Computational Biomedicine
Bacterial Reproduction | Teacher guide

Unit 1: Bacterial Reproduction

Sequence of the unit:

1. Case history: Revealing the data of the case.
2. Diagnosis the species of the bacteria causing the disease of a patient:
    b. How do bacteria reproduce?
    c. Generation time and morphology of bacteria
    d. Bacterial reproduction for additional tests
    e. Confirming the species of the bacteria through Grahm staining
3. **Summary and reflection**: contribution of mathematical models and their constraints.

Case history:

For the past four days, A.R., a young woman of 18, has been experiencing a burning sensation and pain when she urinates, along with an urgent need to frequently use the bathroom. Despite rest and copious drinking there was no improvement, and her symptoms did not subside. She went to the emergency room and by a physical examination of the patient, you identified sensitivity and pain in the lower abdomen. In order to diagnose the illness, you asked her to provide a urine sample and you sent the urine to the hospital laboratory for analysis.

Danit, a scientist, received the urine sample in her laboratory and identified a bacterial infection. She conducted experiments to identify the type of bacteria causing A.R.'s symptoms. Identification of the type of bacteria is crucial so as to later find the appropriate treatment.

Biological background:

Bacteria are single-cell organisms without a nucleus or organelles (such as mitochondria). Their genetic material, usually a single chromosome, is found in cytoplasm. These organisms are called prokaryotes.

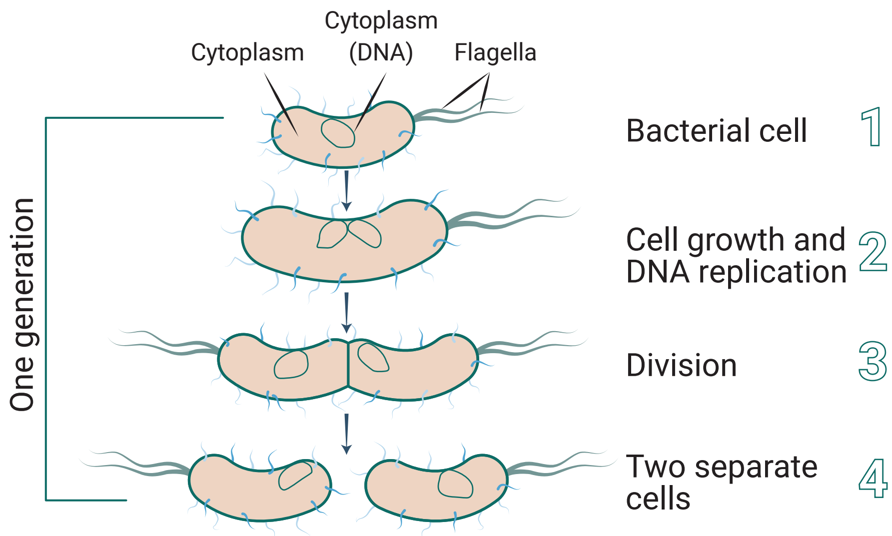
Relative to cells in our body, which are about 20-40 micrometer in size (micrometer is the one thousandth part of a millimeter), the bacterium cell is tiny at 0.2-2 micrometers in size. A single bacterium cannot be seen with a naked eye but can be seen under a light microscope.

Teachers: Here you can illustrate the difference in size between bacteria and cells of our body by comparing it to the difference between the height of a person and a 7-floor building.

Here you can introduce the term Eukaryotes, but it is not necessary for the unit.

Bacteria divide by cell division (see Illustration 1). In this process, the bacterium cell grows, the genetic material of its single chromosome doubles and then the cell divides into two cells. The genetic material in both the bacteria cells created following division is identical to the original cell (see Illustration 1). The amount of time in which a population of bacteria doubles itself is called generation time. Under optimal conditions – with the appropriate temperature, sufficient nutrients, enough oxygen, and so on, each type of bacteria has a typical generation time: there are types whose generation time ranges from one to three hours, while others can even produce a new generation every 20 minutes.

Illustration 1: Bacterial Reproduction by a Process of Cell division



In order to identify the type of bacteria, the bacteria must be grown (increase their number by reproduction) in the laboratory to discover the rate at which they are multiplying.

Teachers: At this point it is possible to ask the students to hypothesize why it is important to identify the species of the bacteria, and how it can be done (for example: observing through the microscope to find differences in morphology, or growing them in test tubes to find differences in generation time).

Activity to identify type of bacteria in the culture

I. Understanding the Bacterial Reproduction Process

We return to the laboratory. To identify the bacteria from which A.R. is suffering, Danit introduced a small sample of A.R.'s urine onto a medium suitable for bacterial growth, and with the aid of a microscope, she followed the bacterial reproduction process for a number of hours.

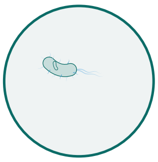
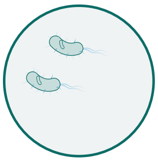
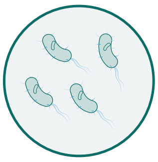
In the video clip that can be accessed through the attached link, watch the bacterial reproduction process as observed under a microscope over a number of hours. The clip is made up of many images photographed through a microscope at different times and combined into one clip. In reality, the process is much slower.

Bacteria growth

In order to calculate the rate of bacterial reproduction, Danit drew a sketch of what she saw under the microscope at different times. In the left-hand column of Table 1, you can see the sketches Danit drew. In order to calculate the rate of bacterial reproduction we will assume that all bacteria cells in the culture divide simultaneously, although this assumption is not accurate.

Teachers: At this stage in the unit students begin to construct a suitable mathematical model for bacterial reproduction. Any model for a phenomenon includes simplifying assumptions which do not relate one by one with reality. In this case, the assumption that all bacteria in the growth medium divide simultaneously is not accurate. In practice, it is more accurate to assume that generation time of all bacteria of the same species in the growth medium is identical. Further on in the unit, students will realize that the model suggested here does not apply for all conditions. This way they can develop the concept that any model includes assumptions and constraints, and therefore is a close description of reality, but not reality itself.

Table 1: The Relationship between the Number of Divisions and the Number of Bacteria Obtained

Sketch of bacteria observed under microscope	Number of divisions	Number of bacteria in sample
	0	1
	1	2
	2	**4**
	3	**8**
	4	16

1. a. Look at Illustration 1, the bacteria growth video clip and at Table 1.
   Describe the reproduction process of bacteria in words:
   b. Complete: After two divisions, there were **4** bacteria in the culture.
   Complete the table.

Teachers: In order to complete the table students can count the number of bacteria on the first column to the left. Some students might find the possibility to use multiplication in 2 or already discover the suitable algebraic expression.

   c. Determine how many bacteria will be in the culture after five divisions. Explain your answer. **32**.

Teachers: At this stage it is more difficult to count. Therefore, there might be students who will feel the need to identify the multiplication in 2 of the former number of bacterial cells (for a more thorough explanation please see: regression rule).
It is also not necessary to find the correct expression for calculating the number of bacterial cells according to the number of divisions (for a more thorough explanation please see: the position in a series).

d. Is it possible that after six divisions, Danit found 96 bacteria? Explain.

It is not possible.

An example of an explanation: According to our hypothesis about how bacteria divide, it is not possible because one has to multiply by 2 to find the next number of bacterial cells. In the table we saw that the amount of bacteria in the 4th division was 16, therefore, in the 5th division it should be 32, and on the 6th – 64.

