**ABSTRACT**

Serial dilution is often a difficult concept for students to understand. In this short dry lab exercise, students perform serial dilutions using seed beads. This exercise helps students gain skill at performing dilutions without using reagents, bacterial cultures, or viral cultures, while being able to visualize the process.

**Key Words:** Serial dilution; simulation; reagents.

Many laboratory exercises and experiments require one to make serial dilutions of bacterial cultures or virus cultures in order to calculate the concentration of bacterial cells or virus particles in the original sample. Dilutions are also often used to make working solutions from stock solutions or in preparing other experimental reagents. In general, dilutions are made by mixing a known amount of sample with a known volume of diluent. Dilutions are more accurate if made through a series of smaller dilutions rather than one big dilution. Serial dilution is the process by which each dilution is made using the same volume of material transferred to a series of tubes containing the same volume of diluent. Serial dilutions are usually made in multiples of 10—termed a “tenfold dilution”—but can be made in any increment you choose, such as twofold, fivefold, or hundredfold dilutions.

In this lab, we will start with an unknown amount of beads and simulate a serial dilution so that you will be able to visualize what is happening and be able to calculate the number of beads in your original sample. The following equations will help you with performing and calculating dilutions.

\[
\text{Dilution} = \frac{\text{sample volume}}{\text{sample volume} + \text{diluent volume}}
\]

For example, 1 mL of sample is mixed with 9 mL of saline. Therefore, the dilution is 1/10. A 1/10 dilution can also be written as 1:10 or \(10^{-1}\).

If you are performing a multistep dilution, multiply all the dilution steps together.

Consider the following serial dilution: \(1/10 \times 1/10 \times 1/10 = 1/1000, \text{ or } 1 \times 10^{-3}\).

If you did not have a serial dilution but made each dilution step different, the equation would be, for example, \(1/2 \times 1/5 \times 1/10 \times 1/2 = 1/200, \text{ or } 1 \times 10^{-3}\).

If the initial concentration of your sample is known, the diluted concentration can be determined by multiplying the starting concentration by the dilution.

For example, if you start with a sample that is 5 mg/mL NaCl and you dilute it 1:1000, then 5 mg/mL \(\times 1/1000 = 5 \times 10^{-3}\) mg/mL, or 5 μg/mL.

If the initial concentration of your sample is unknown, such as the number of bacteria per milliliter or viral plaque-forming units per milliliter, then you must serially dilute and plate your samples on the appropriate medium or host cell. After incubation, count the colonies of bacteria or virus plaques. The best plates to count contain between 30 and 300 colonies or plaques. You then multiply your colony or plaque count by the reciprocal of the dilution factor of the plate you counted.

For example: You dilute a sample 1:100,000, and after plating 1 mL of each dilution you find that there are 75 virus plaques on the 1:10,000 dilution plate (see below). You then multiply 75 divided by the volume plated by the reciprocal of the dilution factor to determine the number of virus particles in your original sample: 75/1 mL \(\times 10,000 = 7.5 \times 10^2\) virus particles/mL (Figure 1).

This simple exercise helps students visualize what is happening during a serial dilution.
Lab Exercise

Materials
- White seed beads: these will represent the diluents
- Green seed beads: these will represent the virus
- Test tubes: these will represent the test tubes
- Small plastic beakers: these will represent the 1-mL pipettes
- Petri dishes: to plate your samples

Figure 2. Supplies for the serial dilution laboratory. In the front are tweezers and a 30-mL plastic beaker. Behind them are the seed beads, size 10/0. In the back row are the test tubes and plastic petri dishes.

Figure 1. Example. You dilute a sample 1:100,000, and after plating 1 mL of each dilution you find that there are 75 virus plaques on the 1:10,000 dilution plate. You then multiply 75 divided by the volume plated by the reciprocal of the dilution factor to determine the number of virus particles in your original sample. Calculation: 75/1 mL + 10,000 = 7.5 x 10^5 virus particles/mL.

Figure 2 shows the supplies you will need to run this exercise. Seed beads (size 10/0) can be purchased from a local craft store or online at http://www.shipwreckbeads.com. For a class of 18 students working in pairs, I used one package or hank of green beads and six packages of white beads. Thirty-milliliter plastic beakers can be ordered from Fisher Scientific, or you can recycle the dosing cups from over-the-counter liquid medicines.

Procedure
1. Obtain five test tubes and fill each with 9 mL of white seed beads (diluent) using the plastic beaker. For this exercise, 9 mL will be represented by filling the plastic beaker to just below the 10-mL mark. Label the test tubes 10^-1, 10^-2, 10^-3, 10^-4, and 10^-5.

2. Remove 1 mL of virus (green seed beads) from the original sample using the plastic beaker and place it in the 10^-1 test tube. For this exercise, 1 mL will be represented by covering the bottom of the plastic beaker with the seed beads. Shake the test tube well to mix the beads. Notice the distribution of green beads mixed in with the white beads. Be sure to mix your beads until they are evenly distributed.

