How to Count Bacteria

There are many methods for counting and this page covers most of them.

Many types of projects are possible when you are able to count bacteria. For example, you could count the bacteria in drinking water, fresh milk, old milk that is slightly sour, buttermilk, yogurt, mud puddle, lemonade, and many other things. Or you may want to know how fast Chlorox kills bacteria. If you add some Chlorox to a culture and count the surviving bacteria at suitable intervals you can plot killing the killing curve and determine whether the killing is single hit or higher order. It will be very informative to plot on normal graph paper and also on semilog graph paper. Don't be alarmed all these things are easy to understand and will eventually be covered in this webpage and its subpages.

This page and its subpages will show you how to count bacteria with precision. This page begins with protocols used by professionals, but at the bottom are ideas you can use when you have no equipment. Beginners can get a good introduction trying the No Equipment Needed page.

Some Typical Bacteria Counts Seen in Experiments

If there are enough bacteria in a liquid culture to make the culture barely cloudy, counting the cells commonly reveals nearly one hundred million bacteria per milliliter (1×10^8 cells/mL). If one mL of an overnight aerated culture of Escherichia coli B is added to 20 mL of fresh broth, and aerated 1.75 hours at 37° C you will have a log-phase culture containing about 200 million bacteria per milliliter (2×10^8cells/mL). Knowing the cell titers with such precision is important in bacteriophage genetics work and other molecular biology projects. See page p.htm for such bacteriophage projects. This page will help you understand all this language and how to do the work. It is not as difficult as it sounds to a novice.

Are you familiar with numbers like 2×10^8 cells/mL. Do you understand and use phrases like "two times ten the eighth cells per ml." It is a very convenient terminology used by all bacteriologists and molecular biologist. This method of expressing numbers is called scientific notation and you can learn about it on page math211. Page b038a gives examples of scientific notation from bacteria experiments.

Counting Bacteria by Dilution and Plating

The example below will help you understand how to dilute and plate bacteria. This example is the way that I usually set up my tubes for assaying the bacteria titer when I am working with log-phage E. coli B in phage experiments. I suggest you print a copy of this diagram and use it at your work bench.

Notice that I transferred 0.1 mL from the bacterial culture into tube #1. Tube 1 which contains 9.9 mL of Diluting Fluid (DF). Note that 0.1 mL + 9.9 mL = 10 mL. Therefore, Tube #1 contains a 100-fold dilution of the original liquid culture. Tube #2 is handled exactly the same way. For tube #3, 0.1 mL was diluted into 1.9 mL making this a 20 fold dilution. Notice I plated 0.1 mL from Tub #3 which is a 10 fold factor. Suppose I count 104 bacteria on the plant. Then the calculation of titer of the original bacteria culture is 100 x 100 x 20 x 10 x 104 => 2.08 x 10^8 cells/mL.

Here are the details of each step:

1. Use a clean, sterile, dry pipet to remove 0.1 ml from the bacteria sample and blow it into the 9.9 ml of dilution fluid in tube #1 and mix thoroughly by blowing lots of bubbles with the pipet for a couple seconds. Discard the pipet into the used jar for later cleaning. Notice tube #1 now contains 1/100 the concentration of bacteria in the original sample because 0.1 ml is 1/100 of 10 ml. Since nearly 0.1 ml of liquid may cling to the outside of the pipet, you must wipe the pipet with Kleenex or toliet paper before inserting the pipet into tube 1.
2. Using another clean, sterile, dry pipet remove 0.1 ml from Tube 1, wipe pipet, blow contents of pipet into Tube 2, continue blowing bubbles for a second or two for good mixing.
3. Using another clean, sterile, dry pipet remove 0.1 ml from Tube 2, wipe, blow contents of pipet into Tube 3, continue blowing bubbles for a second or two for mixing.
4. Plate 0.1 from Tube 3. See below for information on plating.
5. There are many types of pipets, we are using blow out pipets and that is indicated by a frosted ring on the pipet at the top end. Read my page on types of pipets.