Students who found the algebraic expression for bacterial growth: 2n n>0 could suggest another possible explanation: The number 96 cannot result from any integer n in the expression. Although there are exceptions in any model, there is no biological possibility that after 6 divisions there will be 96 bacterial cells in the culture.

Teachers: Until this stage, the questions dealt with doubling bacterial cell numbers step by step with relative ease, if bacterial cell number at the beginning and numbers of cell divisions are known. The next question (question 2) goes a step forward towards larger division numbers that make the calculations more complicated since they involve very large numbers of cells. To make the calculations easier, student should find the mathematical law that best describes the process of bacterial division.

2. Below is Table 2 which shows the results Danit found following additional divisions.

Teachers: In addition to helping the students in finding the algebraic expression, table 2 helps the, to visualize the rapidness of bacterial growth, i.e. that exponential growth is very fast.
Since some students find it hard to understand from the table, it is possible to use another method to demonstrate the phenomenon of exponential growth:
Take a transparent plastic bag, and wheat grains or lentils. Put one grain in the bag and ask the students to multiply by 2. Add 2more grains to the bag, and ask the students to multiply by 2 again. Add 4 grains to the bag. Continue with the question: by the same rule, how many grains should we put in the bag now? 4 time 2 – 8 grains. Now ask the, a challenging question: When will we find it hard to calculate how many grains to add to the bag? When will we reach 1000 grains? This activity demonstrates the difference between intuition and reality.

Table 2: The Relationship between the Number of Divisions and the Number of Bacteria Obtained, with One Bacte rium at the Outset

Number of divisions	**0**	**1**	**2**	**3**	4	**5**	6	...	**10**	...	**n**
Number of bacteria	1	2	4	8	**16**	32	**64**	...	1024	...	

a. Fill in the missing values in Table 2.

Teachers: until the 6th division in the table, the numbers of bacterial cells were calculated in the former questions. For the rest of the divisions, students can use the calculator (by multiplying by 2 or by using ways of calculation mentioned above).
The symbol three dots (…) in the table referrers to the continuation of the series in the same way. Using the dots allows to leap a few positions in the series. In the table there is a leap between the 6th division (position 6) to the 10th division (position 10).

b. Find an algebraic expression that describes the number of bacteria following n divisions.

It is quite possible that the students will encounter difficulties calculating bacterial cell numbers as division numbers increase, for example after the 10^{th} division.

a. Explain to the students that in order to make calculation easier, mathematicians try to find a mathematical law in the numbers.
b. Ask the students to suggest a possible algebraic connection between the number 2 and the number of divisions, that will help them to calculate the number of bacterial cells after each cell division.

Collect the answers of the pupils in the class. It is possible that the following different expressions will be received:

2^n: where n=number of divisions. This is the correct expression to describe the number of bacterial cells after each cell division in a growth medium which began with a single bacterium.
It is possible that some students will suggest the following misconceptions:

$2 \cdot n$: where n=number of divisions. In this case, the expression relates to the number 2, but fails to describe its correct connection to the number of divisions.

$n \cdot 2$: where n=the number of bacterial cells in the former cell division. Students who suggest this expression ignore the fact that in the table n is the symbol of the number of cell divisions. The expression uses the regression rule, i.e. each placement number is calculated through the former placement number: in order to know the number of bacterial cells after the 2nd cell division, we need to know the number of bacteria in the first division and multiply by 2. The expression is correct, but inefficient, since one has to repeat the calculation division after division, and therefore does not help with calculation of large numbers.

c. If the students suggested several expressions, raise the question: How can we determine which mathematical expression is in best accordance with table 2?
   To answer this question, students may suggest to place a division number in the expressions and compare the answer to the number of bacterial cells in the table. For example, if n=3, $2n=2\cdot3=6$, but we know that it is 8 in the table. Therefore, the expression is not in accordance with the data in the table. It is important to ask the students to repeat the test with more examples of division numbers in order to determine it is not the right expression for bacterial growth.

   In case the expression is correct but inefficient, it is possible to challenge the students with the question: What should we do to calculate the bacterial cell number after the 103rd division? Should we continue to multiply by 2 until the 103rd position?

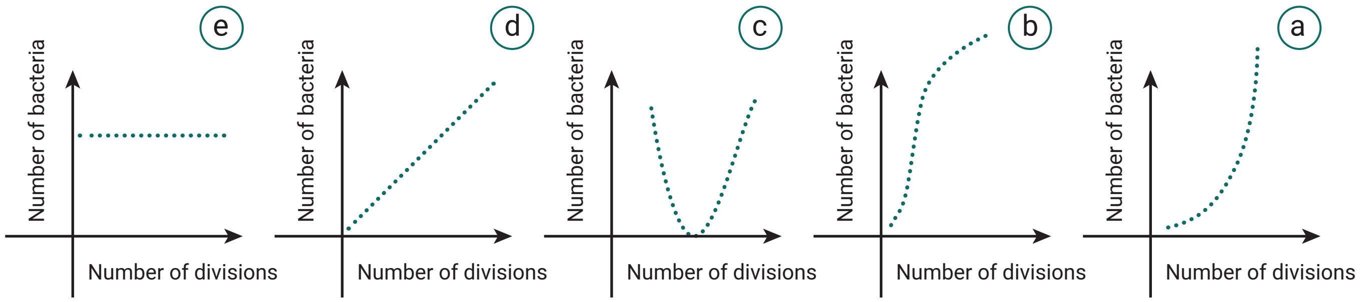
The goal is that the students arrive to the decision that 2^n is the expression in accordance with the data in table 2, where n is the number of divisions. Beginning with one bacterial cell, (as in table 2), the number of divisions is 0, and the number of bacteria is represented by $2^0=1$.

c. Challenge question: The resolution of the human eye is around 2 millimeters (200 micrometers). The length of a single bacterial cell is 0.2 micrometers.
   1. How many bacterial cells one next to the other are needed so we can distinguish them with a naked eye? **At least 1000 bacterial cells 200:0.2=1000**

2. How many divisions should a single bacterial cell go through to reach this number of bacterial cells? **At least 10 divisions according to the expression: 2^n**

3. a. Below are sketches of five graphs.

Teachers: It is possible to return to this question at the end of the unit and identify the exponential part in graph b which resembles graph a.



Think about which of them may fit the description of results of bacterial reproduction obtained by Danit. Note, there may be more than one correct answer.

Present your considerations: Both with respect to the sketches you chose and the sketches you rejected (why are they not correct?).

Answer: Graph A.

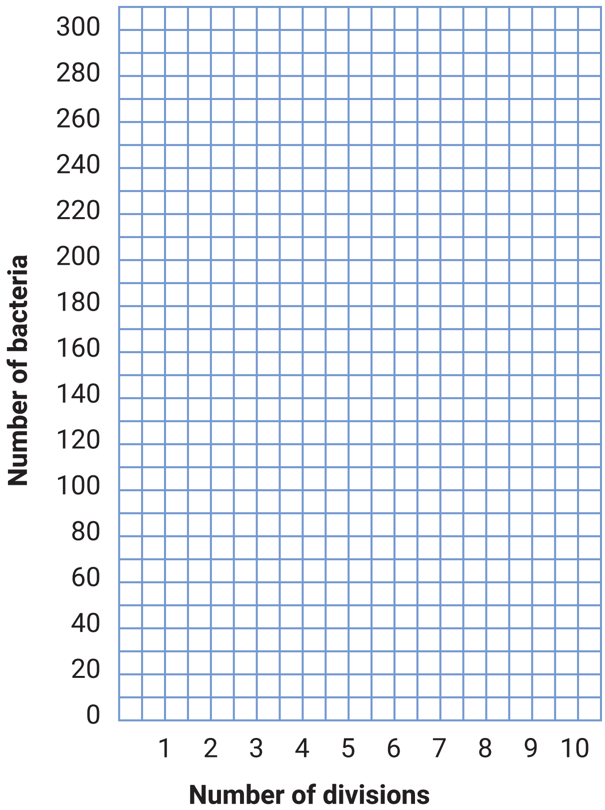
Teachers: At this stage students can discuss the differences between the graphs and suggest several hypotheses, even wrong ones, and even if the students do not agree which one is correct.

