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PIPETTING & DILUTIONS

Students will learn how to pipette during the semester in the microbiology lab, for a variety of purposes. Pipetting is a technique used to measure and transfer a specific volume of liquid to a specific container, and this is essential for accurate results.

ALL OF THESE QUESTIONS

1. Pipette 1.00 mL of a 1.00 M solution of NaCl into a 100 mL volumetric flask and dilute to the mark with distilled water. Calculate the concentration of the solution after dilution.
2. Pipette 1.00 mL of a 1.00 M solution of NaCl into a 10 mL graduated cylinder and dilute to the mark with distilled water. Calculate the concentration of the solution after dilution.
3. Pipette 1.00 mL of a 1.00 M solution of NaCl into a 10 mL graduated cylinder and dilute to the mark with distilled water. Calculate the concentration of the solution after dilution.
4. Pipette 1.00 mL of a 1.00 M solution of NaCl into a 10 mL graduated cylinder and dilute to the mark with distilled water. Calculate the concentration of the solution after dilution.
5. Pipette 1.00 mL of a 1.00 M solution of NaCl into a 10 mL graduated cylinder and dilute to the mark with distilled water. Calculate the concentration of the solution after dilution.

CAUTION: Safety glasses must be worn at all times. Do not drink or eat in the laboratory. Wash your hands before leaving the laboratory.

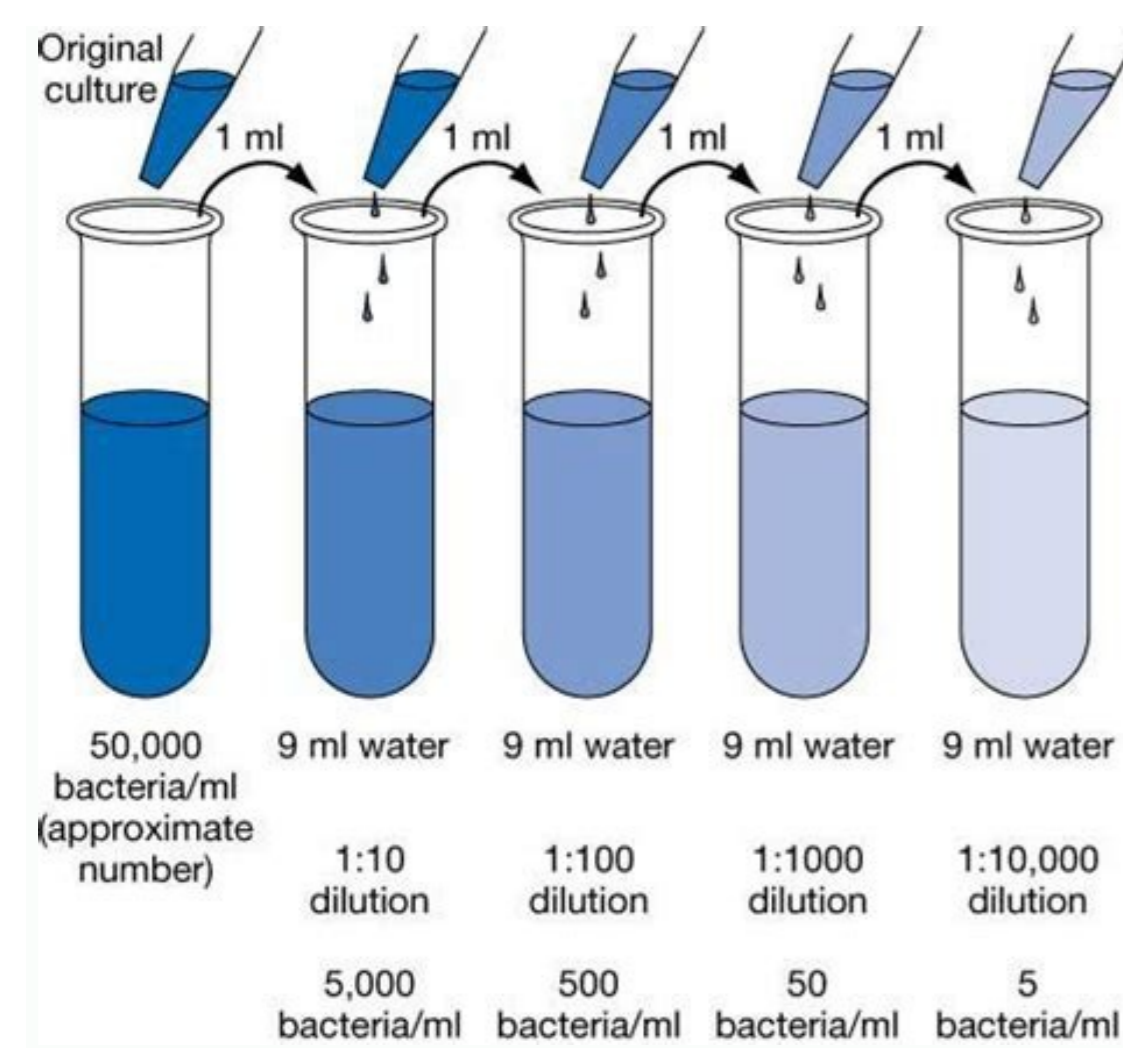
Fall 2011 - Linda Reynolds, Richard Dalgarno

