

The Perfect Brew:

How many yeast cells do we need?

You are a novice home brewer and are very excited to be trying out your very first batch.  You have all the basics ready, but the part of brewing you are still struggling with is the yeast to use.  You have done your research, spoken with some local breweries, and have figured out the strain of yeast to use to give your beer a distinctive flavor.   The only thing you have left to do is to figure out how much yeast to use in your batch.  The amount of yeast can be critical, because these single-celled organisms are responsible for converting the sugar in your mixture into alcohol and producing carbon dioxide as a by-product.   Having too much yeast may make your beer bitter and too carbonated.  Having too little yeast may make your beer have a low percentage of alcohol and perhaps too sweet.  You want to get this just right!

From your research you have learned that the perfect amount of yeast is **1.5 million cells/ml** (to learn more about using yeast in brewing visit this site: <https://wyeastlab.com/yeast-harvesting-re-pitching>).

In this activity, you will learn how to obtain this concentration of cells.

### Learning Objectives:

After this activity, students will be able to:

1. describe the purpose and use of a hemocytometer.
2. describe and label the parts of a hemocytometer.
	1. describe the dimensions of the grid on the hemocytometer.
3. describe how the volume of liquid on the cytometer relates to taking up space.
	1. describe the relationship between cubic millimeter and the microliter.
	2. describe the relationship between cubic centimeter and the milliliter (ml)
4. describe how cells are visualized as dead or alive using Trypan blue.
	1. describe how cells exclude the dye from living cells and why they exclude.
	2. consider the different cell shapes and healthiness of cells.
5. describe and apply the rules of counting cells in a grid.
	1. count the cells in a given grid accurately.
	2. convert the cell count to a concentration.
	3. average the different counts from grids and determine a statistical mean.
	4. perform the calculations to determine the original concentration of cells in a sample.
6. describe the statistical rationale for the 30 to 300 rule.

# Part 1:  Tools for counting?



To count cells, like yeast, we use a specialized instrument called a hemocytometer.  A hemocytometer is a special microscope glass slide with two identical cell counting areas for counting two different samples at a time. The hemocytometer comes with a special cover slip for accurate cell counting. Once you have a liquid solution of your yeast cells, you can use this special slide on a microscope to see the cells. (See more photos like this at<https://www.hemocytometer.org/>)



Before use, the hemocytometer is covered with a special cover slip (thin piece of glass) that spans the two counting chambers. Using a 20-ul micropipette, the cell volume is usually mixed with an equal volume of cell viability stain such as Trypan blue.  It’s important to count the cells that are alive in your sample as they are the cells that will be able to ferment your sugars and produce alcohol.

When you zoom in on the microscope you can see a grid like the example in Figure 1 (there are two of these grids on each hemocytometer slide).  The cell counting area of the hemocytometer is composed of 9 large squares (one of these is marked in red above), and each of the 9 is 1x1 mm.

Figure 1 Diagram of the counting grid on a hemocytometer

*Figure SEQ Figure \\* ARABIC 1 Diagram of the counting space in a hemocytometer.*

The four corner squares are further divided into 16 identical squares (one of these is marked in green above) of 0.25 mm each side.

The middle large square is divided into 25 identical squares, (one of these is marked in yellow), 0.2 mm each side. Each of these 25 squares are further divided into 16 squares each (one of these is marked in blue) 0.05 mm each side.

**Question 1) What is the area of the yellow square shown above?**

**Question 2) Looking at the diagrams above and thinking about the volume of liquid that is covering the yellow square. How deep is the liquid covering the yellow square and how can we calculate this volume?**

To better visualize this volume of liquid, we can see the diagram below:



**Question 3) First let us look at a 1 mm by 1 mm by 1 mm box (shown as the yellow box above), and we define that as a microliter.  How many of these boxes are in a cube that is 1 cm by 1 cm by 1 cm?**

**Question 4) What liquid volume would this box hold?**

# Part 2: Counting cells

We want to ensure that we have a healthy sample of yeast for our brewing experiment.  In order to ensure the cells are healthy, we are using a dye called Trypan blue.  Trypan blue is an acidic dye that cannot get into healthy viable cells.  Dead cells lack a functioning membrane and this allows the Trypan blue to get in, staining the cells blue.  So in order to accurately count living cells, we add Trypan blue dye to our sample and pay attention when we look through the microscope to whether or not the cells are stained a dark blue color.

Another factor to consider, is cells don’t always line up nicely for us. In some cases the cells can be on top of the gridlines of our hemocytometer; if we counted all the cells on the lines we may overcount our sample. So we need to make rules about whether we count cells that are laying on the lines.  To avoid exaggeration of cell count within the large squares, cells touching the right and lower sides should be excluded.

**Question 5) Label the following parts in the following image:  (borders of the grid top, bottom, left, right, dead cells, living cells, borders to count, borders to not count).**

**Question 6)  Examine your image of the hemocytometer grid.  Count the number of viable, healthy yeast cells in our sample and record in the table below.**

What sample image did you count? \_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Total Cell Count | Viable cell count | Non-viable cell count | Average Viable Cell Count |
| 1 |  |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |
| 5 |  |  |  |

**Question 7) Discuss with neighboring students, or look at more than one image and fill in the counts from a few other squares**.

**Question 8) What is the average number of viable cells that you counted?**

**Question 9) Circle the cells that you excluded from your count in your image.**

**Question 10) What are some possible differences between your cell counts and those of your fellow students?**

# Part 3: Calculating Concentration

Now that we’ve counted our cells, we are ready to calculate our estimate for the cell density of our sample. Here’s what we need:

The volume of the space above the yellow square (yellow squares from Part 1 and 2), and the average number of cells from our 5 counts (from Part 2).

**Question 11) What is the volume of a yellow square:**

**Question 12) What is the average number of cells in a small square?**

Next, we need the dilution factor of our sample. We have taken 10 𝛍L of the original sample and mixed with 10 𝛍L of our trypan blue solution. This was done so as to make the process of counting in Part 2 realistic and allow us to see the cells that are alive versus those that are dead. The dilution factor, which we will call D, is the ratio of the diluted solution volume to the original sample volume. It’s given by

$$D = \frac{sample (or stock) solution volume}{total diluted solution volume}.$$

A note on dilution factors: There are a few ways people represent a diluted solution. One way is to a **Sample:Diluent** format. For example, if we added 20 mL of diluent to 5 mL of our sample solution, we could write 5:20.

Another way to represent the situation is with a **Sample:Total** format, so using our example above, we could write 5:25, since there would be 5 mL + 20 mL = 25 mL total volume of the diluted solution.

There is no consensus on which format is used, so be sure you are aware of what format is being used in each instance. In either case for our example, the dilution factor would be

$$D=\frac{5 mL}{25 mL}=\frac{1}{5}=0.20$$

**Question 13) What is the dilution factor for our yeast solution?**

Now we have all the pieces we need to calculate our measured cell density. Cell density is measured as the number of cells per unit volume. In our case, we will use our average number of cells from a small square and the volume (mL) of the small square (Step A). However, since we diluted our stock solution we need to apply the dilution factor, since our sample solution is actually much more concentrated than what we viewed in the hemocytometer. The calculation is:

$$Measured Cell Density=\frac{average number of cells in yellow square}{D\*(volume of yellow square)}.$$

**Question 14) What is the measured cell density of our yeast sample?**

**Question 15) How does this compare to the cell density we need (1.5 million cells/ml)?**

**Question 16) How should we adjust this sample to give us the correct concentration?**