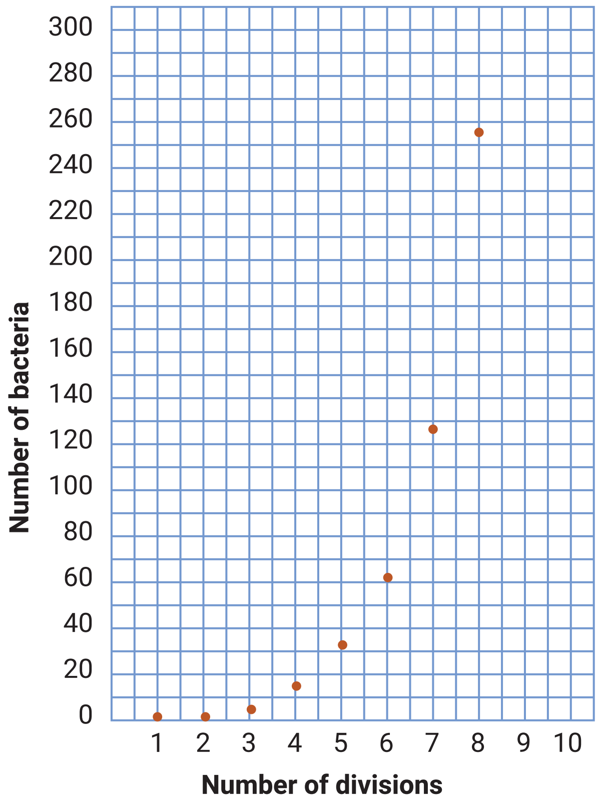
Explanation: As number of division rises up there are more and more bacterial cells. Therefore, we can reject graphs C and E.

At this stage, graphs A, B and D can remain as possibilities. The students can return to this question layer on after they construct a graph on their own.

Nevertheless, there can be considerations concerning the rise in bacterial numbers in relation to the division numbers. For example, in graph D, the number of bacterial cells rises up in equal amounts for every division, which isn't in accordance to the data in table 2. In graph B, as division numbers rise up the rise in bacterial cell numbers slows down, which also isn't in accordance to the data in table 2. Graph A fits the description of results of bacterial reproduction: as division numbers rise up, the number of bacterial cells accelerates.

b. Check your estimates: Plot the results of Danit's experiment on the coordinate system below:





c. Describe the shape of the graph in words (for example: an ascending/descending/constant function, fast/slow/constant rate of change, starting point…).

Teachers: to help the students to describe the graph it is possible to request them to sketch on the same coordinates a linear function, which they are familiar with, and describe the differences between them. These differences will make it easier for the students to understand the pace of change in the exponential function.

A possible answer: the graph doesn't begin in the origin of the coordinates.

When x=0 the number of bacterial cells is y=1. The graph ascends, therefore it is an ascending function, but the pace of change is not constant: in the beginning it is slow and then accelerates.

4. In order to be certain that her results are correct, Danit again performed the experiment, this time with a larger number of bacteria. At the outset of the experiment, she placed 110 bacteria. Danit's results are exhibited in table 3.

Table 3: The Relationship between the Number of Divisions and the Number of Bacteria Obtained, with 110 Bacteria at the Outset

Number of divisions	0	1	2	3	4	5	6	...	10	...	n
Number of bacteria	110	220	440	880	**1760**	3520	**7040**	...	112640	...	110×2^n

b. Fill in the missing values in Table 3.

c. Find an algebraic expression that describes the number of bacteria following n divisions, with 110 bacterial cells at the outset. Explain your expression.
Clue: in Table3a you can first fill in the row: number of bacteria obtained from a single bacterial cell. Then, fill in the row: number of bacterial cells obtained from 11 bacterial cells.

Table3a: The Relationship between the Number of Divisions and the Number of Bacteria Obtained, with One Bacterium or 11 bacterial cells at the Outset

Number of divisions	0	1	2	3		5		...	10	...	n
Number of bacteria obtained from a single bacterial cell	1				16		64	...		...	
Number of bacterial cells obtained from 11 bacterial cells	11				1760		7040	...		...	

Answer: With 110 bacterial cells at the outset, the suited algebraic expression would be: $110 \cdot 2^n$.
Possible explanations:

After 2 divisions, for example, 4 bacterial cells were obtained from a single cell. Therefore, after 2 divisions, 440 bacterial cells were obtained from 110 bacterial cells:

110·2·2=440, or: 110·22=440

Another explanation: The 110 bacterial cells at the outset can be distributed to 110 test tubes, each with a single bacterial cell. In the former questions we found that the number of bacterial cells obtained from a single bacterial can be described with the algebraic expression: 2n. Therefore, when there are 110 test tubes, the suited expression would be: 110·2n. For example, after 5 cell divisions there would be 110·25=3520 bacterial cells, or 2·2·2·2·2·110=3520.

c. Is it possible that after six divisions, Danit found that there were 7,040 bacteria in the culture? Explain.
Yes, with the same calculation method: 110x26 =7040 bacterial cells

Or, if the students already calculated that after the 5th division there were 3520 bacterial cells, then after the 6th division each of the, divided into two cells, therefore:

2x3520=7040

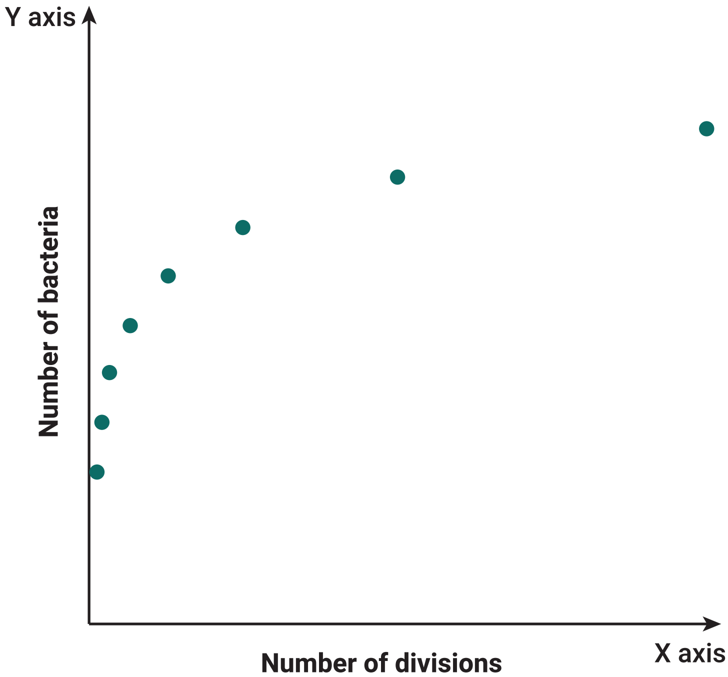
5. **Challenge question**:
Sketch the results of the experiment using an application, Excell or GeoGebra.
Click the link to access a partially-prepared form in Geogebra:

Teachers: sketching the results with the application could help the students visualize the enormous numbers of bacterial cells that are the outcome of many divisions.

