Rate of Infection of a Human T-Cell Line with a Pseudotyped Retrovirus Vector

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INTRODUCTION

Many diseases of the world are spread by viruses. Viruses are infectious agents that can multiply only within the living cells of a host. Once inside a host, viruses spread throughout the host by infecting cell to cell. We chose to study a representative model of the human immunodeficiency virus (HIV), which uses cell-free transmission. Other models of HIV disease infection focus on the human to human transmission, rather than the cell to cell transmission.

To study the factors of HIV infection, we investigated the effect of the viral amount on infection rate on cells. To vary the viral amount of viral infection, we infected Jurkat cells with a modified HIV vector. In the experiment, we varied the viral amount or viral titer. To study the infection rate of retroviruses such as HIV, we used a retrovirus containing a gene of the green fluorescence protein, GFP. Once the virus infects a cell, the GFP gene is incorporated into the genome by using the reverse transcriptase material also carried in the virus. The GFP gene produces fluorescence light, which can be detected under a fluorescent microscope or using flow cytometry (Mitra et al. 1996). In this manner, successfully infected cells can be measured and hence, the number of infected cells can be measured. It is important to note that due to laboratory safety precautions, our retrovirus does not produce additional viral particles through bursting of the host cell – in other words, the initial viral particles introduced to the system are the only viral particles present in the system. We chose to use Jurkat cells as the host cells, which are immortalized human T lymphocyte cells, because as cancer cells, they will proliferate constantly at a predictable rate.

In this paper, we present a group of differential equations that models the factors that influence initial viral infection in a group of cells. We were able to determine a specific rate of infection for all experimental groups, a consistent growth rate of the infected population and uninfected population, and a model of viral decay. We found that the rate of infection is dependent on the initial number of viral particles present to a certain extent, and that there is a significant difference in the growth rate of cells that are uninfected and infected.

METHODS

Viral Titer

- To determine the viral titer, we used retroviruses produced from PT67 packaging cell lines, which are capable of infecting both human and mice cells to infect mouse NIH3T3 cells. The virus was infected at various dilutions (1:10, 1:100, 1:1000, and 1: 10,000).

- To determine the viral titer, we used the following equation:

  [%GFP*starting cell #]/[dilution*total volume]

  The titer was determined to be 455,500 infecting units/mL.

Viral Infection Protocol

- The retroviruses produced from the same PT67 cells used in the viral titer experiment were transduced into Jurkat cells, an immortalized line of human T lymphocyte cells. The cells were infected with varying titers of virus using a spinfection protocol, and polybrene was added to aid in infection efficiency.

Finding Number of Infected Cells

- When the infected host cells (Jurkats) express this protein after the gene has been integrated into their genome, the cells will emit fluorescence in the FITC channel when excited with a 488nm laser. Thus, infected cells can be determined through positive expression of GFP, as determined by a flow cytometer.

Finding Number of Live and Dead Cells

- Cell staining with propidium iodide was carried out to determine live and dead cell counts. Propidium iodide is a non-membrane permeable intercalating agent that stains DNA in the PERCP channel, and is useful for staining dead cells because the DNA will only be stained if the cell is dead and leaking DNA into the environment.

Using the Differential Equations to Build the Model

- Rather than use the ordinary differential equation solver on Matlab, we opted to use Euler's method, whereby the time derivative for each population is multiplied by a small time step and then added to the previous population number.

Finding Rate of Infection

- K was the only term that required iteration to obtain. When k is increased, the model dramatically underpredicts populations, and when k is decreased, it overpredicts.

