Connecting Industry to Mathematics Instruction
NSF ATE Award # 1954291

Solutions: Student Activity Sheet - Virus Maximus

In partnership with WAKE TECH, WAKE COUNTY PUBLIC SCHOOL SYSTEM, WakeEd PARTNERSHIP

Virus Growth and Vaccine Production

Hemagglutinin (**HA**) is an antigenic glycoprotein found on the surface of influenza viruses. It is responsible for binding the virus to the cell that is being infected.

Overview: Below are sets of data showing viral HA protein percent yield for three different virus strains being considered for use in the upcoming flu vaccine production process, and a control. Calculate which strains, if any, showed a significant **difference** ($p<0.05$) in HA percent yield compared to the control. Based on the results, make a recommendation to manufacturing on which strain(s) to use, or to stick with the control strain. The sample for each run is 100.

Part 1:

1. Look at the data and compare each strain to the control. Estimate which strain you think is the least effective. Why do you think it is the least effective? Strain A b/c yield is similar to the control
2. Estimate the strain that you think will be the most effective. Why do you think it is the most effective? Strain B or C is acceptable because both have yields much greater than the control.

Control		Strain A		Strain B		Strain C	
Run	HA Percent Yield	Run	HA Percent Yield	Run	HA Percent Yield	Run	HA Percent Yield
1	41%	1	43%	1	54%	1	59%
2	38%	2	34%	2	61%	2	55%
3	37%	3	39%	3	56%	3	53%

3. What would be used as the population parameter for this situation? Control Strain

4. What is your hypothesis and what alternate hypothesis will you be testing? Hypothesis is that there is no difference between the control and Strain A, B or C. (H_o: $p = .38$) could be $p <= .38$

Alternate Hypothesis: There is a difference between the control and strains.

(H_A: $p \neq 0.38$) could be $p > .38$

5. Using the median values for the control and each strand as the "mean", complete the following table.

Link to [Detailed Answer Key] using both Desmos and Graphing Calculator***

Strain	H_o	H_a	Calculations to find Probability (show work - What you typed into Desmos)	Probability	Significant?
A	H_o:$p = .38$	H_a: $p \neq 0.38$	0.4183	p= 0.837	0.837 not < 0.05 Not significant
B	H_o:$p = .38$	H_a: $p \neq 0.38$	0.0001044	p= 0.0002	0.0002 < 0.05 Yes Significant
C	H_o:$p = .38$	H_a: $p \neq 0.38$	0.00023	p= 0.0005	0.0005 < 0.05 Yes Significant

6. Based on your calculated probability, which strain do you feel can be discarded? Why?
Strain A because $p > 0.05$

7. Looking at the other two strains does the differences in their probability justify using one over the other or do you feel that they are both similarly effective? Explain your conclusion with complete sentences.
Both Strains B and C have significant p values and either would be appropriate to use, although B appears to be slightly better the p - values are so close that you can not rule either out.

8. In the context of this question, why would a probability of 0.03 be considered significant when 0.20 is not?
Since the significance level it 0.05 ($\alpha = 5\%$), the p-value of 0.03 is less than the significance level, while the p-value of 0.20 is not less than 0.05. P=0.03 means that the probability of the observed HA yield happening if the control value is true is so small that it is unlikely to happen while the 0.20 is above our threshold so we do not reject our null hypothesis.
The probability measures the likelihood of the observed percentages in a strain if the percentages were the same as the control group (Example: They find 56% in Strain A when it is really 38% in the control group.) If the probability of that happening is very small (less than the significance level) then we can reject the null hypothesis and know that 38% is not accurate for that strain

