

Biol 213L: Genetics Lab (Fall 2000)

Sample Lab Report for Lab 1

Even those of you who just answered the questions no doubt noticed that if you strung those answers together and added the appropriate headings, you'd get something very close to a real lab report. I've taken that step below, to give you my conception of what a complete lab report might look like. Mine is single spaced to save paper. Please double space your own reports!

What to look out for while reading the sample lab report

Introduction

- The problem stated at the beginning of the Introduction was general and abstract, something anyone could appreciate without much background. The problem stated at the end of the Introduction was specific and concrete, the actual problem that prompted the experiments. That problem should be the focus of all that follows

Materials and Methods

- Use subsection headers. Especially in complicated lab reports, like you'll write for the fly lab, headers make what you write much easier to read.
- Many of you did not understand how to arrive at the volumes used to make the original mixture, using the absolute number method. You will have to be comfortable with this kind of calculation, so please take a look in my example report.
- I gave the actual equations I used to calculate numbers. I did not merely describe the procedure in words. The derivation of the equations, I admit, is more than I expected in your report, but I wanted you to see where they came from.

Results

- Focus on the scientific problem. The problem isn't to learn how to dilute (though I hope you do). The problem is what are the volumes used to make the mixture of *E. coli*.
- Note that I justified why I was throwing out some data, then I threw it out.
- Even though the initial volumes I got from the ratio method added up to 200 μl , I did not present this as confirmation that everything was all right. Volumes obtained from the ratio method will ALWAYS add up to the total volume of the initial mixture. It's built into the equation.

Discussion

- Discussions should do more than sling words around. Whatever idea you float, test it against reality. It isn't enough to say that pipetting error might have produced erroneous results. How much pipetting error would be required to produce the results you obtained? Is that amount of pipetting error likely, given your measurements of precision and accuracy?
- Just as it isn't enough to say that pipetting error might be a problem, it also isn't enough to say that the pipetting error according to your measurements was +/- 2%. That error applies to a single pipetting. It is necessary to go through the dilution experiment and see how much this error could accumulate.

Lab Report 1 (Sample)

Serial Dilution of a Bacterial Culture

Introduction

Genetic analysis often requires the measurement of the frequency of events that are not likely to occur except in a large sample. For example, one in perhaps a million trypanosomes may undergo a mutation that enables it to evade our immune system and cause sleeping sickness. To quantitate the rate of mutation, it is necessary to know the number of total trypanosomes, but how can you count millions of microorganisms?

Dilution can bring large numbers of organisms to a manageable number. Dilution is achieved by mixing a small volume of a culture with a large volume of diluent. Micropipettors are very useful in transferring the small volumes required, but, like any machine, defects or misuse may limit their accuracy and precision.

Since we will rely on a P1000 micropipettor to perform dilutions, we sought to measure the degree to which we could rely on it, determining how accurate and precise it is. To test our ability to use dilutions within a biological context, we were given a mixture of two strains of bacteria. Our job was to determine the volumes of the pure cultures used to form that mixture.

Materials and Methods

Calibration of a micropipettor

We calibrated our P1000 micropipettor in two ways. First, we assessed the accuracy of the micropipettor, that is degree to which measurements accord with reality, by comparing the volume delivered by the P1000 with the volume delivered by a conventional 1 ml pipet, which we took to be 1 ml. In brief, 1 ml of water was delivered by the pipet to a test tube, and the P1000, set to 1 ml, was used to suck up that volume of water. The setting of the P1000 was adjusted until it sucked up all the water and no air.

Second, we assessed both the accuracy and the precision of the micropipettor, the latter being the degree to which measurements accord with one another, measuring the true volume of water delivered as judged by its weight. The P1000 set to 1 ml was used to deliver water to a pan balance ten times. After each delivery, the weight of the water was measured and, using the equivalence of 1 g water = 1 ml, the volume was calculated. The accuracy of the micropipettor was assessed by comparing the average volume delivered to 1.0. The precision of the micropipettor was assessed by noting the range between the lowest and highest measurements.

