

## How Many HeLa?

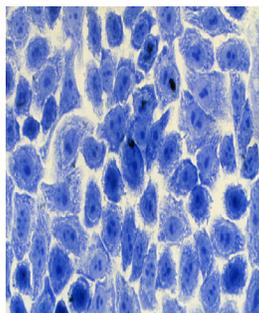
Dividing cells expand exponentially.

Cells that can grow *in vitro*, like HeLa cells, may divide rapidly and indefinitely. With this Snack, you can figure out how long it would take for HeLa cells to grow to cover the city of San Francisco.

*Note: The **To Do and Notice** section of this activity will take you through the steps necessary to calculate the amount of time needed for a sample of HeLa cells to grow to cover the city of San Francisco. As you work, be sure to note your results at each step, and carefully label your findings. You'll find a sample solution for each calculation in the **What's Going On?** section, below, and background information about the HeLa line of cells in **Going Further**.*

### Tools and Materials

- [Printed HeLa images with scale bar](#), or compound microscope and slide of fixed, cultured HeLa (or other) cells
- Scientific calculator or access to an Internet search engine, such as Google
- Pencil and notepaper to record results



## Assembly

None needed.

## To Do and Notice

The three images included with this Snack show three different fields of view of a coverslip on which HeLa cells were grown in a single layer. These three rectangular areas were randomly selected, thereby representing a random sample of the total number of cells on the coverslip. A scale bar in the bottom right-hand corner of each image is marked in micrometers ( $\mu\text{m}$ ). Choose one image (it doesn't matter which) and find the area, or *field of view*, by using the scale bar to estimate its length and width. The result will be the same for all three images.

$$\text{area} = \text{length} \times \text{width}$$

There are 1,000 micrometers ( $\mu\text{m}$ ) in one millimeter (mm), and 1,000,000  $\mu\text{m}^2$  in one  $\text{mm}^2$ . Convert the area in  $\mu\text{m}^2$  to  $\text{mm}^2$ .

The cells shown in the images were stained with a dye that colors proteins and nucleic acids blue. The dark blue dots in the centers of the cells are DNA, which is in the cell nucleus. The pale blue space surrounding the nucleus is the cytoplasm. Choose any of the three images and use the scale bar to estimate the diameter of one HeLa cell. Estimate the diameter of at least three cells in your sample and calculate an average diameter. Be careful to measure the whole cell, not just the round nucleus. Once you have this information, try estimating the area of one HeLa cell. You can assume that the cells are roughly circular with a radius,  $r$ .

$$\text{diameter} = 2 \times r$$

$$\text{area of a circle} = \pi \times r^2$$

Next, using all three images, determine the average number of cells in a field of view. To do this, count the cells in each of the three fields of view, add them together, and divide by 3.

Density is expressed as number of cells/ $\text{mm}^2$ . To calculate the average density of the cells on the coverslip, divide the average number of cells in a field of view by the area of the field of view.

Remember that the images are a small subset of the cells on the entire coverslip. The coverslip used for these images measures 22 mm by 22 mm. Calculate the area of the coverslip. Then calculate the total number of cells,  $N$ , on the coverslip by multiplying the density of the cells by the area of the coverslip.

$$N [\text{cells}] = \text{density of cells} [\text{cell}/\text{mm}^2] \times \text{area of coverslip} [\text{mm}^2]$$

Growth rates for cultured cells are often measured in “doubling time,” the length of time it takes for a population of cells to double in size. The doubling time of HeLa cells grown in culture is approximately 16.2 hours. The relationship between doubling time and the number of cells at a given time,  $N_t$ , is expressed by this equation:

$$N_t = N_0 \times 2^{t/16.2}$$

where  $N_0$  is the number of cells at time 0, and  $t$  is the time in hours.

Imagine that the cells on the coverslip were allowed to grow continuously in culture and would not run out of space or nutrients; assume, also, that no cells will die. Use the equation above to calculate the number of cells there would be after one day, or 24 hours, given the generation doubling time of 16.2 hours.

$N_0$  is the number of cells you calculated to be on the coverslip. You will need to use the exponent function on your scientific calculator to calculate  $2^{t/16.2}$ , or you can use Google or other Internet search engine: just type “ $2^{(t/16.2)}$ ” into the search field.