Detailed Answer Key

Strain	**Detailed Answers with Graphing Calculator**	Significant?
A	Use normal distribution calculator "Mean" = .38 Stand dev = $\sqrt{\frac{(.38)(1-.38)}{100}}$ = .04853 **Graphing Calculator:** normalcdf(.39, 10000000, .38, .04853) = .418356 Since it is two sided 2(p) = .837	no, 0.837 not < 0.05
B	Use normal distribution calculator "Mean" = .38 Stand dev = $\sqrt{\frac{(.38)(1-.38)}{100}}$ = .04853 **Graphing Calculator:** normalcdf(.56, 10000000, .38, .04853) = .0001 Since it is two sided 2(p) = .0002	yes 0 .0002< 0.05
C	Use normal distribution calculator "Mean" = .38 Stand dev = $\sqrt{\frac{(.38)(1-.38)}{100}}$ = .04853 **Graphing Calculator:** normalcdf(.55, 10000000, .38, .04852) = .00023 Since it is two sided 2(p) = .0005	yes 0.0005 < 0.05

Strain	**Detailed Answers with Desmos**	Significant?
A	normaldist(.38, .04853) Mean, Standard Deviation Find Cumulative Probability (CDF) Min: .39 Max: ∞ = 0.418372760588 Since this is two sided 2(p)= 2(.418) = .837	no, 0.837 not < 0.05
B	normaldist(.38,.04853) Mean, Standard Deviation Find Cumulative Probability (CDF) Min: .56 Max: ∞ = 0.00010402083659 Since two sided: 2(p) = 2(.0001)= 0.0002	yes 0 .0002< 0.05
C	normaldist(.38,.04853) Mean, Standard Deviation Find Cumulative Probability (CDF) Min: .55 Max: ∞ = 0.000230035230554	yes 0.0005 < 0.05

	Since Two sided: 2(p) = 2(.00023) = 0.0005	

Part 2:

When counting the amount of virus cells, which is called calculating viral titer, dilution is necessary to give a countable number. Shown below is an image of a sample of a virus showing the stages of dilution from a high concentration of the white virus cells to a low concentration of the cells. (The white is the virus cells and the purple is the dilution agent)

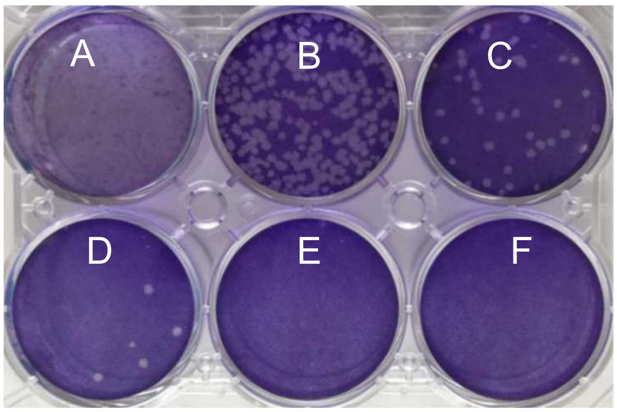
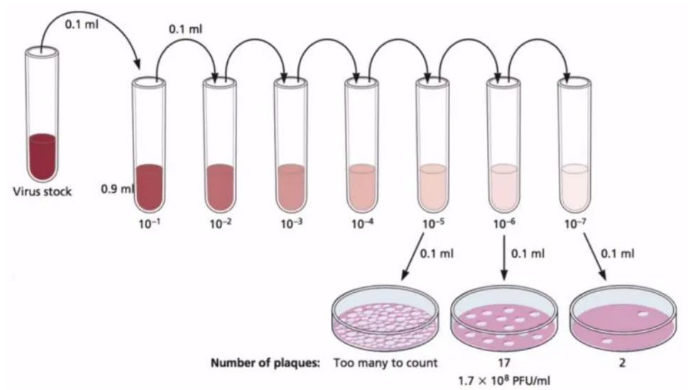


Figure 1. Plaque-forming titer assays.