BOX MODEL
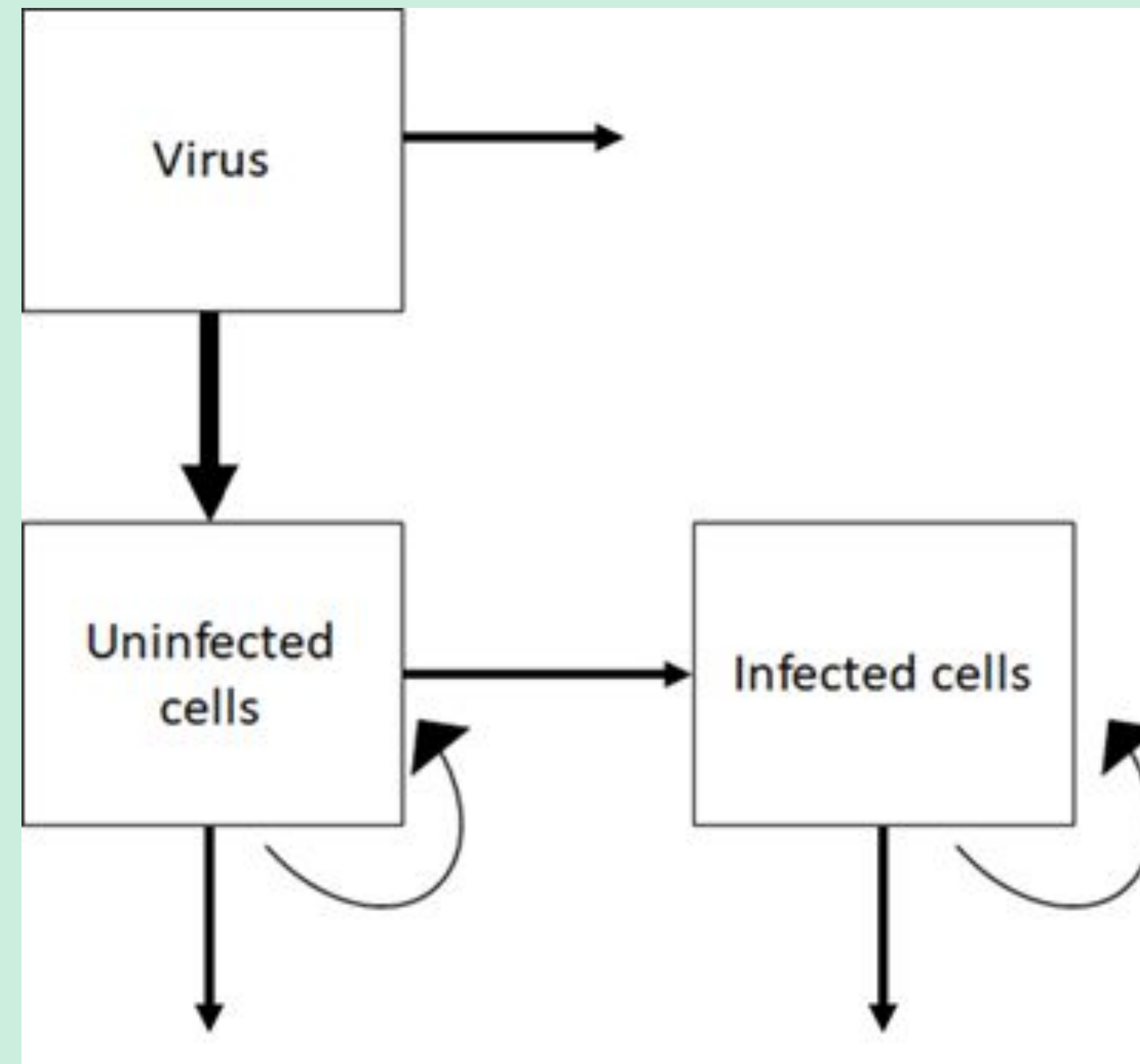
Figure 1. Box model of a viral infection model in which viral infection occurs only at time 0 and cells are able to continuously replicated, either infected or uninfected, without constraint

EQUATIONS

$U(0)$ = Initial cell count

$I(0)$ = 0

$V(0)$ = Initial viral particle count

The changes in U, I, and V in our system are reflected in the following differential equations:

$\frac{dU}{dt}$ = + uninfected cell growth - newly infected cells

$\frac{dI}{dt}$ = + uninfected cell growth + newly infected cells

$\frac{dV}{dt}$ = - viral particle uptake - viral particle degeneration

The following are the actual differential equations used:

$$\frac{dU}{dt} = r_u U - kUV$$

$$\frac{dI}{dt} = r_i I + kUV$$
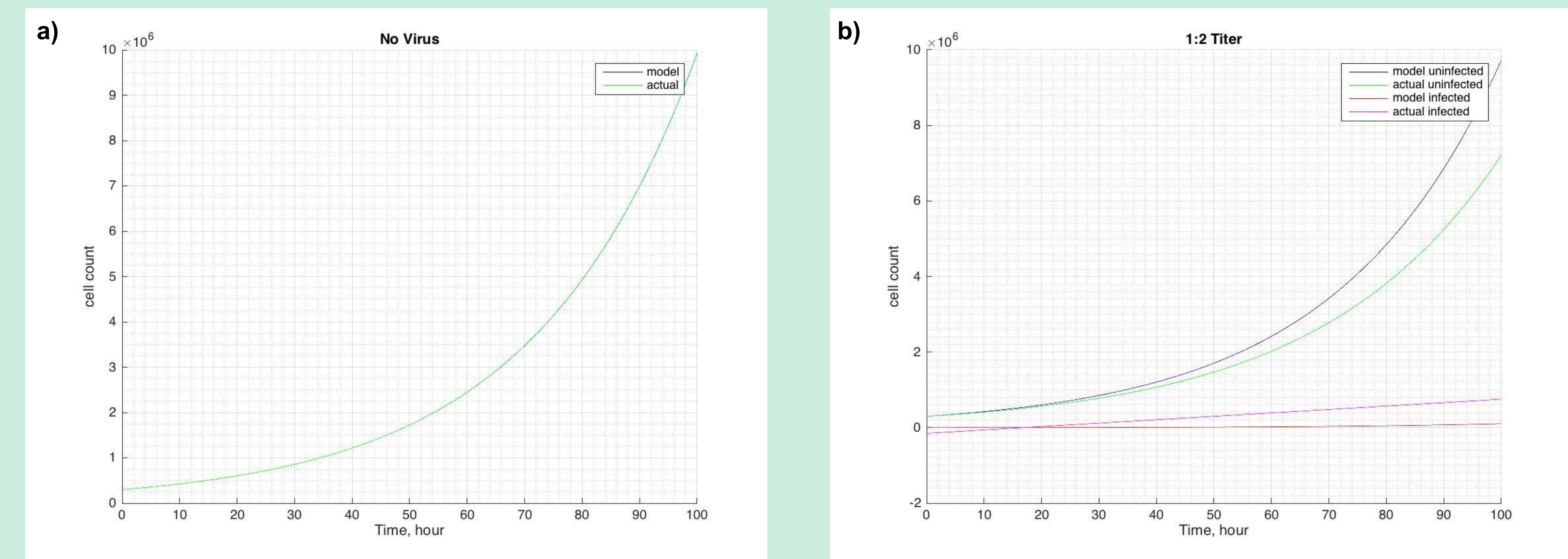
$$\frac{dV}{dt} = -r_v V - kUV$$