6. **Challenge question:**
a. Which of the following statements correctly describes the bacterial reproduction process?
- A relationship between the number of bacteria and the number of divisions is not evident.
- The number of bacteria influences the number of divisions.
- The number of divisions influences the number of bacteria. The correct answer
- As the number of bacteria increases, the number of divisions decreases.

b. Ronen drew the following sketch.
He noted the number of bacteria following each division on the X axis and the number of divisions on the Y axis.

Teachers: this question is to enrich the students and is not needed to understand the unit itself. It is important to consider whether to introduce it to the students or not since for some it might be too challenging.



c. Does Ronen's sketch fit the descriptive statement of the bacteria reproduction process you selected in question "a" above? Explain.

Answer: The graph Ronen drew may be mathematically correct but in graphs that present results of scientific research, the influencing factor (the factor that was manipulated or the independent variable) is placed on the X axis and the influenced factor (the factor that was measured or the dependent variable) is shown on the Y axis. In this way, the graph describes the influence of the change in the independent variable (the factor having the influence) on the values of the dependent variable (the factor that is influenced). This allows us to identify in a glance the characteristics of the phenomenon. For example, we know that the change in the number of bacterial cells is slow in the beginning (the first cell divisions) and then becomes very fast; but in Ronen's sketch the change is very fast in the beginning and slows later on. The graph should describe a situation in which as the number of divisions increases, the number of bacteria increases in a growing pace.

Teachers: When the students notice that the variables are not positioned on the same coordinates, it is possible to pose questions that will guide them to the solution: Why is this the accepted norm? What would we see if the variables are not on the correct coordinates? How different would we have analyzed the graph?

In contrast, from the mathematical point of view, there is no constraint in situating the variables on the opposite coordinates. In our example, flipping the variables on the coordinates could be convenient if, for example, we would like to know how many divisions are needed to reach a certain number of bacterial cells.

In the graph that Ronen sketched, the number of divisions is situated on the y axis, and the number of bacteria is situated on the x axis.

To receive a suitable algebraic expression, we can adjust the variables in the expression to Ronen's graph:

$$x = 2^y$$

To receive an algebraic expression in which y is isolated, we would have to use algorithms:

$$y = \log_2 x$$

Algorithms are not part of the curriculum in grade 9. Therefore, the following explanation is only for teachers:

Exponentiation has three components:

The base is the number to be exponentiated.

The exponent is the number of times the base is multiplies by itself.

And, obviously, there is the result.

$$Base^{Exponent} = Result$$

The action of finding the exponent when we know the base and the result is called Logarithm, and is written in the following way:

$$Log_{Base}\ Result = Exponent$$

For example:

therefore $3^2 = 9$ therefore $\log_3 9 = 2$

$2^3 = 8$ therefore $\log_2 8 = 3$

In the same way, the connection between the variables in Ronen's graph is $x = 2^y$ therefore $\log_2 x$.

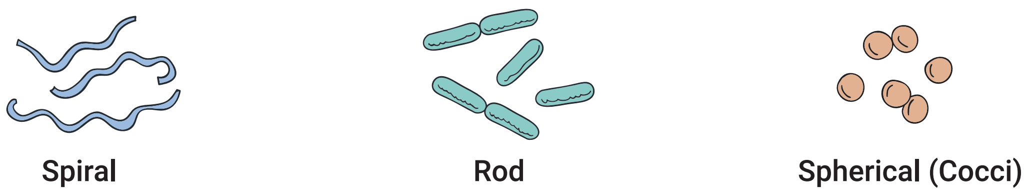
b. Initial Identification of Bacterium Type in the Culture Using Generation Time and Bacterium Shape

Generation time: The time required for the population of bacteria to **double** in number. Each species of bacteria has its typical generation time. Generation time is usually defined in minutes. Although not all bacteria of the same species divide simultaneously in the culture, their generation time is identical.

Bacteria can be divided into three main groups according to their external shape (see Illustration 2).

- **Spherical (cocci)** which have round cells
- **Rod** whose cells are elongated and cylindrical, in the shape of rods
- **Spiral** whose cells are spiral shaped

Illustration 2: Typical bacteria shapes

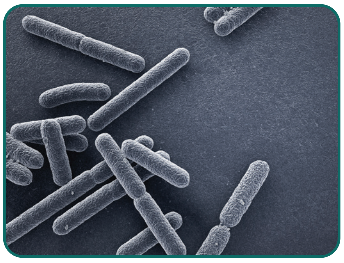
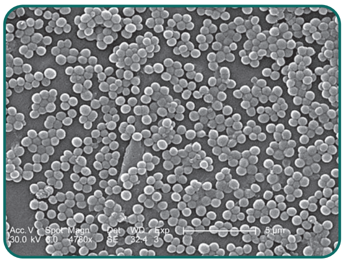


7. a. View again the attached video clip which Danit filmed, and try to identify the shape of the bacteria in the culture

   b. Below is Table 4 showing data about generation time and shape of different types of bacteria. Based on the shapes of bacteria in the table, what is the possible type of bacteria in A.R.'s urine sample?

   Answer: Since they are rod shaped, the type could be either Pseudomonas eruginaosa or Escherichia coli.

Table 4: Generation Time and Cell Structure of Bacteria Types

Bacterium type	Generation time (minutes)	Bacterium shape	Photo taken with electronic microscope	Information on bacterium
Escherichia coli	20	Rod		One of the common, naturally-occurring bacteria types in human intestines. When bacteria pass from the intestines to the urinary tract, they can cause a urinary tract infection. It is the most common bacteria found in urinary tract infections.
Staphylococcus aureus	24	Spherical (Coccus)		A type of bacteria commonly found on the skin of the body (armpits, groin, digestive system). These bacteria are not usually harmful but when they enter the body and the urinary tract, they can cause complicated infections. This bacterium is responsible for a small percentage of urinary tract infections.
Pseudomonas aeruginosa	30	Rod		A type of bacteria found in the environment (on the ground or in water). When it enters the body, for example, the lungs, blood or urinary tract, it can cause infections. This bacterium is responsible for a small percentage of urinary tract infections. This bacterium is present in 7-10% of patients hospitalized with a urinary tract infection.

8. a. Danit took the bacteria from A.R.'s urine sample and inserted 100 bacteria into a test tube. After an hour, she checked the number of bacteria in the test tube and found that there were 800 bacteria.

I. Calculate the number of divisions (duplications) that took place within the hour.

3 divisions

Different ways to find the correct answer:

1. **In the outset of the experiment there were 100 bacterial cells. After the first division there were 220, after the second: 440, and in the third – 800.**

2. It is possible to use the algebraic expression:

$$2^n \cdot 100 = 800$$

$$2^n = 8$$

$$n = 3$$

II. Calculate the generation time of the bacteria that were in A.R.'s culture. Select the correct answer and explain the way you calculated.

- 12 minutes
- 15 minutes
- 20 minutes
- 30 minutes

The correct answer: 20 minutes. We found that during one hour (60 minutes) there were 3 divisions. Therefore, each division probably lasts 20 minutes, which is the generation time.

b. According to the data on generation time in table 4, which type of bacteria is found in A.R.'s urine sample?