Assignment B: Math of Microbiology worksheet (10 pts.) Name _____

This assignment is intended to help you continue reviewing math involved in science and to specifically challenge you with some microbiologically-related problems. Many of these problems come from real-world situations in biotechnology industry, research, and medicine. You should feel free to work with classmates or seek any help you need to become comfortable with these types of calculations. This assignment will be graded worth 10 pts (1 pt each)... but ultimately you will have a 30 pt quiz administered during the final exam time in order to prove your mastery of these mathematical skills. Some of these we have already worked on in class, some we have not.

1. An object weighs 35.7 grams. How many micrograms is the object? How many nanograms? How many kilograms? Write your responses in scientific notation. (for assistance, review the final Appendix in your lab manual, pg 214)
2. You need to make 625ml of a 7.5% (w/v) sodium nitrite (NaNO2) solution as a food preservative to prevent microbial growth. How many grams of NaNO2 (f.w.=68.995 g/mol) will you need? What is the molarity (M) of this solution?
3. You can purchase the antibiotic tigocycline as an IV drip stock solution at 10 milligrams per ml concentration. The safe, effective dose for children is 1500 micrograms per kg body mass. How many ml of the stock solution must you administer to a 20.5 kg child?
4. You perform 9 steps of a 1:10 (10 fold) serial dilution on a 24 hr growth of liquid bacteria culture. You plate 200 microliters of the last three dilution tubes in the series and find that the plate made from the most dilute tube gives you 41 colonies. What is the concentration of the bacteria (cells/ml) in the original liquid growth tube?
5. Starting with 7.2×10^8 bacteria/ml, you autoclave the sample for 15 minutes and find you have 4.1×10^3 bacteria/ml remaining alive. What is the Decimal Reduction Time (DRT or D-value) for this species under autoclave conditions?

- Answers:**
1. $35.7 \text{ g} = 35,700,000 \text{ } \mu\text{g} = 35,700,000,000 \text{ ng} = 0.0357 \text{ kg}$
 2. $625 \text{ mL} \times 7.5\% = 46.875 \text{ g } \text{NaNO}_2$
 $46.875 \text{ g } \text{NaNO}_2 \div 68.995 \text{ g/mol} = 0.681 \text{ mol}$
 $0.681 \text{ mol} \div 0.625 \text{ L} = 1.09 \text{ M}$
 3. $10 \text{ mg/mL} \times 20.5 \text{ kg} \div 1500 \text{ } \mu\text{g/kg} = 1.37 \text{ mL}$
 4. $200 \text{ } \mu\text{L} \times 41 \text{ colonies} = 8200 \text{ colonies}$
 $8200 \text{ colonies} \times 10^9 = 8.2 \times 10^{12} \text{ cells/mL}$
 5. $D = \frac{\log(N_0) - \log(N)}{k}$
 $D = \frac{\log(7.2 \times 10^8) - \log(4.1 \times 10^3)}{2.303 \times 15}$
 $D = \frac{8.856 - 3.613}{34.545} = 0.149 \text{ min}$