**Table 1.** Constants, symbols, and the value used for each

Parameter	Symbol	Value Used
Uninfected replication rate	r_u	0.035/hr
Infected replication rate	r_i	0.02/hr
Viral degeneration rate	r_v	-0.1733/hr
Probability of infection interaction	k	**10^{-8}/count*hr

** count represents one viral or cell unit

MATHEMATICAL MODELS

Figure 2. Mathematical modeling of different viral infections over time for a) no virus or b) a 1:2 initial viral titer
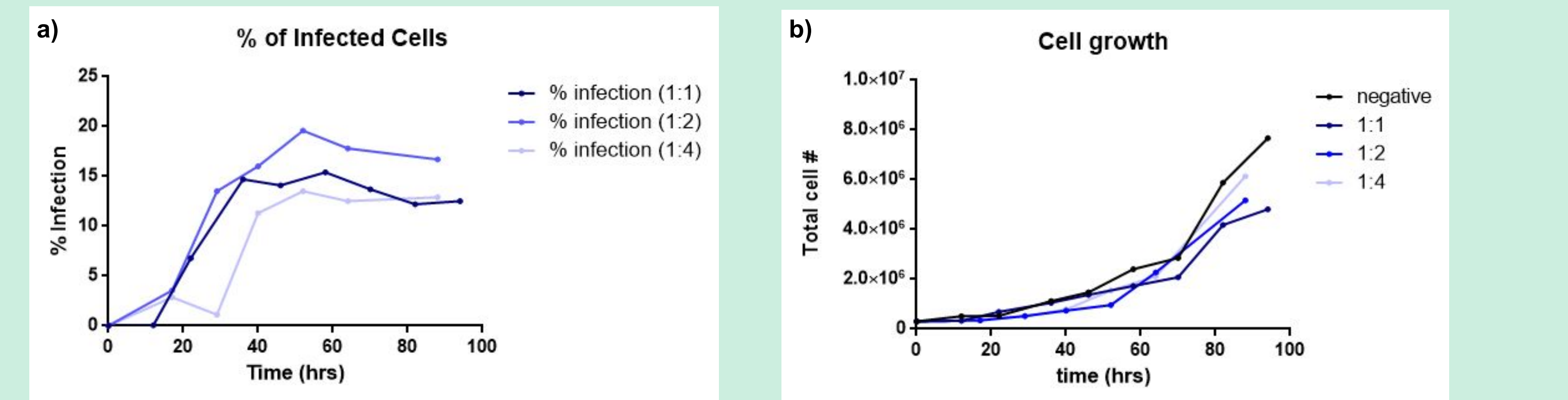
Figure 3. Graphs of the raw data comparing the a) percentage of infected cells over time with varying initial infection viral titers and b) cell growth over time after viral infection

Titer	Uninfected (100hrs)	Infected (100hrs)	Uninfected (1000hrs)	Infected (1000hrs)
1:1	0.9984	0.9505	0.9953	0.5196
1:2	0.9995	0.9080	0.9989	0.5196
1:4	1	0.9521	0.9999	0.5196

Table 2. Correlation Coefficients Between Model and Actual Data

CONCLUSIONS

- We found that in the two highest dilutions we used, the rate of infection was relatively similar, but a decrease in the rate of infection could be seen in the lowest titer dilution. In addition, we found in our experiments that the highest viral titer surprisingly did not produce the highest infection efficiency. Rather, a two-fold dilution lower generated the highest percentage of infected cells.

- We did, however, find a correlation between the number of viral particles present at initial infection and the resulting rate of cell growth. As expected, the more virus particles present, the slower the cell are able to bounce back from the infection and resume normal growth rates.

- We noticed that the infected cells had a slower growth rate after infection, despite being the exact same cells as the uninfected cells. This suggests that something in the infection and genome integration process hinders the cell proliferation ability and rate.

- While the model for uninfected cells holds in the short run (under 100 hours) and long run (under 1000 hours), but the model for infected cells only holds in the short run.