Calculate the area these cells would cover in  $\text{mm}^2$ , assuming that they have the same density that you calculated above.

$$\text{area of } N \text{ cells at time } t [\text{mm}^2] = N [\text{cells}] / \text{density} [\text{cells}/\text{mm}^2]$$

Calculate the number of cells there would be after one week (168 hours), and the area they would cover. To get a better sense of how big an area this would be, you may want to convert  $\text{mm}^2$  to square meters ( $\text{m}^2$ ). There are  $10^6 \text{ mm}^2$  in  $1 \text{ m}^2$ .

Calculate the number of cells there would be after three weeks (504 hours), and the area they would cover. To get a better sense of how big an area this would be, you’ll want to convert  $\text{mm}^2$  to square kilometers ( $\text{km}^2$ ). There are  $10^{12} \text{ mm}^2$  in  $1 \text{ km}^2$ .

Calculate the number of cells there would be after four weeks (672 hours), and the area they would cover.

The city of San Francisco is approximately 10 km by 10 km. How many HeLa cells would it take to cover the city? About when do you think the cells on the coverslip would grow to cover the city?

## **What's Going On?**

Immortalized cell lines, which are cells that can live and proliferate semi-indefinitely *in vitro*, are an extremely valuable tool for scientists. Because they divide rapidly and can be genetically manipulated, cell lines can be used to ask and answer many questions about the structure and function of cells, proteins, and genes.

When working with cultured cells, it’s important to know the general rate of proliferation and be able to calculate density and cell number so you can time and standardize your experiments by using the same number and density of cells for each condition or treatment. Cell density also affects cell growth and proliferation, so making sure your cells are happy and growing well requires being able to determine their density.

Although it’s critical to know the approximate number of cells you’re using in a given experiment, you don’t have to count each cell. That would be impossible! Instead, counting one or more random subsets of a larger sample will give you a good estimate of cell numbers. Random sampling and estimation are important techniques in almost every aspect of scientific research.

***A sample solution to the calculations in this Snack:***

***Q: Find the area of an image, or field of view, by using the scale bar to estimate its length and width. The result will be the same for all three images.***

A: You can fit approximately 6 scale bars across the image, and approximately 4.5 scale bars down. So the image measures about 300  $\mu\text{m}$  wide, and about 225  $\mu\text{m}$  tall. Therefore, the area of the image is about  $300 \mu\text{m} \times 225 \mu\text{m} = 67,500 \mu\text{m}^2$ .

***Q: Convert the area in  $\mu\text{m}^2$  to  $\text{mm}^2$ .***

A: To convert your estimate to  $\text{mm}^2$ , multiply by  $10^{-6}$ . In this case, the area is about  $0.068 \text{ mm}^2$ .

***Q: Choose any of the three images and use the scale bar to estimate the diameter of one HeLa cell. Estimate the diameter of at least three cells in your sample and calculate an average diameter.***

A: Answers will vary depending on which cells you choose to measure, where and how you measure them, and how close your estimate is. Between 20 and 30  $\mu\text{m}$  is a reasonable estimate for the average diameter of one cell.

***Q: Try estimating the area of one HeLa cell. You can assume the cells are circular.***

A: Answers will vary based on your estimate of the average diameter of a cell. Reasonable estimates for the area of one cell are between  $300 \mu\text{m}^2$  and  $700 \mu\text{m}^2$ .

***Q: Next, using all three images, determine the average number of cells in a field of view. Count the cells in each of the three fields of view, add them together, and divide by 3.***

A: Answers will vary depending on how accurate your method of counting is and whether you choose to count cells on the edges of the image that are only partially visible. A reasonable estimate of the average number of cells in one field of view is about 150 cells.

***Q: Density is expressed as number of cells/ $\text{mm}^2$ . Calculate the average density of the cells on the coverslip by dividing the average number of cells in a field of view by the area of the field of view.***

A: A reasonable estimate of the density of the cells on the coverslip is  $2,200 \text{ cells}/\text{mm}^2$ .

***Q: The coverslip used for these images measures 22 mm by 22 mm. Calculate the area of the coverslip. Then calculate the total number of cells on the coverslip by multiplying the density of the cells by the area of the coverslip.***

A: The area of the coverslip is  $484 \text{ mm}^2$ . An estimate of the total number of cells on the coverslip based on a density of  $2,200 \text{ cells}/\text{mm}^2$  is  $1,064,800$  cells.

***Q: Calculate the number of cells there would be after one day, or 24 hours, given the generation doubling time of 16.2 hours.***

A: Answers will vary depending on how many cells you calculate as your starting number. The calculations below are based on using  $1,064,800$  for  $N_0$ .