Adam Soliman

Dilution Worksheet

11) If I add 25 mL of a 0.15 M NaCl solution to 150 mL of a 0.1 M NaCl solution, what will be the molarity of the solution? (24788)

$$\frac{25 \text{ mL} (0.15 \text{ M})}{150 \text{ mL}} = 0.125 \text{ M}$$

22) If I add 100 mL of a 0.15 M NaCl solution to 150 mL of a 0.1 M NaCl solution, what will be the molarity of the solution? (24788)

$$\frac{100 \text{ mL} (0.15 \text{ M})}{150 \text{ mL}} = 0.100 \text{ M}$$

33) How much 0.15 M NaCl solution do you need to dilute 250 mL of 10 M NaCl? (24788)

$$\frac{250 \text{ mL} (10 \text{ M})}{0.05 \text{ M}} = 50,000 \text{ mL}$$

44) I have 50 mL of a 1.5 M NaCl solution. If I boiling away and the volume of the solution is 250 mL, what will be the molarity of the solution? (24788)

$$\frac{0.375 \text{ L} (1.5 \text{ M})}{0.250 \text{ L}} = 2.07 \text{ M}$$

55) How much water (mL) do you need to add to 500 mL of a 2.4 M NaCl solution to make a 1.0 M solution? (24788)