Determination of volumes of pure cultures in a mixture

We were given a microfuge tube containing 200 μ l mixture of two strains of *E. coli*: JC158 (lac^+ , able to metabolize lactose) and AB3517 (lac^- , unable to metabolize lactose). The total number of cells in the mixture was estimated by diluting the culture and plating a known volume on MacConkey plates. MacConkey plates contain lactose and a pH indicator. JC158 (lac^+) metabolizes lactose, reducing the pH of its surroundings, producing rise to red colonies. AB3517 (lac^-) does not alter the pH and produces cream-colored colonies.

In order to estimate the dilution necessary to plate a countable number of cells, we assumed that the original mixture of stationary phase cells contained 2×10^9 cells/ml. This is a typical concentration for cultures that have just ceased growth [Elhai et al, 2000]. The dilution scheme can be seen in Table 1. The dilutions were plated as previously described (Elhai et al, 2000) and counted after 26 and 54 hours of incubation.

The volumes used to make the original mixture were calculated in two ways. The first method (Absolute Number Method) reversed the dilution scheme to arrive at an estimate of the concentrations of the Lac⁺ and Lac⁻ strains in the original mixture ($C_{\text{Mix(JC)}}$ and $C_{\text{Mix(AB)}}$, respectively). From these and the estimated concentrations of the pure cultures, the desired volumes (V_{JC} and V_{AB}) were calculated. The method proceeded in three steps. First, the concentration of the plating suspension (C_{Plating}) was calculated for both strains separately as:

$$(1) C_{\text{Plating}} = N_{\text{Plate}} / V_{\text{Plating}} = N_{\text{Plate}} / (0.1 \text{ ml})$$

where V_{plating} (0.1 ml) is the volume taken from a dilution for plating and N_{Plate} represents the number of red or cream-colored colonies found on the plate. Then, the accumulated dilution factor (F) was used to calculate the concentration of cells (C_{Mix}) in the original mixture:

$$(2) C_{\text{Mix}} = F \cdot C_{\text{Plating}}$$

Finally, this concentration and the estimated concentration (C_{init}) of the original pure culture was used to obtain the volume (V_{pipet}) and number of cells (N_{pipet}) taken from that culture:

$$(3) C_{\text{init}} \cdot V_{\text{pipet}} = N_{\text{pipet}} = N_{\text{Mix}} = C_{\text{Mix}} \cdot V_{\text{Mix}} = C_{\text{Mix}} \cdot 0.2 \text{ ml}$$

These three equations were combined to give an expression to calculate V_{Pipet} , the quantity desired:

$$\begin{aligned} (4) V_{\text{Pipet}} &= C_{\text{Mix}} \cdot V_{\text{Mix}} / C_{\text{init}} && \text{Rearrange (3)} \\ &= F \cdot C_{\text{Plating}} \cdot V_{\text{Mix}} / C_{\text{init}} && \text{Substitute for } C_{\text{Mix}} \text{ from (2)} \\ &= F \cdot N_{\text{Plate}} \cdot V_{\text{Mix}} / (C_{\text{init}} \cdot V_{\text{Plating}}) && \text{Substitute for } C_{\text{Plating}} \text{ from (1)} \\ &= (0.2 \text{ ml}) \cdot F \cdot N_{\text{Plate}} / (2 \cdot 10^9 \text{ cells/ml} \cdot 0.1 \text{ ml}) && \text{Put in known values} \\ &= F \cdot N_{\text{Plate}} / (10^9 \text{ cells/ml}) && \text{Note that units are ml} \end{aligned}$$