Escherichia coli

Teachers: The calculated generation time was 20 minutes, which matches the generation time of Escherichia coli. Therefore, this is the type of bacteria found in A.R.'s urine sample. There might be some students who will suggest also S. aureus, since 24 minutes are close to 20 minutes. At this stage, it is possible to leave the decision open. Another possibility is to encourage the students to explain their different hypotheses (for example, to refer to the other columns in the table). Cross linking the information from the table will lead to the correct type of bacteria.

c. To ensure that the generation time you calculated fits the type of bacteria you identified, return to the video clip in which Danit assembled all the pictures taken under the microscope at different times. Does the data in the video match the type of bacteria you identified in A.R.'s urine sample? Explain your answer.

Yes, the video clip shows that the number of bacterial cells is doubled every 20 minutes.

d. Does your hypothesis regarding the type of bacteria, which was based on their shape, match your suggestion for the type of bacteria you found when looking at generation time (question 8b)?

Yes, because table 4 shows that Escherichia coli bacteria have a rod shape. Table 4 shows two rod shaped bacteria: Escherichia coli and P. aeruginaosa. While the generation time of Escherichia coli matches the generation time that Danit found in the urine sample, the generation time of P. aeruginaosa is 30 minutes. Therefore, Escherichia coli is more probably the bacteria in A.R.'s urine sample.

According to the data you have up to this point, there is a suspicion that Escherichia coli bacteria are in A.R.'s urine sample. However, in order to confirm this, Danit must conduct additional experiments that require a larger number of bacteria. To do so, she will have to multiply the bacteria by growing them for additional time.

c. Growing Bacteria for Additional Tests of Bacteria Type

Danit needs at least 1010 bacteria per milliliter (ml) for the additional tests she wants to conduct in order to confirm the type of bacteria A.R. is suffering from. Based on the data she collected up to this point, she performed the following calculation:

Generation time = 20 mins.

Number of bacteria per ml at outset = 5000

Desired final number of bacteria per ml = 10^{10}

According to Danit's calculation, after seven hours, there should already have been 10^{10} bacteria per ml in the culture.

Teachers: The method for calculating the time needed to obtain 1010 bacteria in the culture can be found in the Appendix: Several methods for calculating the time required to obtain 1010 bacteria

Milliliter (ml) unit of volume = One thousandth of a liter or a cubic centimeter (a cube, each of whose sides is one centimeter in length).

It is customary to write **large numbers** as a multiple of numbers from 1 to 10 (not including 10) and a power of 10 in the following manner: $1 \cdot a < 10, a \cdot 10^n$

This type of expression is called scientific notation.

Example: 24,730,000,000,000 would be written in scientific notation as $2.473 \cdot 10^{13}$

Writing **large numbers** in a uniform manner helps to read the number, to carry out calculations, to compare numbers, to create estimates and to recognize the degree of accuracy. This type of notation is customary in scientific writing. Later on, we will see that small positive numbers are written in a similar manner.

The power of 10 in the scientific notation of a number corresponds to the place value of the first digit on the left side of the number. Therefore the power is a number lower by 1 than the number of digits in the integer part of the number.

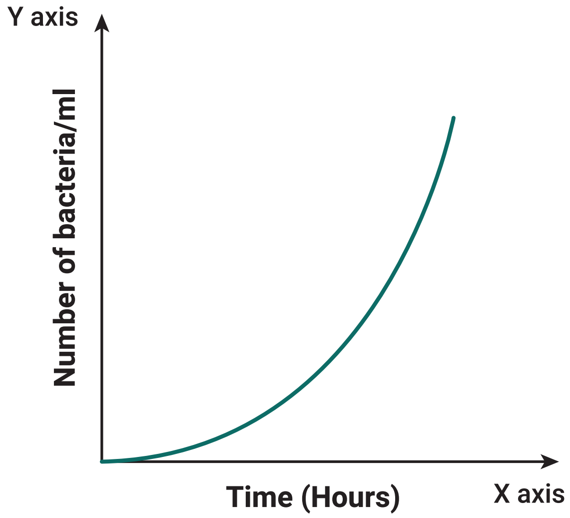
Example: The number 8,400,000 has seven digits. The place value of the number 8 (the first digit on the left) is 10^6, and its scientific notation is $8.4 \cdot 10^6$, in which the corresponding power of 10 is 6.

Danit grew the bacteria for 10 hours, but when she counted the bacteria after 10 hours, she discovered that there were only 10^9 bacteria per ml. To understand what happened she again took 5000 bacteria per ml, grew them and checked every hour how many bacteria there were in the growth medium.

9. Hypothesize what the results will look like during the 10 hours process and plot the hypothesized graph of the number of bacteria throughout the 10 hours on the coordinate system below.

Teachers: Here the students are requested only to sketch the graph. The emphasis should be on the shape of the graph and not on precise numbers.

An expected sketch the students would suggest:



At this stage, any suggestion should be accepted. Further on in the unit the students will discover that bacterial numbers stop rising at some stage of bacterial growth in the culture.

It is probable that students will sketch the shape of the graph they are familiar with so far, in which the number of bacteria continues to rise (resembling the sketch shown on the coordinates above). Perhaps they think that the unexpected result Danit obtained in her experiment is a mistake, or they don't think that the number is large enough to influence the shape of the graph, or any other reason. Perhaps some students will sketch a plot that at some stage parallels to the x axis or even declines (since Danit found a lower number of bacteria than expected). Here it is important to pay attention to the reasoning of the students (not only to the shape of the graph), and to discuss it.

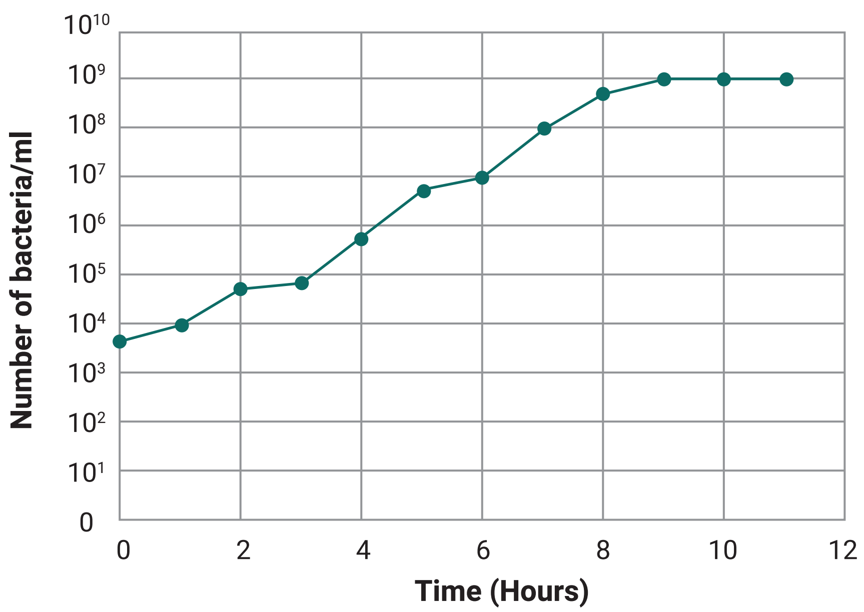
The results of the experiment in which Danit counted the number of bacteria per ml in the test tube over time are presented in Table 5.