Suppose 125 colonies grow in the petri plate. Multiply the dilution factors by the count to obtain the titer of the bacteria sample: 100 x 100 x 20 x 125 ==> 250,000,000 bacteria per milliliter in the original sample. 250,000,000 = 250 million = 2.5 x

10^8 bacteria per milliliter. It is important to understand Scientific Notation which is explained on page b038a.htm.

resume here

Beginners usually assume each colony grew from a single bacteria, but experts prefer say each colony originated from a colony-forming unit. Dead bacteria do not form colonies. Some bacteria occur as single cells. Other species hang together in chains or clumps of 2 or more bacteria. A piece of dirt with 10 bacteria on it will form a single colony. Molecular biologists do everything with as much precision as possible. Therefore, they prefer to work with bacteria which do not form chains or clumps.

Other Methods for Counting Bacteria

Counting Chambers

Counting chambers automaticly fill with a certain volume. There are several kinds of counting chambers but they all consist of a special microscope slide with a coverglass.

- Fill chamber with bacterial liquid culture according to manufactuer's instructions.
- Place slide under microscope
- Count the bacteria according to the instruction sheet.
- Calculate the number of bacteria per milliliter of orginal sample from the known volume of the counting chamber according to the instruction sheet.
- One disadvantage of this method is that you can't tell which bacteria are alive. Therefore the count includes dead bacteria. Thus this method is useless in disinfection studies.

Most Probable Number.

- Dilute the bacteria so that each tube contains less than one bacteria on average
- Count the number of tube which grew
- Using a special method calculate the Most Probable Number
- to be continued and revised. page b038b will give more details on the MPN method.

Membrne Filters

- Water is filtered on a filter which has holes smaller than bacteria

- The filter is then placed on a dish of agar and bacteria grow on the top of the filter.
- Count the bacteria and calculate the cell titer.

One big advantage of this method is that huge volumes of water can be filtered to show a few bacteria per liter. The filter method also allows one to rinse the filter by following the sample with sterile clean wter so that anything interfering with the growth of bacteria is rinsed away.

Photometers and Spectrometers

- Light of a suitable wavelength is passed through a tube of the culture.
- If there are lots of bacteria, less light gets through the culture.
- A meter reads the amount of light passing through the culture
- By consulting a standard curve which you have prepared by reading the meter and plating the bacteria, you can estimate the number of bacteria.
- This method is very useful in industrial fermentations where you need to know the number of bacteria now rather than tomorrow after the plates have grown colonies.

How to Plate Bacteria

- Pour plate or spreading plate.
- hine light through the culture, cells will block light according to the number of bacteria
- cell counters move culture thru a hole and by conductance the cells are counted. Cost tens of thousands of dollars.

Colony forming units are single bacteria or clumps of bacteria which are able to form colonies on the medium used. 0.1 ml of the samle is taken the pipet adjusted to zero and wiped with kleenex or toliet paper and blown into tube #1. 0.1 ml into 9.9 ml is a 100-fold dilution. If the titer of the sample was 10 to the eighth, it is 10 to the sixth in tube #1.

Methods for Kids not having Equipment

Don't be discouraged if don't have the equipment mentioned above. You can make your equipment and have useful results. Your home built equipment produce larger errors but who cares; you are having fun and learning. Indiana Biolab offers many items from its stocks for kids and amateurs.

Comparing Bacteria Counts of Water Samples.

Suggested water samples: tapwater, well water, rainfall caught in a sterile vessel, rainbarrel water, ditch water, pond water, river water, lake water, ocean water, swimming pool water. Remember to use sterile tools at all stages of your study.

- Put a drop of water of well water on a sterile plate of TGY or other agar.
- Spread it uniformly over the surface of the agar with any non-absorbent sterile tool. A glass rod bent into an L-shape is ideal. The bottom of a teaspoon will also work.
- Incubate the plate at 30C or room temperature (r.t.).
- Examine the plate as often as you like. At room temperature, it will probably take a day or two for single cells to grow into colonies large enough for you to see. Different species of bacteria grow at different speeds and some species will take many days.
- Count the colonies. There are about 18 drops per mL, the size of drop depends on the orifice. Small tips make small drops and it can take 30+ drops to make one milliliter.

Calulation of bacteria titer: If your pipet gives 18 drops per mL and you counted 10 colonies, then the bacterial count for your sample is 18 x 10 = 180 bacteria per milliliter. Keep in mind that on TGY agar most common bacteria will grow and the colonies you find will be mostly harmless bacteria. There are agars which permit the growth of coliforms or the coliforms give colored colonies.

