

	1.	2.	3.	4.		
Dilution factor	Infected wells	Uninfected wells	Cumulative infected	Cumulative uninfected	Ratio	% Infected
100	3/4	1/4	13	1	13/14	92.86
400	3/4	1/4	10	2	10/12	83.33
1600	2/4	2/4	7	4	7/11	63.64
6400	2/4	2/4	5	6	5/11	45.45
25600	2/4	2/4	3	8	3/11	27.27
102400	1/4	3/4	1	11	1/12	9.09
409600	0/4	4/4	0	15	0/15	0

Largest dilution factor for which % infected is >50

Dilution factor closest to 50% without being equal to or over 50

1. Count total infected wells from bottom of the table to the top

2. Count total uninfected wells from top of the table to the bottom

3. Calculate a ratio of total infected wells to total uninfected for each dilution, using the numbers calculated in (1) and (2) as numerator and denominator.

4. Express ratio calculated in (3) as a percentage to determine cumulative percent positive wells for each dilution.

5. Calculate proportionate distance (PD):

$$PD = \frac{(\% \text{infected} > 50) - 50}{(\% \text{infected} > 50) - (\% \text{infected} < 50)} = \frac{63.64 - 50}{63.64 - 45.45} = \frac{13.64}{8.19} = 0.750$$

6. Calculate TCID<sub>50</sub>:

$$\log \text{TCID}_{50}/\text{ml} = \log(\text{inoculum volume})^{-1} + \log(\text{dil. factor} > 50) + PD \log(\text{serial dil. factor})$$

$$\log \text{TCID}_{50}/\text{ml} = \log(0.1)^{-1} + \log(1600) + (0.75)\log(4)$$

$$\log \text{TCID}_{50}/\text{ml} = 1 + (3.204 + 0.452) = 4.656$$

$$\text{TCID}_{50}/\text{ml} = 10^{4.656} = 45289$$