

## Plaque Assay – Calculation of the infectious dose in a material

Watch the video and answer the questions below: <https://www.youtube.com/watch?v=tbx6fqYdNWc>

1. What is the ratio of people who die from contracting Nipah virus?
  - a.  $\frac{1}{2}$  people
  - b.  $\frac{3}{4}$  people
  - c.  $\frac{1}{4}$  people
  - d.  $\frac{4}{4}$  people
2. What was the indirect evidence suggesting that Pteropodidae bats harboured Nipah virus?
  - a. They had virus in their secretions
  - b. They had antibodies directed toward the virus
3. The virus is sent to the lab to determine \_\_\_\_
  - a. Genetic make-up
  - b. Mutation rate
  - c. Both A and B

Now watch the video to watch: <https://www.youtube.com/watch?v=tGVDYGxqtJ0>

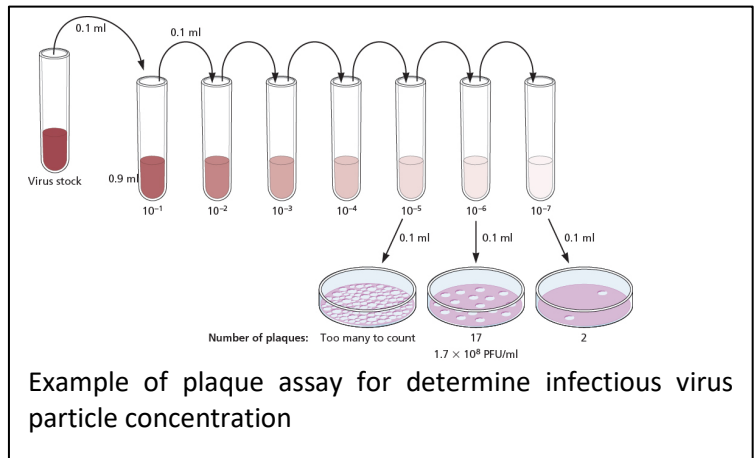
And read the situation below. Then answer the question.

There is an outbreak of a new respiratory virus in Malaysia with a high case fatality rate (almost 50%). The outbreak was most likely a spillover event stemming from human contact with a virus infected flying fox bat. It seems that this new respiratory virus has a higher transmission rate ( $R_0$ ) than influenza or SARS. Early estimates of confirmed case have now placed the virus in 5 major cities across the world. Confirmed case are rapidly increasing and the WHO has placed you on a task force investigating the infectivity of this new virus now called **HIRV (Highly Infectious Respiratory Virus)**.



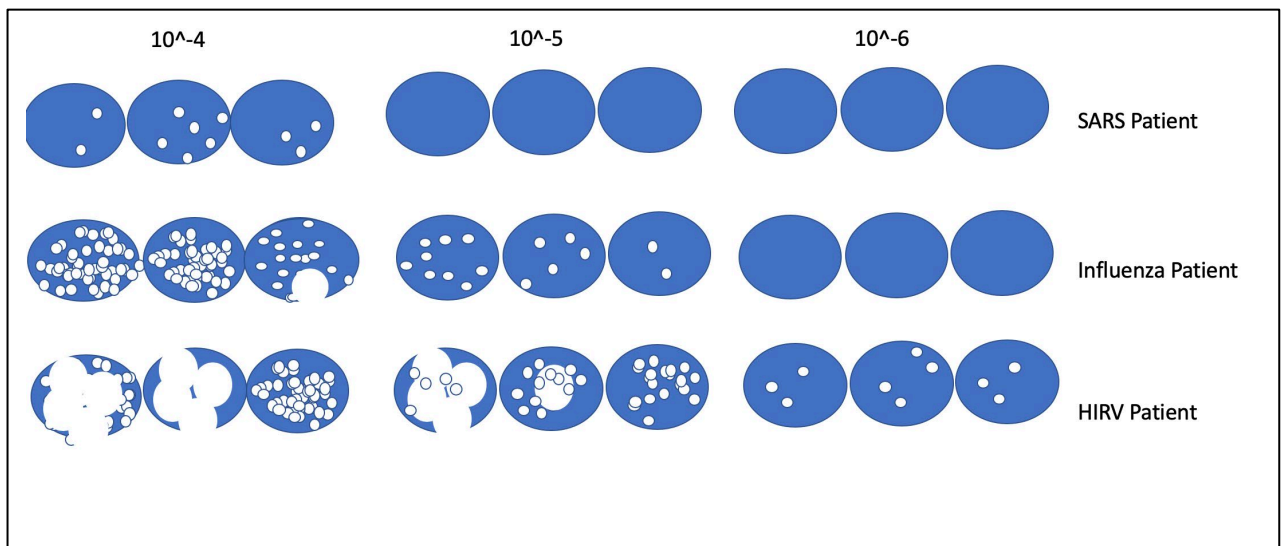
Flying Fox

Working with Dr. Kelvin, you hypothesize that the virus is spreading faster due to higher amounts of infectious virus being shedding from the respiratory tract of infected humans. You have collected respiratory sputum and nasal wash samples from infected persons and want to test your hypothesis. If you can show that there is a higher amount of infectious virus being shed from infected persons you can help stop the contagion through the implementation of appropriate public health policies.



You have 1 ml of nasal wash from patients infected with SARS virus, influenza virus, and HIRV. You follow the standard plaque forming assay protocol to determine the number of infectious particles per ml. You have performed a 1 in 10 dilution series for each sample. After 6 serial dilutions, you added 100 ul from you  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  dilutions to MDCK cells growing in cell culture plates. Similar to the example below.

You acquired the following results:





Use the formula below to calculate the number of PFU/ml from each patient's nasal washes.

$$\frac{\text{Average\# Plaques}}{D \times V} = \text{PFU/ml}$$

D = Dilution factor

V = Volume of diluted virus  
added to the well

**What were the viral titers?**

- A.) SARS:  $4 \times 10^5$  pfu/ml; Influenza:  $5.67 \times 10^6$  pfu/ml; HIRV:  $4.33 \times 10^7$  pfu/ml  
B.) SARS:  $3.67 \times 10^5$  pfu/ml; Influenza:  $5.33 \times 10^6$  pfu/ml; HIRV:  $3.33 \times 10^7$  pfu/ml