection steps, see the link below.

Elasmobranch Blood Project - AZA SAFE: Sharks and Rays (safesharks.org)