3. Remove 1 mL of sample from the 10^-1 test tube and place it in the 10^-2 test tube. Shake the test tube well to mix the beads. Notice that there are fewer green beads in the test tube.

4. Remove 1 mL of sample from the 10^-2 test tube and place it in the 10^-3 test tube. Shake the test tube well to mix the beads.

5. Remove 1 mL of sample from the 10^-3 test tube and place it in the 10^-4 test tube. Shake the test tube well to mix the beads.

6. Remove 1 mL of sample from the 10^-4 test tube and place it in the 10^-5 test tube. Shake the test tube well to mix the beads.
The American Biology Teacher

Serial Dilution Simulation Lab

The researchers have used serial dilutions in their work and ask students to look for a journal article in the course syllabus. After the lab, 50% of the students felt that the lab had helped them a lot with their understanding of serial dilutions and 50% felt that the lab had helped them somewhat. Most students were more comfortable performing a serial dilution and more comfortable calculating the results after the lab. None of the students felt that the lab did not help at all. A number of students commented that it had helped a lot to visualize what was happening. All the students felt that I should keep this lab in the course syllabus.

This semester, we ran the exercise during the first lab session. In three subsequent lab sessions in which the students had to perform serial dilutions for each exercise, not one of the students made a mistake in performing a serial dilution. Also, none of the students asked me to explain the lab exercise to them again after instructions were given at the beginning. This is in contrast to previous years, when a number of students needed individual help before they could start the serial dilutions or calculate the results. This simple exercise helps students visualize what is happening during a serial dilution and leads to a better understanding of the technique. It also helps them understand the concept of serial dilution without using living organisms and without making mistakes in their experiments.

### Sample Problem

Diagram a series of twofold, fivefold, and tenfold dilutions using four tubes. For each of the dilution series, calculate the number of virus particles in your sample if after making the dilutions you plated 1 mL from the fourth tube and then after incubation you counted 50 plaques on the fourth dilution plate.

### Further Suggestions

You can experiment with alternatives to seed beads, but I found that the seed beads include white and brown rice, larger beads, or glow-in-the-dark beads. You can make up numerous dilution problems as a homework assignment. To follow up the dry lab, I have students serially dilute Methylene Blue, measure the absorbance of their samples at 665 nm, and see how close they have come to a set of dilution tubes I have made. Also, as a follow-up, I will have students look for a journal article in which the researchers have used serial dilutions in their work and ask them to explain why the serial dilutions were beneficial to the experiment. I usually have the students do this as homework, but you might consider having examples in the lab for them to look through and have a group discussion. This is a good way to see if the students have grasped the concept of serial dilutions. Two examples of journal articles that can be used are provided in the References below (El-Shibiny et al., 2005; Godden et al., 2008). Finally, depending on the course, students can apply what they learned to enumerate bacteria from food or milk.

### Assessment

I developed this laboratory for use in an upper-level undergraduate virology course. Students taking this course are junior and senior college students who have already taken general microbiology, biochemistry, and/or genetics. I used this exercise on the first day of class to introduce the students to a technique that they would be using throughout the semester. Before and after the lab, I gave a short assessment survey to the students. Before the lab, 90% of the students had performed a serial dilution before but only 50% were comfortable with the concept and with calculating the experimental results of a plaque assay. After the lab, 50% of the students felt that the lab had helped them a lot with their understanding of serial dilutions and 50% felt that the lab had helped them somewhat. Most students were more comfortable performing a serial dilution and more comfortable calculating the results after the lab. None of the students felt that the lab did not help at all. A number of students commented that it had helped a lot to visualize what was happening. All the students felt that I should keep this lab in the course syllabus.

This semester, we ran the exercise during the first lab session. In three subsequent lab sessions in which the students had to perform serial dilutions for each exercise, not one of the students made a mistake in performing a serial dilution. Also, none of the students asked me to explain the lab exercise to them again after instructions were given at the beginning. This is in contrast to previous years, when a number of students needed individual help before they could start the serial dilutions or calculate the results. This simple exercise helps students visualize what is happening during a serial dilution and leads to a better understanding of the technique. It also helps them understand the concept of serial dilution without using living organisms and without making mistakes in their experiments.

### References


CYNTHIA KELER (cynthia.keler@delval.edu) is Associate Professor of Biology at Delaware Valley College, 700 East Butler Ave., Doylestown, PA 18901. TABITHA BALUTIS, KIM BERGEN, BRYANNA LAUDENSLAGER, and DEANNA RUBINO are currently students in their junior year at Delaware Valley College.

Table 1. Record your results in the table and calculate the number of virus particles in your original sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percentage of viruses</th>
<th>Concentration of original sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁵</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7. Label five petri dishes 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵.

8. Take 1 mL from each dilution tube and place it into the corresponding petri dish.

9. Count the number of virus particles (green beads) in each dish and record your data in Table 1. Use the tweezers to remove the green beads and place them into your beaker as you count them. Remember, the best plate to use is one that contains between 30 and 300 virus particles (green beads).

10. Calculate the number of virus particles in your original sample using the following equation: number of beads/mL plated × 1/dilution factor = # virus particles/mL.