<u>TCID 50 protocol</u>

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 CO_2 for approx. 5 days.

5. Check daily.

TCID 50 Calculation

Example

Dilution		Control 1 2 3 4 5 6 7 8 9 10 11 12	% Death
10^{-1}	A	(-) (-) (+) (+) (+) (+) (+) (+) (+) (+) (+) (+)	**100%**
10^{-2}	B	(-) (-) (+) (+) (+) (+) (+) (+) (+) (+) (+) (+)	**100%**
10^{-3}	C	(-) (-) (+) (+) (+) (+) (+) (+) (+) (+) (+) (+)	**100%**
10^{-4}	D	(-) (-) (+) (+) (+) (+) (+) (+) (+) (+) (+) (+)	**100%**
10^{-5}	E	(-) (-) (+) (+) (+) (+) (+) (+) (+) (+) (+) (+)	**100%**
10^{-6}	F	(-) (-) (+) (+) (+) (+) (+) (+) (+) (+) (-) (-)	**80%**
10^{-7}	G	(-) (-) (-) (-) (-) (-) (-) (-) (-) (-) (-) (-)	**0%**
10^{-8}	H	(-) (-) (-) (-) (-) (-) (-) (-) (-) (-) (-) (-)	**0%**

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/ 2.37×10^{6}
4. Calculate TCID 50/ml. Divide by the ml of viral innoculum added to row A
       Example above: according to our protocol=.008 ml
       TCID 50/ml= 2.37×10^{6}/ .008= 2.9×10^{8}
5. Calculate PFU/ml. Divide by constant.
       Example above: 2.37×10^{6} x .69 = 2.0×10^{8} PFU/ml