The second way (Ratio Method) of calculating the volumes used to make the original mixture relied on the assumption that the ratio of Lac⁺ to Lac⁻ cells is the same throughout the experiment, both in the original mixture and on the plates of dilutions. If that's so, then:

$$(5) N_{\text{Plate(JC)}} / N_{\text{Plate(Total)}} = N_{\text{Mix(JC)}} / N_{\text{Mix(Total)}}$$

$$(6) N_{\text{Plate(AB)}} / N_{\text{Plate(Total)}} = N_{\text{Mix(AB)}} / N_{\text{Mix(Total)}}$$

But the number of cells in the mixture is the same as the number of cells in the pipet tips that produced that mixture ($N_{\text{Mix}} = N_{\text{Pipet}}$). Since for any solution, the number of cells can be calculated from the concentration and volume of that solution:

$$(7) N = C \cdot V$$

if follows that:

$$(8) N_{\text{Plate(JC)}} / N_{\text{Plate(Total)}} = N_{\text{Pipet(JC)}} / N_{\text{Mix(Total)}} = (C_{\text{Pipet(JC)}} \cdot V_{\text{Pipet(JC)}}) / (C_{\text{Mix(Total)}} \cdot V_{\text{Mix}})$$

$$(9) N_{\text{Plate(AB)}} / N_{\text{Plate(Total)}} = N_{\text{Pipet(AB)}} / N_{\text{Mix(Total)}} = (C_{\text{Pipet(AB)}} \cdot V_{\text{Pipet(AB)}}) / (C_{\text{Mix(Total)}} \cdot V_{\text{Mix}})$$

Presuming that the pure cultures of JC158 and AB3517 are at the same concentration, then any volume taken from them (C_{Pipet}) and any mixture of them (C_{Mix}) will also have the same concentrations of total cells. If so, then the equations simplify to:

$$(10) \frac{N_{\text{Plate(JC)}}}{N_{\text{Plate(Total)}}} = \frac{V_{\text{Pipet(JC)}}}{V_{\text{Mix}}}$$

$$(11) \frac{N_{\text{Plate(AB)}}}{N_{\text{Plate(Total)}}} = \frac{V_{\text{Pipet(AB)}}}{V_{\text{Mix}}}$$

and solving for V_{Pipet} and substituting in the known value of V_{mix} :

$$(12) V_{\text{Pipet(JC)}} = (0.2 \text{ ml}) \cdot \frac{N_{\text{Plate(JC)}}}{N_{\text{Plate(Total)}}}$$

$$(13) V_{\text{Pipet(AB)}} = (0.2 \text{ ml}) \cdot \frac{N_{\text{Plate(AB)}}}{N_{\text{Plate(Total)}}}$$

Thus the two volumes can be calculated from the known number of colonies.

Results

Calibration of micropipetter

In order to assess the accuracy of our P1000 micropipetter, we compared the volume delivered by a conventional 1 ml pipet to that sucked up by the micropipetter set to 1 ml. The micropipetter took up all the water in the test tube and a bit of air besides. When set to 0.96 ml, the micropipetter took up precisely the water available. At first, I obtained different amounts of air with each trial, but once I and others in my group figured out how to use the micropipetter, the results were the same, regardless of who pipetted and how fast the plunger was released.

As an independent measure of accuracy of the P1000 and to determine the precision of measurements with this micropipetter, the weight of 10 1 ml aliquots of water were measured (Table 2). The calculated volumes of the aliquots ranged from 0.98 to 1.02 ml, a precision of 2%. The average calculated volume was 1.00 ml. In this regard, the experiment disagreed somewhat with the accuracy determination based on taking up water delivered by a conventional pipet. From this experiment I conclude that the micropipetter faithfully delivers the volume within 2% of that set.

Determination of volumes of pure cultures in mixture of *E. coli*

To reconstruct the events that produced the mixture of *E. coli* strains given to us, we diluted and plated the mixture on indicator plates. 26 hrs after plating, the both red (Lac^+) and cream-colored (Lac^-) colonies could be seen. The colony counts obtained at this time differed somewhat from that obtained at 54 hrs, but since the two types of colonies were more difficult to distinguish at 54 hrs, counts from the earlier time was used.