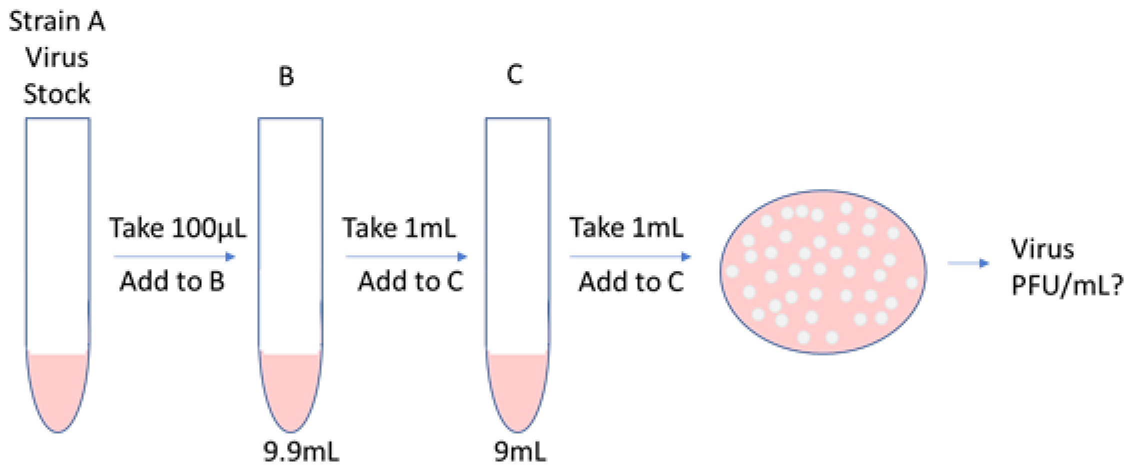
Bacteriophage Plaque Assay

1. Which dilution sample do you think would be the best to count the number of cells? Why? Reminder: Dr. Johnstone gave parameters in the video for how many cells needed to be present in a sample. **Video Link** Petri Dish C: students should pick the one that is between 30 and 300 cells that is countable

Pictured below is the experimental overview and results of stocks using the control and three strains from Part 1. Each dot in the dish represents one virus unit called a “plaque forming unit (PFU)”.

Calculate the amount of PFUs per milliliter (PFU/mL) for each viral stock being tested. µL means 1 microliter. A microliter is a unit of volume equal to 1/1,000,000th of a liter.

Note: 1mL=1000µL. You will notice that on the diagrams the units are sometimes in mL and other times in µL.



<u>Calculations for Strain A:</u>

First you must calculate the dilution factor- To do this you are starting with your virus stock (all the grown virus cells) and taking a small amount of the stock to Tube B and mixing it together. You then will take a small amount of sample in Tube B and mix it into Tube C. Finally you take a small amount of sample from C and count the virus cells. You will then use this number to calculate how many virus cells were in the original Stock.

Take 100 µL from the stock and place in Tube B:

First convert 100µL into mL: $\frac{1mL}{1000\mu L} = \frac{x}{100\mu L}$ cross multiply to get x = 0.1 mL

Find the total amount of solution in Tube B: 9.9 mL + 0.1mL = 10mL

In Tube B there was 0.1 mL of stock so the total ratio of stock to solution is $\frac{0.1}{10} = 0.01 \; or \; 10^{-2}$

Take 1mL from the Tube B and place in Tube C:

Find the total amount of solution in Tube C: 9mL + 1mL = 10mL

In Tube C there is 1mL from Tube B so the total ratio of Tube B to Tube C is $\frac{1}{10} = 0.1 \; or \; 10^{-1}$

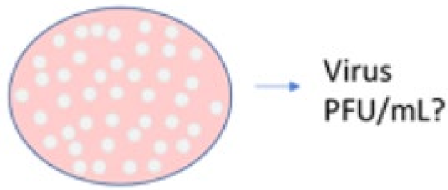
Total Dilution and Final Count

The total dilution is then $10^{-2} \cdot 10^{-1} \cdot 1 = 10^{-3}$ you need to multiply by the amount taken from each test tube. The amount taken from C is 1mL so we multiply by 1.

Count the virus cells in your diagram above: 39 cells

Final count: 39 PFU ÷ 10^{-3} = 39,000 PFU

Convert to scientific notation: 39,000 = 3.9 x 10^{4} PFU/mL



Take 1mL from the stock and place in Tube B:

No need to convert since you start with mL.

