

## BLEEDING CHICKEN EMBRYOS

Fertile eggs are purchased through Hogan Animal Facility. You must have ACUC number on grant and fill out the form in person.

The hatcheries belong to Dr. Engel's lab (in Dr. Holmgren's lab) or Dr. Lamb's lab and permission should be obtained from them for their use. You must check the humidity, temperature and turning action of the hatchery occasionally as this piece of equipment has been known to behave strangely in the past. When you use the former one, you have to rotate eggs at least once a day by hand. Always keep the apical side down. The day that the eggs are put into the hatchery is "Day 1" although this nomenclature is not the only one used when speaking about developmental stage. We have used Lillie's Development of the Chick as a source of information on developmental staging. You can start the incubation at any time you want by keeping eggs at 4°C.

Generally washing labeling etc. of embryonic blood cells is much like the adult with slight modifications.

1. Place egg "pointed" end down in egg cup or 50 ml beaker.
2. Gently tap around the cap of the egg and remove a disc of shell.
3. Below the membrane you will see the embryo (whatever stage) floating upon the yolk.
4. The blood network is readily visible. Bleeding is done by nicking a peripheral artery/vein and collecting the blood into a pulled 200  $\mu$ l glass capillary or p200 being careful to draw up as little egg white as possible. Watch out for yolk. Once it is broken everything gets messy and you must discard the egg.
5. Collect the blood into NKM or PBS containing 1 mg/ml heparin on ice.
6. Spin down blood at #1 setting in clinical. This light spin will pellet the red cells but leave a lot of cell junk in suspension.
7. Wash blood cells in NKM. There is no need to worry about removing lymphocytes--they do not appear until the seventeenth day. However leukocytes, thrombocytes and phagocytes are present but only at about 0.4% of cells in blood.
8. On early days of development (3-6 days) I normally use around  $10^6$  cells in 100  $\mu$ l of 50  $\mu$ Ci/ml  $^{35}$ S methionine/DME for labeling. In later days I use up to  $10^8$  cells.
9. RNA is isolated as from reticulocytes from adult cells with care to remove as much hemoglobin as possible.

### *General Outline of Stages*

Day 3/4. Embryo appears as a whitened area surrounded by a slightly red vascular system. Blood islands may be visible around periphery (avg.  $1-2 \times 10^6$  cells/egg).

Day 4/5. Embryonic heart can be seen beating. Vascularization is becoming more extensive (avg.  $5 \times 10^6$  cells/egg).

Day 5. Very extensive network of veins etc. Very easy to bleed. (Avg. Day 6 -8  $\times 10^6$  cells/egg -by day 9 I don't even bother to get "every last drop." I can easily get  $10^9$  cells in 1 dozen).