$$\frac{500 \text{ mL} (2.4 \text{ M})}{1.0 \text{ M}} = 1200 \text{ mL}$$

Greater than 300 and less than 30 is a high degree of error. This recipe pairs nicely with my macaroni salad recipe! Author: Tracey Cordie The Best Pizza Dough This easy pizza dough recipe is the key to making an extraordinary homemade pizza. And if you don't have... The purpose can be determination of bacterial, fungal, or viral counts (indirectly). Image Source: Chromoscience Objectives of Serial Dilution The objective of the serial dilution method is to estimate the concentration (number of organisms, bacteria, viruses, or colonies) of an unknown sample by the enumeration of the number of colonies cultured from serial dilutions of the sample. In serial dilution, the density of cells is reduced in each step so that it is easier to calculate the concentration of the cells in the original solution by calculating the total dilution over the entire series. Serial dilutions are commonly performed to avoid having to pipette very small volumes (1-10 µl) to make a dilution of a solution. By diluting a sample in a controlled way, it is possible to obtain incubated culture plates with an easily countable number of colonies (around 30-100) and calculate the number of microbes present in the sample. 10-64. To work the problem, you need 3 values—a colony count from the pour or spread plates, a dilution factor for the dilution tube from which the countable agar plate comes, and the amount of the dilution that was plated on the agar plate. Enjoy! Author: cookingmama Easy Coleslaw Dressing A creamy coleslaw dressing that can be made with ingredients you already have! You can pour it immediately... Author: GarlicQueen Grandma's Baked Beans My mother has made these beans for years. Thank you very much for your cooperation. 5.7×10^5 CFU/mL. Fresh rosemary and thyme... Use the total dilution for the tube from where the plate count was obtained. Home » Basic Microbiology » Serial Dilution- Definition, Formula, Calculator, Procedure, Uses Serial dilution, as the name suggests, is a series of sequential dilutions that are performed to convert a dense solution into a more usable concentration. This results in the highest dilution to have the most inaccuracies and the least accuracy. Because serial dilution is performed in a stepwise manner, it requires a more extended period of time which limits the efficiency of the method. Serial dilution only allows the reduction of bacteria/cells but not the separation of bacteria/cells like in other techniques like flow cytometry. This technique also requires highly trained microbiologists and experts in aseptic techniques. In contrast, for a less contaminated sample, a low dilution factor might be sufficient. Serial two-fold and ten-fold dilutions are commonly used to titer antibodies or prepare diluted analytes in the laboratory. The dilution factor in a serial dilution can be determined either for an individual test tube or can be calculated as a total dilution factor in the entire series. The dilution factor of each tube in a set. For a ten-fold dilution, 1 ml of sample is added to 9 ml of diluent. Typed or printed answers will not be accepted. 1. If you count 60 colonies after generating a dilution factor of 10^{-8} and plating 100 microliters, what is the starting concentration? 6.0×10^{10} CFU/mL. Author: Betty Crocker Kitchens Good Old Fashioned Pancakes This is a great recipe that I found in my Grandma's recipe book. Davidson, Estimation method for serial dilution experiments, Journal of Microbiological Methods, Volume 107, 2014, Pages 214-221, ISSN 0167-7012, Cullen, J. The agar solidifies, with the bacterial cells locked inside of the agar. Serial Dilution Applications/Uses Serial dilution is performed in a number of experimental sciences like biochemistry, pharmacology, physics, and homeopathy. This protocol is specific for bacterial counts (colony-forming units, CFUs), but can be modified for fungi (CFUs) and viruses (plaque-forming units, PFUs for viral counts). If you start out with a 10^{-3} pre-made dilution, and pipetted 100 microliters into a 9.9 mL saline tube then plated 100 microliters of that solution, what would be the final dilution factor? Some of which are: An error might occur during the propagation of the sample, and the transfer inaccuracies lead to less accurate and less precise transfer. coli in nutrient broth all the way to soil samples and hamburger meat. In coffee, we add a certain amount of cold press coffee and add water over it to obtain a desired concentration of coffee. Another example of serial dilution is the dilution of acids and bases in chemistry to obtain a required concentration. Serial dilution of culture to determine the number of bacteria in a given sample through a plating technique is also an essential example of serial dilution. Judging from the weathered look of this... Now, for the total dilution factor, Total dilution factor for the second tube = dilution of first tube \times dilution of the second tube. The following is a step-by-step procedure to working dilution problems, and includes some practice problems at the end. (2016). Retrieved from Ben-David, Charles E. What was the starting concentration? In simple words, serial dilution is the process of stepwise dilution of a solution with an associated dilution factor. In biology, serial dilution is often associated with reducing the concentration of cells in a culture to simplify the operation. The procedure described above produces a set of pour plates (sample spread on top of solidified agar) can be used also. This cannot be done with a fluid solution since 1) one cannot identify purity of the specimen, and 2) there is no way to enumerate the cells in a liquid. Author: JoAnn Hague Apple Crisp II A simple dessert that's great served with ice cream. The procedure of Serial dilution References Basic Practical Microbiology-Manual. Air contaminants can contribute significantly to a really low count and a high count can be confounded by error in counting too many small colonies. Author: Ina Garten Home Style Meatloaf When it's comfort that you seek, nothing satisfies quite like a classic meatloaf. The second tube now has a total dilution factor of 10⁻². The same process is then repeated for the remaining tube, taking 1 ml from the previous tube and adding it to the next 9 ml diluents. As six tubes are used, the final dilution for the bacteria/cells will be 10⁻⁶ (1 in 1,000,000). 