Find the total amount of solution in Tube B: 1mL + 9mL = 10mL

The total ratio of stock to solution is: $\frac{1}{10} = 0.1 = 10^{-1}$

Take 1mL from the Tube B and place in Tube C:

Find the total amount of solution in Tube C: 1mL + 9mL = 10mL

The total ratio of Tube B to Tube C is: $\frac{1}{10} = 0.1 = 10^{-1}$

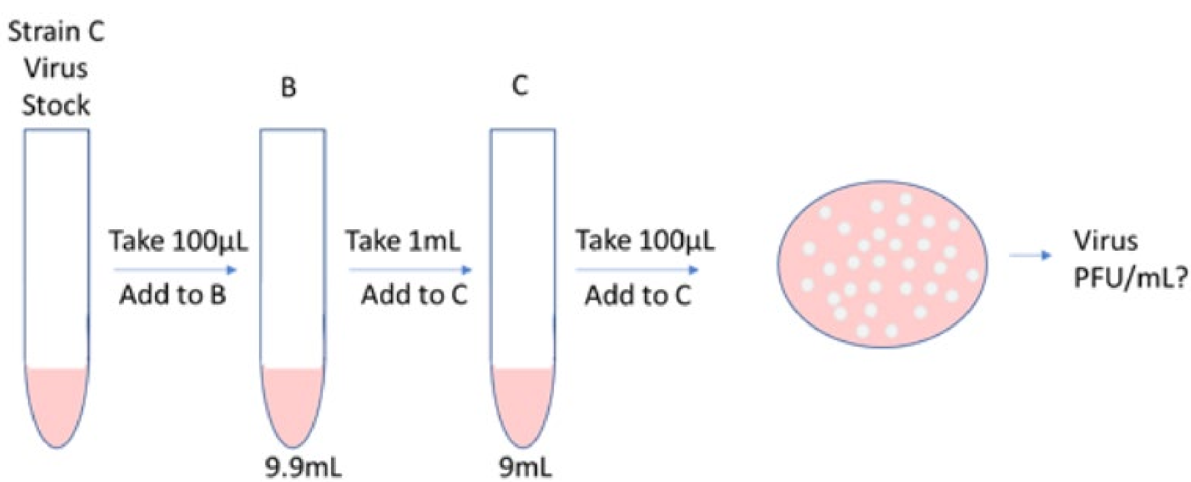
Total Dilution and Final Count

The total dilution is: $10^{-1} \cdot 10^{-1} \cdot 1 = 10^{-2}$

Count the virus cells in your diagram above: 42 cells

Final count: $42 \div 10^{-2} = 4{,}200$

Convert to scientific notation: 4.2×10^{3}



Take 100 μL from the stock and place in Tube B:

First convert 100μL into mL: $\frac{1mL}{1000\mu L} = \frac{x}{100\mu L}$ cross multiply to get x = 0.1 mL

Find the total amount of solution in Tube B: 9.9 mL + 0.1mL = 10mL

The total ratio of stock to solution is: $\frac{0.1}{10} = 0.01\ or\ 10^{-2}$

Take 1mL from the Tube B and place in Tube C:

Find the total amount of solution in Tube C: 1mL + 9mL = 10mL

The total ratio of Tube B to Tube C is: $\frac{1}{10} = 0.1 = 10^{-1}$

Total Dilution and Final Count (Note: there is not 1 mL of tube C used, but 100μL and you must convert to mL)

The total dilution is: $10^{-2} \cdot 10^{-1} \cdot 10^{-1} = 10^{-4}$

Count the virus cells in your diagram above: 31 cells

Final count: $31 \div 10^{-4} = 310{,}000$

Convert to scientific notation: 3.1×10^{5}

Conclusion:

Seqirus produces a flu vaccine that requires maximal viral HA protein yield and virus production for manufacturing the vaccine efficiently. Using the information from #1 and #2, make a recommendation to Dr. Johnstone for which strain Seqirus should use to produce the vaccine. Remember that you are presenting your written conclusion to a scientist and you should use complete sentences and proper grammar in your explanation of why you picked a certain strain.

From Part 1 both Strand B and C are acceptable, but in Part 2, Strain C produces a much higher final count. Therefore I suggest using Strain C.