Please be aware that if you made two plates, it is unlikely both plates would have same number of colonies. As a rough estimate of the expected variation (Variance) take the square root of the actual count. Let us suppose you got 100 colonies. The square root of 100 is 10 (because 10 times 10 = 100). 100 plus or minus 10 gives 110 to 90 as a likely range for the true number of bacteria per drop of the original source. Multiplying by 20 (20 drops per mL), we obtain 1800 to 2200 bacteria per mL of the source.

Keep in mind that besides the expected statistical variation mentioned above, there may be systematic bias errors. Examples of bias include:

- Some bacteria will remain on the spreader - causing your count to be too small
- Some bacteria were unable to grow under your conditions - causing your count to be too small
- Contamination from dust in the room - causing your count to be too large

- some bacteria may be clumped together and grow a single colony - causing your count to be too small
- Your dropper may yield more or fewer drops than 20 per mL
- Try to think of more systematic bias errors
- Try to think of random errors such as drops differing in size from drop to drop or some plates being inferior to others (somewhat unlikely).

Few science fair contestants consider statistics. This simple introduction will enable you to impress some judges. More important; it will help you understand the important of statistics in medical research and the planning of your project.

You are likely to find many kinds of microbes growing including molds.

Keep in mind that you are actually assaying Colony Forming Units (CFUs). Examples of CFUs include a single bacterium, several bacteria on a particle of meat or dirt, etc.

You can use eyedroppers or short pieces of glass tubing. 1/10 ml pipets and test tubes are not very expensive.

Simple Introduction to Dilution for Cell Counts

If you used city tap water in the above experiment you probably got no bacteria. If you used well water in the above experiment probably got many bacteria. If you used water from a swimming pool, you probably got many bacteria per drop and it is likely the plate was covered and could not be counted.

If you got so many colonies that they ran together, then your count is not accurate because they are hard to count and very likely some colonies consisted of 2 or 3 bacteria landing so close together that they grew as a single colony.

Therefore, you may consider buying some test tubes and pipets as they are not so expensive.

You can use small glass bottles, or other convenient vessels and count drops to make serial dilutions. You could put 49 drops of DF in a container and add one drop of well water, mix by swrilling and plate one drop. If 80 colonies grow, that indicates 50 x 80 = 4000 bacteria per drop of well water or 80,000 per mL if you dropper makes 20 drops per mL.

Examples of Common Bacteria Titers Foods and Waters

Recall that one mL of water weighs one gram. Most foods sink in water, which means they are a little heavier than water.

Source	Typical Titer bacteria per mL or gram
overnight aerated culture of Esherichia coli B	3 to 5 x 10^8
	x 10^8
	x 10^8

I will add more to this page as time passes

Put this hard stuff in a separate section and subpages. It is also true that all killing and growth in bacteria cultures follows a logarithmic function, not a linear function. If those words are new to you, you have not completed a good algebra course. Do not fear this page and its subpages will teach you all you need to know. This page is a remarkable opportunity for you to learn some very interesting mathematics and biology. This biomath is very interesting to me. I use it to count bacteria in cultures, molecules in cells, the number of particles involved in a reaction, etc. From these numbers I can make guesses about how something happens inside a test tube or inside a cell (I can propose possible mechanisms for a reaction).

Please do not give up. I have some easy and hard stuff in this page. Work on the easy stuff and someday you will understand the hard stuff. You can be very proud of yourself if read some of these pages.

If you know there are 2 x 10^8 bacteria per mL of your culture, we say the titer of the culture is "2 times 10 to the 8th". That is 200 million cells per mL. Recall one mL is about 20 drops of water. If all this is new to you try reading about Scientific Notation(LINK). Scientific notation is easy. "2 times ten to the eighth" means 2 * 10 * 10 * 10 * 10 * 10 * 10 * 10 * 10. Notice there are eight 10s. 10 to the eighth is very familiar to any bacteriologist as a bacterial culture having that titer is just barely turbid.

First installed 1998 Nov 30 Revision #4 1999 Sep 29 37°C
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Some Rules to Guide your Experiments.

When I dilute 1 mL of overnight aerated E. coli B cells into 20 mL of Tryptone Broth (TB) and aerate 1.75 hours at 37 90° C, the cell titer is usually 2 x 10^8 cells/mL +/- 5%.