3.2×10^5 CFU/mL. Author: SweetCravings Ultimate Chocolate Chip Cookies We named this recipe "Ultimate Chocolate Chip Cookies," because it's got everything a cookie connoisseur... It won't take long to make at all, and it's quite... Most specimens have high enough numbers of microorganisms that the specimen has to be serially diluted to quantitate effectively. Serial Dilution Formula/Calculations Serial dilution involves the process of taking a sample and diluting it through a series of standard volumes of sterile diluent, which can either be distilled water or 0.9 % saline. Then, a small measured volume of each dilution is used to make a series of pour or spread plates. Depending on the estimated concentration of cells/organisms in a sample, the extent of dilution is determined. We use all-purpose... The agar plate allows accurate counting of the microorganisms, resulting from the equal distribution across the agar plate. Colonies grow within the agar, as well as on top of the agar and below the agar (between the agar and the lower dish). Author: dakota kelly Best Hamburger Ever These burgers are the best on the grill in the summertime. This easy white bread recipe... Author: Dora Basic Homemade Bread If you'd like to learn how to bake bread, here's a wonderful place to start. Serial Dilution Examples A simple example of serial dilution performed in our daily life is tea or coffee. Look at all plates and find the one with 30-300 colonies (or plaques), preferably. There are 200 colonies on a plate, you generated a dilution factor of 10^{-9} and plated 100 microliters. Author: Ree Drummond Food Network The Best Steak Marinade We use this marinade for rib steaks, but can be used for almost any cut of beef. It is believed that dilution increases the potency of the diluted substance by activating its vital energy. Author: Janet Caldwell Best Chocolate Chip Cookies Crisp edges, chewy middles. In order to continue enjoying our site, we ask that you confirm your identity as a human. 2.0×10^{12} CFU/mL. It is a common practice to determine microbial counts for both liquid and solid specimens—suspensions of E. The society of General Microbiology. //www.biolom.com/dateien/Bethyl-Serial-Dilutions.pdf 3A Dilution Worksheet and Problems Sources 6% - Serial-Dilutions.pdf 3% - 1% - 1% - 1% - 1% - 1% - Author: Taste of Home Perfect Pot Roast Feed your family with Ree Drummond's Perfect Pot Roast recipe from Food Network. If duplicate plates (with same amount plated) have been made from one dilution, average the counts together. Thank you for your participation! Apple Crisp Perfect and Easy I adapted this from a 50-year-old peach crisp recipe. The final dilution factor for your plate was 10^{-4} , and there were 57 colonies on your plate, what was the starting concentration? Example: For the first tube, dilution factor = 10⁻¹ (1 ml added to 9 ml) For the second tube, dilution factor = 10⁻¹ (1ml added to 9 ml) Total dilution factor = previous dilution \times dilution of next tube = total dilution of 10⁻¹ \times 10⁻¹ = 10⁻² Online Serial dilution calculator AAT Bioquest, Inc. This provides an initial dilution of 10⁻¹. The dilution is thoroughly mixed by emptying and filling the pipette several times. The pipette tip is discarded, and a new pipette tip is attached to the pipette. Now, 1 ml of mixture is taken from the 10⁻¹ dilution and is emptied into the second tube. (Merck Omni Calculator) Endmemo (Handymath) (Toctris Bioscience) (Physiology Web) (Selleck Chemicals) (ApexBio Technology) (CiteAb) (Fluffy Frog) (Functional Biosciences) (CUSABIO) (Procedure of Serial Dilution) The following is the procedure for a ten-fold dilution of a sample to a dilution factor of 10⁻⁶: The sample/culture is taken in a test tube and six test tubes, each with 9 ml of sterile diluents, which can either be distilled water or 0.9% saline, are taken. A sterile pipette is taken. 1 ml of properly mixed sample/culture is drawn into the pipette. The sample is then added to the first tube to make the total volume of 10 ml. J. & MacIntyre, H. On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment. Journal of applied phycology, 28(1), 279-298. A set of serial dilutions is made, a sample of each is placed into a liquefied agar medium, and the medium poured into a petri dish. Serial Dilution Limitation/Problems Even though serial dilution is a useful technique in laboratories, it faces some challenges. Author: Taste of Home The Best Canning Salsa This is a great use for tomatoes from the garden and nothing tastes better than homemade. Although it works well with peaches, it works even... L. In this case, the dilution factor for that test tube will be: After the first tube, each tube is the dilution of the previous dilution tube. Jam-packed with all kinds of stuff, and no... Author: Diane Kester Easy Meatloaf This is a very easy and no fail recipe for meatloaf. For e.g., if a water sample is taken from an extremely polluted environment, the dilution factor is increased. Serial dilution is used in microbiology to estimate the concentration or number of cells/organisms in a sample to obtain an incubated plate with an easily countable number of colonies. In biochemistry, serial dilution is used to obtain the desired concentration of reagents and chemicals from a higher concentration. In pharmaceutical laboratories, serial dilution is performed to receive the necessary concentration of chemicals and compounds, as this method is more effective than individual dilutions. In homeopathy, homeopathic dilutions are used where a substance is diluted in distilled water or alcohol. Author: Betty Crocker Kitchens 5 points each (-2 points if units are missing) The Online Practice must be printed off of Blackboard, and answers must be written in student's own handwriting, with work shown when applicable, for credit. If you count 32 colonies after making a dilution 10^{-3} and plating 100 microliters, what is the starting concentration? Author: UNIVSTUDENT Perfect Roast Chicken For the perfect roast chicken dinner every time, try this popular recipe from Ina Garten, Food Networks...

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