H. Morales 8/22/06

TCID 50 protocol

1. Put A6 cells in 96well plates at 5000cells/well in ASF +10% FCS (100ul total) for about 2 days.

2. In a separate V bottom 96 well plate, do dilutions of virus:
   A. 1:25- Add 8ul of virus in 192ul of ASF-A6 or ASF
   B. 1:50- Add 60ul of 1:25 in 60ul ASF-A6 or ASF
   C. 1:500- Add 12ul of 1:50 in 108ul ASF-A6 or ASF
   D. 1:5000- Add 12ul of 1:500 in 108ul media and so on until dilution H. $5x10^7$
   *Note: Make sure you resuspend the wells very well before going to the next dilution!!

3. Transfer 100ul of each dilution to the plate with A6 to make a total of 200ul per well.
   *Remember to leave columns 1 and 2 without virus, these are the control.

4. Leave in incubator at 37C with CO2 for approx. 5 days.

5. Check daily.

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TCID 50 Calculation

Example

1. Calculate Proportionate Distance (PD) between the two dilutions in between 50% death: (% next above 50%)- 50% / (% next above 50%) – (% next below 50%)

Example above:

PD= 80%-50% / 80%-0%= 30/80= .375

2. Calculate 50 % end point. Log lower dilution= dilution in which position is next above 50%

Example above:

Log lower= 10^{-6} or -6

3. Add PD and Log lower dilution

Example above: -6 + .375 =-6.375

Log TCID50= 10 – 6.375 or 1/ 2.37 x10^{6}

4. Calculate TCID 50/ml. Divide by the ml of viral innoculum added to row A