Table 5: Results of Experiment, Counting the Bacteria Cells at Different Times

Time (hours)	Number of bacteria/ml in the test tube
0	$5X10^3$
1	$1X10^4$
2	$5X10^4$
3	$8X10^4$
4	$5X10^5$
5	$5X10^6$
6	$1X10^7$
7	$1X10^8$
8	$5X10^8$
9	$1X10^9$
10	$1X10^9$
11	$1X10^9$

10. On the coordinate system below, Danit plotted the number of bacteria obtained at different times, which is summarized in Table 5 and the results are presented on Graph 1.

גרף מספר 1: תוצאות ניסוי של גידול החיידקים בתרבית במשך 11 שעות



b. Look at Graph 1 and table 6 and fill in the blaks:

After 9 hours the number of bacterial cells in the culture was **1X10**.

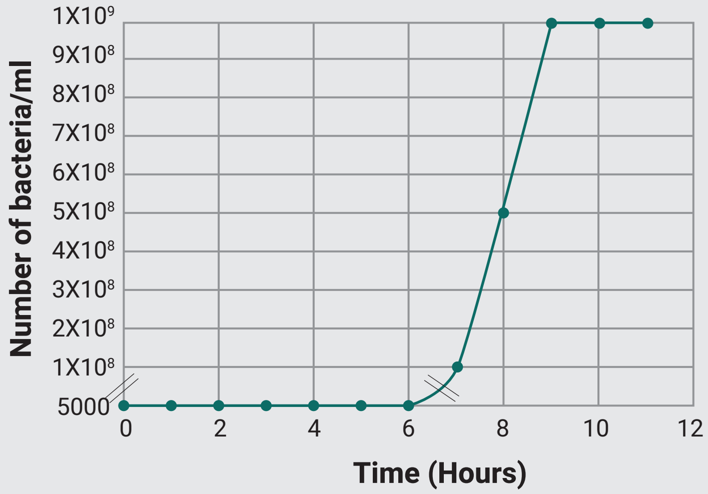
After an additional hour the number of bacterial cells remained unchanged and this trend remained the same for the next **2** hours of the experiment.

c. Point out two differences between Graph 1 and the graph you hypothesized (question 9).

Teachers: There are several types of differences between the two graphs. If the students mention that graph 1 is not as steep as the graph they sketched, it is possible to explain that the reason is the logarithmic scale, where the first scale mark is in the digits, the second is in the tens, the third is in the hundreds etc.

An explanation for the logarithmic scale of the graph

In culture conditions which allow bacterial growth, bacterial populations are continuously doubled, therefore the changes in the number of bacteria are very large. If you plot a graph with a regular Y axis, it will not be possible to see the changes among all the points on the graph. As can be seen on the graph below, despite the changing number of bacteria/ml over the first six hours, it is impossible to see the differences because the values are relatively small such that the points representing them are very close to the value of 5000 bacteria on the Y axis.



In addition, since very quickly the size of the population increases in several magnitudes, it will be difficult to plot all the points to the area of the graph.

Sometimes students find it difficult to understand how much space is minimized with a logarithmic scale. This can be demonstrated in several ways:

– The exponent tells us how many digits the full number has. It is possible to request the students to write the full numbers in table 5.
– Here, again, it is possible to demonstrate the large changes with a transparent plastic bag, and wheat grains or lentils (as in question 2, table 2). This time, ask the students: When will there not be enough room in the bag for all the grains?
– Use examples from their everyday life: The graph is too tall and we would like to shorten it like a photograph.

In such cases, when the differences between the measured values are very large, it is customary to plot the graph using a logarithmic scale, as can be seen in Graph 1 above: in this kind of scale the values presented on the Y axis are expressed by the use of powers of 10 (the exponents) instead of the full numbers (the results). This way, the first scale mark represents number 10 (10^1), the second scale mark represents the number 100 (10^2), the third – 1000 (10^3) etc. In this type of scale, the distances between the scale marks remain constant, although each one of them represents scaled up magnitudes.

Please note that the y axis in the above graph is marked //, which represents a break in the axis.

It is also possible to discuss with the students about connecting the dots of the graph to a continuous line.

Students may suggest several answers. For example:
- **The number of bacteria stopped growing after 8 hours**
- **There are more moderate inclines as well as more steep inclines in the numbers of the bacteria**
- **The change is not constant**

c. Choose one of the differences you pointed out and explain it.
An explanation for the change of trend in the graph (The change is not constant):
All the former graphs were drawn according to a theoretical mathematical expression; therefore, the change had a constant trend. In contrast, graph 1 is based on the results of an experiment conducted in real. Biological systems demonstrate variability that stems from the research methods or environmental conditions. Therefore, the change is not in accordance with the theoretical mathematical expression, and consequently not constant.

d. Assuming the bacteria are constantly reproducing it would be expected that the graph describing the reproductive process of bacteria would be one with an ascending function throughout. In Graph 1, it appears that during the first eight hours, the function does indeed ascend but starting from the eighth hour it appears that the function is constant (with a slope of 0). Try to hypothesize what happened in the culture after nine hours. Why didn't the number of bacteria increase?
A variety of answers are to be expected. For example: Something in the components of culture medium was changed, Danit added something that disturbed the growth of the bacteria.

e. Danit thought that the bacteria stopped reproducing. In order to examine her hypothesis, she observed the culture under a microscope and saw that even after nine and ten hours there were bacteria that were dividing in the culture. How was it possible that there were bacteria in the culture that were reproducing but the overall number of bacteria in the culture did not increase? Propose hypotheses.

Teachers: In sections "d" and "e" students are invited to raise hypotheses of the biological processes that lead to the different shape of the graph. At this stage, they are not expected to know the answer. The explanation is brought on here, but it is recommended to reveal it to the students only after they bring up their own hypotheses.

An explanation to the constant number of bacterial cells in the culture although they continue to divide (the plateau of the graph):

Under optimal (ideal) growth conditions, where bacterial cells density is not high, there are abundant nutrients and the substances secreted by the bacteria are mixed in with the fluid around them, the bacterial population doubles at regular intervals: from one bacterium two are obtained, which will become four bacteria, from those, eight, and so on (the number of bacteria after n generations will be 2n).

In practice, when bacteria are grown in the laboratory in a closed container there is an increase in the density of bacteria and a deterioration in growth conditions. There may be several reasons for the change in conditions:
- The nutrients diminish and become a factor which limits the rate of division.
- The bacteria secrete waste into the growth medium which accumulates and changes the growth conditions and harms the reproduction process of the bacteria.

These changes in the growth medium cause the rate of bacterial reproduction to decrease and the rate of death to increase up to the point where they are equal. Under these circumstances, the population size remains stable, which is expressed in a graph as a horizontal line (with a slope of approximately 0).

Even when bacteria reproduce in their natural environment, for example in the human body, the conditions are not optimal and there are various factors which limit their rate of reproduction.

11. One possible hypothesis is that the bacteria stopped growing in numbers. At this stage, new bacteria were being created, but there was also an equal number of bacteria that were dying. Choose the appropriate answers and fill in the blanks:

After nine hours, the **difference** between the number of new bacteria created and those dying bacteria in the culture increases/**remains unchanged**/decreases and is equal to: ___**0**______ bacteria.

In total, the number of live bacteria in the culture increases/**remains unchanged**/ decreases and is equal to: ____**$1x10^9$**____ bacteria.

The ratio between the number of bacteria created and those dying increases / **does not change** / decreases and is equal to: __**1**___.

12. In light of what you learned about the growth of bacteria in the laboratory, try to think about how Danit would obtain the number of 10^{10} bacteria per ml which she needs in order to definitively identify the type of bacteria in A.R.'s sample.

