

CHICKEN INJECTION AND BLEEDING

Solutions:

-Acetyl-Phenylhydrazine 100 mg/ml

Place 1.5 ml Abs. ETOH in a glass tube

Add 200 mg 1-Acetyl-Phenylhydrazine

Mix and warm until dissolved

Add 0.5 ml H₂O.

-3:1 Abs. ETOH/H₂O

-NKM 140 mM NaCl for 1L: 8.12g NaCl

3 mM KCl

0.22g KCl

3 mM MgCl₂

0.61g Mg Cl₂

3 mM KH₂ PO₄

0.41g KH₂ PO₄

Injection Schedule:

Day 1: 0.05 ml/lb

Day 2: 0.04 ml/lb

Day 3: 0.075 ml/lb

Day 4: 0.02-0.04 ml/lb

Day 5: Bleed.

Procedure:

1. On day 5, bleed into NKM with Heparin to 1 mg/ml (Prepare mix fresh)
2. Filter through gauze to remove coagulated blood and any large debris.
3. Spin down in clinical at number 3. Remove serum supernatant.
4. Resuspend in 50 ml NKM to rinse and split into two 30 ml Corex tubes. Spin at 4 K RPM in the HB4 rotor at 4°C.
5. With a long pasteur pipet, first remove the top white layer of lymphocytes (buffy coat), then remove the supernatant.
6. Resuspend cells and combine in one 50 ml Corex tube at original volume of blood. Make a 1:1000 dilution of cells and count in hemocytometer.
7. (Optional) Spin cells and resuspend to 10⁹ cells/ml or convenient volume.

Comments:

1. Use alternate legs each day.
2. Inject no less than 20 hours apart (if chicken is kept alive, allow to recover one week).
3. Unruly feathers may be wet with 75% EtOH when sterilizing area of skin to be injected.