Sample Preparation

Invertebrate Hemolymph Collection & Preparation: Horseshoe Crabs

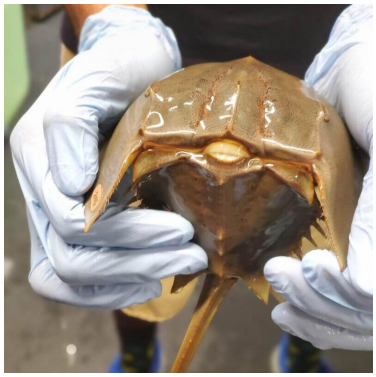
Chemistry assays can be run using either hemolymph "serum" (no anticoagulant) or plasma (heparin).

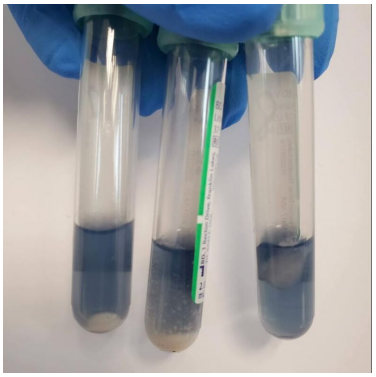
Hemolymph Collection:

1. Insert needle into the pericardial sac/heart using the plain syringe (no additive) and withdraw ~ 2 mL hemolymph red top tube
2. Holding the hub of the needle, remove the syringe and replace with the syringe containing 1.5 mL of anticoagulant; draw back to ~ the 2 mL mark and then withdraw the needle
3. Plain syringe contents. Red Top Tube
4. Anticoagulant syringe contents. 4 mL vial, invert to mix
5. Quickly transfer 200 µL of the cells into the vial containing formalin, using the pipette to mix the contents.

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; waiting until the full exam procedure is done, whether that is 10 minutes or an

hour or longer, will negatively impact the quality of the sample. Thrombocytes will begin to aggregate in heparin samples fairly quickly and other cell morphology distortions may occur.



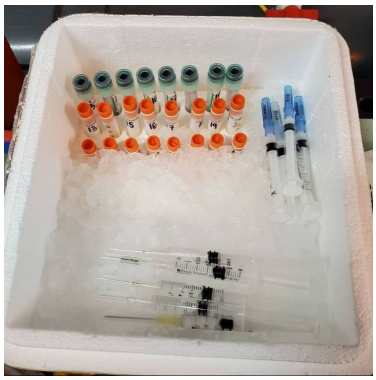


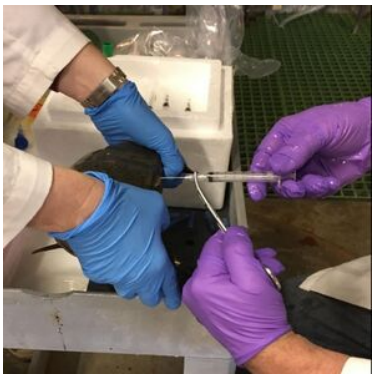
Hemolymph for Hemocyte Counts: Crustacean Anticoagulant Method

For the larger horseshoe crabs, we have been using the same anticoagulant formula (easy to prepare and very stable), by prefilling 3mL syringes with 1.5mL anticoagulant. The filled syringes, with 1 inch 22-gauge needles, are placed in a crushed ice bucket. With the crab restrained, remove the cold syringe from the ice and draw hemolymph to the 2.0 mL mark.

Immediately transfer the contents to a cold empty tube, mix quickly and immediately transfer 200uL hemolymph/anticoagulant into a cold vial prefilled with 800uL of 10% formalin.

If chemistry tests are needed, we use a 2-syringe technique by first drawing hemolymph into a plain cold syringe (transfer contents to a cold heparin tube), hold the needle in place with hemostats, and switch to the second syringe prefilled with anticoagulant.





Hemolymph for Hemocyte Counts: Citrate Method

Based on literature and our experience, an important pre-analytical factor is keeping all items on ice (syringes, needles, blood tubes and formalin tubes). We've found that even picking up the syringes too soon, where they warm a bit in your hands, can lead to rapid cell aggregation. So, we keep the iced supplies close at hand when sampling.

We used to draw the hemolymph directly into a syringe pre-filled with crustacean formula for anticoagulant but more recently have found that using small citrate blood tubes works very nicely and is far less complicated. An aliquot of cold citrate whole hemolymph is immediately fixed in cold 10% neutral buffered formalin.

For best results, keep all tubes in crushed ice, remove the caps just before sample collection, and keep the tubes in ice during sample transfers and pipetting.

1. Draw hemolymph into cold syringe
2. Immediately transfer contents to a) small citrate tube and b) serum or heparin tube
3. Immediately transfer 1 part citrate hemolymph to a tube containing 4 parts formalin (e.g., 200uL hemolymph + 800uL formalin) and mix by pipetting. At this point, the cells will fix quickly, and the sample is stable for a long time (weeks, months)
4. The serum or heparin tubes are held in the cooler until returning to the lab where they are centrifuged to remove the serum or plasma for chemistry assays.