Students can suggest, for example:

- **To grow a few cultures (containers) in parallel**
- **To add to the culture more growth medium without bacteria**
- **Every now and then, she can take out a certain amount of bacteria from the culture (to decrease the density)**

Teachers: the last suggestion is possible but inefficient since taking out bacteria prevents from arriving at the desired amount.

d. Final Identification of Bacteria Type in the Culture Using Gram Staining

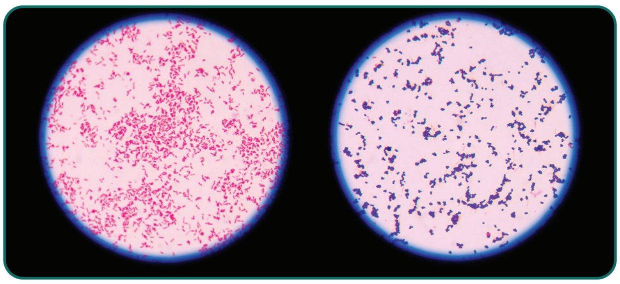
After a number of attempts, Danit found the way to obtain the number of bacteria she needed for the purpose of performing additional tests.

The generation time of Escherichia coli bacteria is close to the generation time of Staphylococcus aureus (see Table 4). In order to be sure that in A.R.'s bacterial culture there are *Escherichia coli* and not Staphylococcus aureus, Danit performed a special coloring procedure for bacteria called Gram staining. See the Gram staining example in Illustration 3. It is known that in this type of staining, *Escherichia coli* bacteria become pink while Staphylococcus aureus bacteria turn purple.

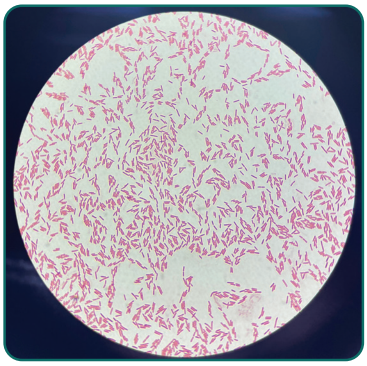
Illustration 3: Bacteria under a microscope after Gram staining.

Bacteria that turn purple (right hand side) are called Gram positive, and bacteria that turn pink (left hand side) are called Gram negative.

Illustration 3: Bacteria under a microscope after Gram staining.
Bacteria that turn purple (right hand side) are called Gram positive, and bacteria that turn pink (left hand side) are called Gram negative.



Below is the picture of what Danit saw when she looked under the microscope at A.R.'s bacteria that she grew, after using the Gram-staining procedure.



13. Did the staining results of the bacteria confirm Danit's hypothesis that in A.R.'s culture there was an infection due to *Escherichia coli* bacteria? Explain.

    Yes, the results confirm her hypothesis since the photograph shows that the bacteria were stained pink as Gram-positive bacterial cells stain, among which are *Escherichia coli*.

Points summarizing the unit and to reflect on

At the beginning of this activity you built a simple mathematical model to predict the **rate of bacterial reproduction** and thus can be used to calculate how many bacteria are expected after X divisions. Later on, using the model you also found the generation time of the bacteria.

- At which stages in the unit did you use **mathematical tools**? (For example: diagrams and graphs, tables, algebraic expressions, numerical calculations, statistical measures)
- Did the mathematical tools help predict the number of bacteria there would be at every point in time?
- For which stage of bacterial reproduction in the laboratory was the mathematical model appropriate?
- What are the gaps you found between your model and the results obtained in the laboratory?
- Try to summarize what you learned in this unit about the scientific research process and the stages of developing scientific discoveries.

The mathematical model you built helped you to offer a prediction regarding the rate of reproduction of bacteria under specific conditions, but we saw that when the conditions changed the model did not accurately predict the number of bacteria. The reason for this is that in the initial model we did not relate to all the processes that take place in the bacterial culture.

In order for there to be a model that will correctly describe the rate of bacterial reproduction even after they remain in the culture for a long time, there is a need to build another model that will also take into account the additional processes that occur in the bacterial culture in the laboratory.

In the next unit, you will try to find the appropriate treatment that will help A.R.

Definitions and explanations

Regression rule

A way to calculate terms in a series so that any term is calculated by **performing an arithmetic action on the previous term in the series**.

For example, to find the term in the series 10,20,30 … one has to add 10 to the previous term.

n (position in the series	1	2	3	...	n
	10	20	30	...	

The algebraic expression, then, would be: (n-1)+10

Therefore, to calculate the term in position 4 in the series: 30 + 10 = 40

This type of series is called recursive series, which means that something is repeated again and again.

Calculation by position

A way to calculate terms in a series by their position.

For example, to find the term in the series 10,20,30 …

n (position in the series	1	2	3	...	n
	10	20	30	...	

The algebraic expression, then, would be: n·10

Therefore, to calculate the term in position 12 in the series: the position (n) should be multiplied by 10: 12·10 = 120

$2^0=1$

One can refer to 2^0 as 2^{3-3} or $^{27-7}$, and in general as 2^{n-n}

According to exponential rules: $a \neq 0$, $\frac{a^n}{a^m} = a^{n-m}$

Therefore: : $a \neq 0$, $\frac{a^n}{a^n} = a^{n-n} \quad = a^0$

According to arithmetic rules, any number divided by itself equals 1.

Therefore: $a \neq 0$, $\frac{a^n}{a^n} = 1$

And we receive: $a \neq 0$, $\frac{a^n}{a^n} = a^{n-n} \quad = a^0 = 1$

Ascending function, descending function

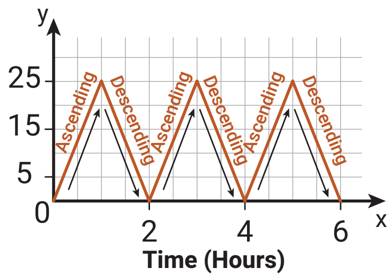
From the "Integrated Mathematics for seventh Grade", part C unit 13, page 24

A function is Ascending or descending according to the change in y values. Observing the x axis from left to right (i.e. rising x values):

If for all x values in a certain domain - y values increase, then the function is said to be an ascending function.

If for all x values in a certain domain - y values decrease, then the function is said to be a descending function.

For example: The following function is ascending and descending alternately.



Constant pace, changing pace

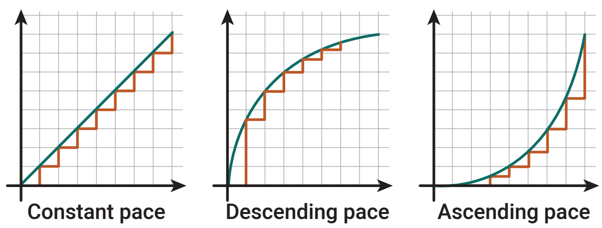
From the "Integrated Mathematics for seventh Grade", part C unit 14, page 42

In a series of following steps of the same width

If the height of the steps continues to rise – the pace accelerates.

If the height of the steps continues to decline – the pace slows down.

If the height of the steps remains unchanged – the pace is constant.



Axis break

Usually, when constructing graphs, it is important to maintain equal distances between the origin of the coordinates and the scale marks, as well as between the scale marks themselves: the distance between each scale mark on the axis should remain constant to represent a constant difference between the numbers written on it.

