9/10/19 Meeting with Lauren

About: protocol for egg-passaging influenza

1. Determine titer through TCID50 or EID50 (in eggs instead of cells, more stringent way of determining titers but takes a lot more effort). Do TCID50 instead
   1. HA titer first to see if there is any virus there (only takes around an hour)
      1. Serial dilutions of virus in turkey red blood cells
      2. Hemagglutinin binds to receptors on outside of blood cells. Viral particles link blood cells together and cause them to clump into a dot (hemagglutination). Find dilution where clumps no longer form
      3. Tells you number of particles
   2. TCID50 (takes 3-4 days)
      1. Serial dilutions of virus in MDCK cells
      2. Look for cell death by eye. Find dilution that doesn’t kill cells anymore
      3. Tells you number of infectious particles
      4. Start by injecting 1000 TCID50 (go up to 5000 if no infection, but never above 10,000 because then you get defective interfering particles)
2. Find SPF (serum pathogen free) egg supplier
   1. Eggs should arrive at 10 days old
   2. Gives you ~2 days to infect them (maybe can infect up to day 15, but start to hatch around day 20)
3. Biosafety: need to update protocol. Might have to have backup plan in case eggs hatch
   1. Want to wear eye protection/face mask, lab coat
4. Lauren highly recommends getting an egg-rocker incubator
5. For candling: can use a flashlight with a cup on the end to concentrate light
6. Injection protocol:
   1. Mark edge of air sac with pen by candling (looking at egg with flashlight)
   2. Use Dremel tool to remove shell and seal with hot paraffin to sterilize the injection site
   3. Eggs should still be warm during injection because temperature difference sucks the inoculum into the egg
   4. Inoculum of ~50uL, inject with needle. Then seal with paraffin
   5. Put on rocker for 48hr in incubator
   6. Kill egg at 4 degrees overnight (or at least 2 hr)
7. Harvest virus:
   1. Use egg punch (cracker) to remove perfect circle of shell from top of egg
   2. Collect allantoic fluid: use 5mL pipette as tool to hold back membrane and 10mL pipette to suck up allantoic fluid
   3. Put in 15/50mL tube to spin for 15 min at highest speed, then take supernatent
   4. Can use sucrose gradient to purify virus (probably do this before antigenic characterization assays but not between passaging steps or for sequencing)