After 24 hours, there would be about  $2.97 \times 10^6$  cells, or 2,970,000 cells.

*Q: Calculate the area these cells would cover in mm<sup>2</sup>, assuming that they have the same density that you calculated above.*

A: After 24 hours, these cells would cover about  $1.35 \times 10^3$  mm<sup>2</sup>, or 1,350 mm<sup>2</sup>.

*Q: Calculate the number of cells there would be after one week (168 hours), and the area they would cover.*

A: After one week, there would be about  $1.41 \times 10^9$  cells, or 1,410,000,000 cells. These cells would cover about 641,000 mm<sup>2</sup>, or 0.641 m<sup>2</sup>.

*Q: Calculate the number of cells there would be after three weeks (504 hours), and the area they would cover.*

A: After three weeks, there would be about  $2.47 \times 10^{15}$  cells, or 2.47 quadrillion cells. These cells would cover about  $1.12 \times 10^{12}$  mm<sup>2</sup>, or 1.12 km<sup>2</sup>.

*Q: Calculate the number of cells there would be after four weeks (672 hours), and the area they would cover. Convert mm<sup>2</sup> to square kilometers (km<sup>2</sup>).*

A: After four weeks, there would be about  $3.25 \times 10^{18}$  cells, or 3.25 quintillion cells. These cells would cover about  $1.48 \times 10^{15}$  mm<sup>2</sup>, or 1,480 km<sup>2</sup>.

*Q: The city of San Francisco is approximately 10 km by 10 km. How many HeLa cells would it take to cover the city? About when do you think the cells on the coverslip would grow to cover the city?*

A: San Francisco is approximately 100 km<sup>2</sup>. It would take approximately  $2.2 \times 10^{17}$  cells to cover the city. Based on the time points calculated above, it would take between three and four weeks for the cells on the coverslip to grow to cover the city.

## **Going Further**

If you want to visualize this process more clearly, you can make a graph of the exponential growth of the cells, with time on the x-axis and the number of cells on the y-axis. You may want to calculate a few additional time points to make the curve clearer, as well as use semi-log graph paper.

If you have access to slides of cultured cells, this Snack can be done by first finding the size of the field of view on your microscope (see the **What's the Size of What You See?** Snack), and using this measurement to estimate the density of the cells on your slide. To do the calculations on cell division, you will need to look up the doubling time for the cells you are observing.

## **Background: About the HeLa Cell Line**

The HeLa line, the first human immortalized cell line, was started in 1951 with cancerous cervical tissue taken from an African American woman named Henrietta Lacks. Lacks died of cancer shortly after the tissue sample was taken, but her cells continued to grow in laboratories, and they were used to create and test the polio vaccine.

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At the time, ethical guidelines did not require researchers to obtain permission to acquire and study tissue samples. The scientist who took the sample and created the HeLa line, George Gey, did not tell Henrietta or her family that he had taken the sample, or that the cells grew faster and more robustly than any cells previously studied. It would be decades before the Lacks family found out about the huge scientific impact the cells had made, and the industry the cell line had become. HeLa cells have since been used in countless studies, relied on by thousands of labs across the world, and sold by multiple biological research supply companies.

There are now hundreds of immortal cell lines, many of which come from samples of cancer tissues taken from patients. Cancerous cells usually carry mutations that allow them to divide indefinitely, avoiding the normal controls of the cell cycle. These cells can ignore signals that tell them to stop dividing. Some cell lines divide more rapidly than others.

The story of Henrietta Lacks and the HeLa cell line is a valuable introduction to an exploration of ethics in medical research, how they have evolved over time, and the risks and benefits different groups of people take on as research subjects. Rebecca Skloot's book *The Immortal Life of Henrietta Lacks* is an engaging and accessible entry point to this story and topic.

### **Teaching Tips**

In this Snack, students' answers can and should vary. This is the nature of random sampling and estimation.

### **Resources**

For information on the size, scale, and rates of division of various cells, see: Phillips, R., and Milo, R. (2015) *Cell Biology by the Numbers*, Garland Science

For information on ethical guidelines for clinical research in the US: <http://clinicalcenter.nih.gov/recruit/ethics.html>

More information on cancerous cells and cell division: [https://www.exploratorium.edu/imaging\\_station/research/cancer/story\\_cancer1.php](https://www.exploratorium.edu/imaging_station/research/cancer/story_cancer1.php)