In some cases, the first dots begin in high values in relation to the scale marks. In these cases, it is custom to minimize the area of the graph and to "break the axis": two short lines (// or ≈) are marked on the origin of the coordinates (0,0) between the first scale mark and the second one, to emphasize that the gap between them is not the constant gap between all the scale marks in the graph.

Connecting dots in the graph

The line which connects any two dots that represent measurements that were performed in the experiment, is not based on any measurements at all. The dots on this line are the result of interpolation (approximation of results that were not measured and are in position between two measurements). Nevertheless, connecting the dots together helps to easily identify the trend of the phenomenon being researched. This is especially common in sciences when the variable on the x axis is continuous (for example: time, concentration), so there is a meaning to any dot that was not measured (for example1hour and 15 minutes).

Constant function and slope=0

Any function described with a straight line is a constant function, in which as values in x axis change there is a constant change also in y values. The slope of a constant function indicates the rate of the change in y. A function which is described by a straight line parallel to the x axis in a graph is a constant function with a slope=0. This slope indicates that y values remain unchanged as the values of x axis change.

Several ways to calculate thetime needed to receive 10^{10} bacterial cells

The appropriate way to calculate is:

$$5000 \cdot 2^n = 10^{10}$$

Teachers:

1. During the discussion it is important to encourage the students to suggest several ways to solve the problem.
2. Several ways are suggested below. There is no need to discuss all of them, and there may be students who will suggest other ways as well.

Generally, the stages of the solution are as follows:

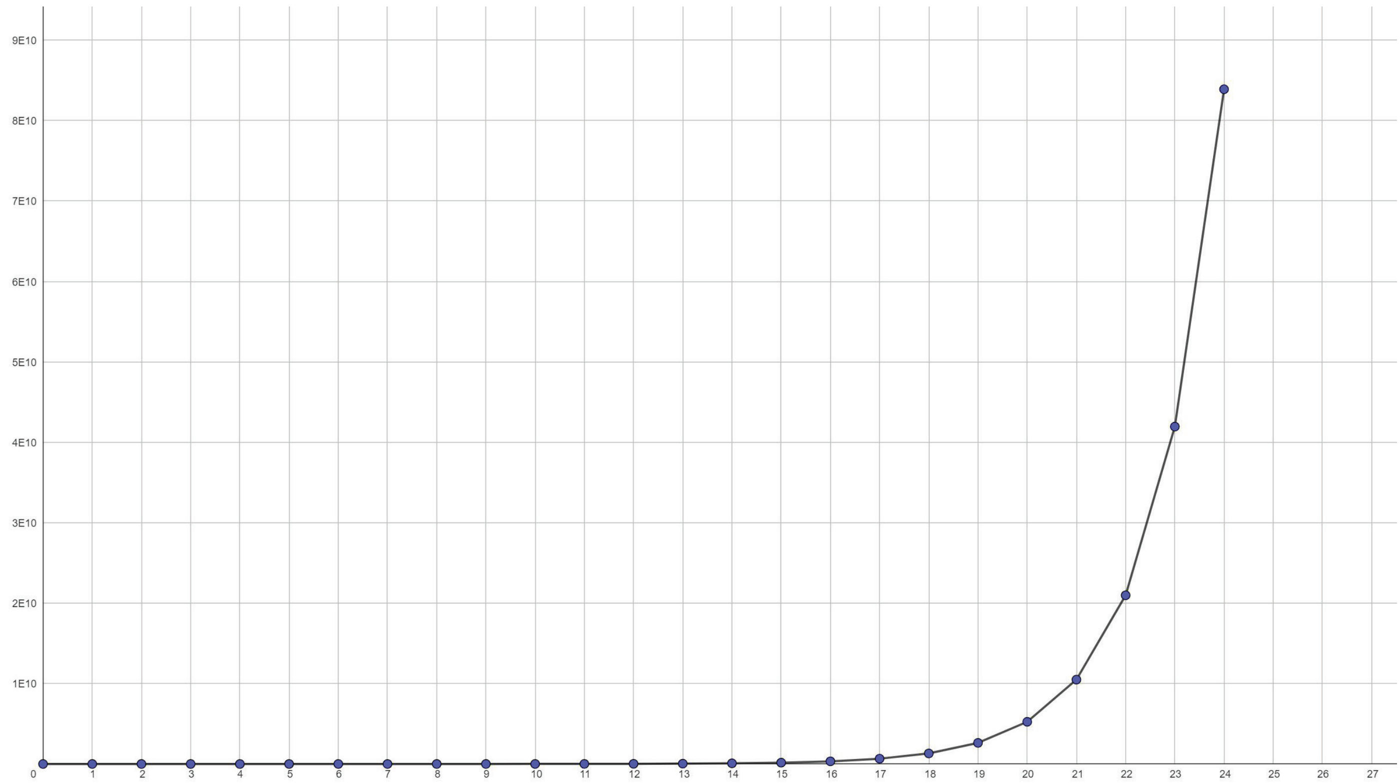
a. Find the exponent n, i.e. the number of divisions needed to receive 1010 bacterial cells. The result is: n=21

b. Find the time needed to receive 10^{10} bacterial cells, knowing that the generation time is 20 minutes, that are 1/3 hour: Divide by 3 the number 21 (the result received in "a"), or multiply it by 1/3, to receive the result: 7 hours.

Strategies for stage a:

- **Trial and error**: the students will use a calculator to find the exponent n=21.
- **Graph**: Students can construct a graph to estimate n value, and then try values in proximity to these values. Constructing the graph requires changes in the definitions of the axes, to make it possible to see the graph and "find" the correct dot.

For example:



	A	B	C
1	החזרה	קוטר ההילה	
2	0	5000	
3	1	10000	
4	2	20000	
5	3	40000	
6	4	80000	
7	5	160000	
8	6	320000	
9	7	640000	
10	8	1280000	
11	9	2560000	
12	10	5120000	
13	11	10240000	
14	12	20480000	
15	13	40960000	
16	14	81920000	
17	15	163840000	
18	16	327680000	
19	17	655360000	
20	18	1310720000	
21	19	2621440000	
22	20	5242880000	
23	21	10485760000	
24	22	20971520000	
25	23	41943040000	
26	24	83886080000	

:In column A
First row-0, second
row: =a2+1

:In column B
=5000*2^a2
(=5000·2^{a2} :כלומר)

Mark a2 and b2
and drag downwards
from the right low corner

- Using arithmetic rules and exponent rules

$$5000 \cdot 2^n = 10^{10}$$
$$5 \cdot 10^3 \cdot 2^n = 10^{10} \qquad / : 10^3$$
$$5 \cdot 2^n = 10^7 \ /: 5$$
$$2^n = 2000000$$

Using logarithms:
Note that logarithms are
not part of the curriculum in
junior high school

$$n = \frac{\ln 2000000}{\ln 2}$$
$$n = 21$$

Trial and error with calculator

From this stage on, Stage b is identical to all strategies: dividing the result by 3 or multiply by.-1/3

References:

Graph 1 and table 5:

Duffy G., Whiting R.C., .Sheridan J.J., The effect of a competitive microflora, pH and temperature on the growth kinetics of Escherichia coli O157:H7' Food Microbiology, 1999, 16, 299-307

Table 5 photographs:

Staphylococcus aureus

Content Providers: CDC/ Janice Carr/ Deepak Mandhalapu, M.H.S., Public domain, via Wikimedia Commons

Pseudomonas eruginaosa

https://phil.cdc.gov/Details.aspx?pid=229

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