Two dilutions of the mixture were plated. The mixture diluted 10^4 -fold gave rise to colonies that overlapped, making counting problematic. Both dilutions were scored (Table 3), but the 10^4 -fold dilution yielded fewer than 10-fold as many colonies as the 10^5 -fold dilution and the latter results were used in later calculations.

Concentrations of JC158 and AB3517 in the original mixture were calculated from the number of red and cream-colored colonies (Table 3). From these, initial volumes of 115 μl and 52 μl were calculated for JC158 and AB3517, respectively, as described in Materials and Methods. 69% of total colonies on the plate were red and 31% cream colored, permitting a calculation of the original volumes of 138 μl and 62 μl , respectively, according to the ratio method (see Materials and Methods). The volumes obtained from the absolute number method and from the ratio method differ by about 20%.

How can we decide amongst these two estimates? One matter of concern is that the sum of the volumes calculated by the absolute number method is 167 μl , 17% lower than the true volume of 200 μl . Another way of expressing this discrepancy is that the total cell concentration calculated for the original mixture should be equal to that of each of the original pure cultures in stationary phase, which we took to be 2×10^9 cells/ml. The calculated total cell concentration was 1.7×10^9 cells/ml (Table 3), 15% lower than anticipated.

Discussion

We were presented with a mysterious tube filled with two strains of *E. coli* in unknown proportions. Our goal was to discover the volumes of two pure cultures used to make the mystery tube. I believe that about 140 μl of JC158 and 60 μl of AB3517 were used. These values were obtained by the ratio method. The failure of a second independent method to confirm these values cast some doubt on my conclusions. However, this method is probably less trustworthy.

The absolute number method requires knowledge of the cell concentrations of each of the two pure cultures. I took these both to be 2×10^9 , the figure given as the cell density at which *E. coli* ceases growth (Elhai et al, 2000). The ratio method relies on the assumption that both strains will dilute with equal fidelity and the same fraction of cells of both strains will grow into colonies on MacConkey agar. I know of no property of these strains that would lead to differential plating efficiency, and indeed, the ratios of the two types of colonies is roughly the same at the 10^4 and 10^5 dilutions. On the other hand, the assumption that the pure cultures have concentrations the same as that at which *E. coli* ceases growth is very suspicious. Certainly, starved *E. coli* must die off at some rate, and we have no idea how long the strains were starved before being given to us. If 15% of the cells had died, then the viable cell concentration would be 1.7×10^9 cells/ml, agreeing with the value derived from the absolute number method. The ratio method also requires that the two pure cultures are of equal concentration. This would be true so long as the cultures die at approximately the same rate.

The ratio method is more trustworthy also because it is insensitive to pipetting error. Only the ratio between the two cell types is important. However, pipetting error is unlikely to be significant even in the absolute number method. If each of the liquid transfers used to dilute the mixed culture (Table 1) were off by 2%, the volume of the diluent always being 2% too big and the volume of the culture always being 2% too small, the resulting error would still be too small. Recalculating the dilutions shows that the worst that could happen is that the concentrations of the original mixture would be underestimated by 16% (see Table 4), still less than the 20% observed discrepancy. It would take something beyond routine pipetting error to account for the difference between the two calculations.

Table 4: Estimate of greatest expected error from pipetting

Mixture	Dilution assuming 2% error (worst case)			No error	
	Volume of culture	Volume of diluent	Dilution factor	Accumulated dilution factor	Accumulated dilution factor
Dilution A	9.8 μl	1010 μl	104.1	104.1	100
Dilution B	9.8 μl	1010 μl	104.1	$1.08 \cdot 10^4$	$1.00 \cdot 10^4$
Dilution C	98 μl	918 μl	10.37	$1.12 \cdot 10^5$	$1.00 \cdot 10^5$
Dilution D	98 μl	918 μl	10.37	$1.16 \cdot 10^6$	$1.00 \cdot 10^6$