SI Microbiology - Full Discipline Demo

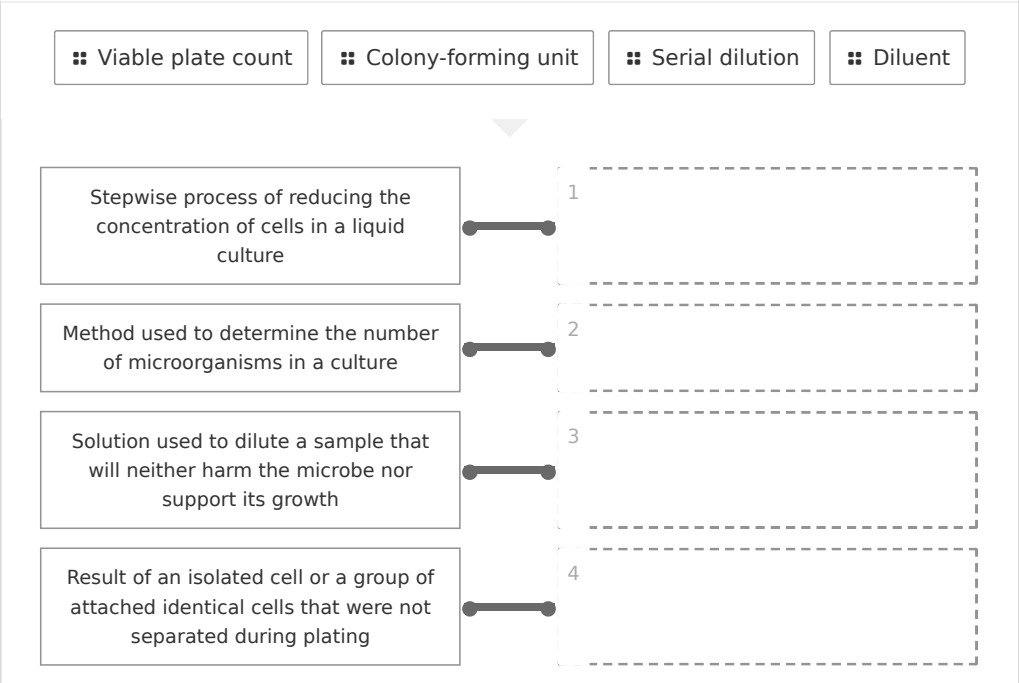
Serial Dilution and Viable Plate Counts

Final Report - Answer Guide

Institution	Science Interactive University
Session	SI Microbiology - Full Discipline Demo
Course	SI Microbiology - Full Discipline Demo
Instructor	Sales SI Demo

Test Your Knowledge

Match each term with the best description.



Correct answers:

1 Serial dilution 2 Viable plate count 3 Diluent

4 Colony-forming unit

Classify each statement as true or false.

The volume of the sample added to a plate is important for calculations and should not vary between plates.

If more than one dilution produced a plate that falls within the 30-300 CFU range, only the most diluted plate is used in the calculations.

Only plates containing colonies within the 30-300 range are counted and recorded for accuracy.

The volume of agar used in a plate is important for calculations and should not vary between plates.

True	False
1	2

Correct answers:

1

The volume of the sample added to a plate is important for calculations and should not vary between plates.

Only plates containing colonies within the 30-300 range are counted and recorded for accuracy.

2

If more than one dilution produced a plate that falls within the 30-300 CFU range, only the most diluted plate is used in the calculations.

The volume of agar used in a plate is important for calculations and should not vary between plates.

Exploration

A plate is classified as viable if it contains between _____ CFUs.

- 10-30
- 30-300 ✔
- 20-100
- 40-400

To determine the number of CFUs in the original culture, the number of colonies on the viable plate is _____ the total dilution factor of that plate.

- added to
- multiplied by ✔
- divided by
- subtracted from

Exercise 1

Why is a dilution series necessary when performing a viable plate count to estimate microbial numbers in a sample? Do your results support the need for a dilution series? Reference Data Table 2 in your explanation.

Dilution series are necessary because most undiluted cultures contain more organisms than can be directly counted. The results recorded in Data Table 2 support this practice as the original *S. cerevisiae* solution contained an estimated 4,750,000 CFU/mL which would produce too many colonies to count if plating a 0.2 mL sample as performed in the procedures.

Why was it important to carefully measure the volume of sample added to agar plates but not the volume of agar used in the plates in this exercise? What would be the effect of accidentally adding 0.3 mL instead of 0.2 mL of sample to one of the agar plates?

The volume of agar used in a plate is not important as long as all plates are the same diameter. The volume of the sample added to a plate is important for calculations and should not vary between plates. If 0.3 mL of sample was accidentally added to a plate, the colony numbers would

increase by 50% over those that would have developed from the specified 0.2 mL of sample volume, leading to an overestimation of microbe CFUs in the original culture sample.

How would your results have been impacted if you used 1/2 of the packet of S. cerevisiae for the procedures instead of only 1/4? Would you have produced at least one viable plate? Reference your actual results recorded in Photos 1-6 and Data Table 2 in your explanation.

Note to instructors: students responses will vary based on results recorded in Photos 1-6 and Data Table 2 but should be consistent with doubling the number of CFUs in those results.

Using 1/2 packet of S. cerevisiae instead of only 1/4 would have resulted in twice as many CFUs in each of the dilutions and most likely resulted in the plates in Photos 4-6 each being classified as viable in Data Table 2, unlike the actual results where only the 10^{-4} and 10^{-5} were classified as viable. However, the 10^{-4} plate, even with twice as many colonies, would still be used in the final calculations, as it would remain the least diluted viable plate.

Photo 1: 10-1 Dilution
(SAMPLE ANSWER BELOW)

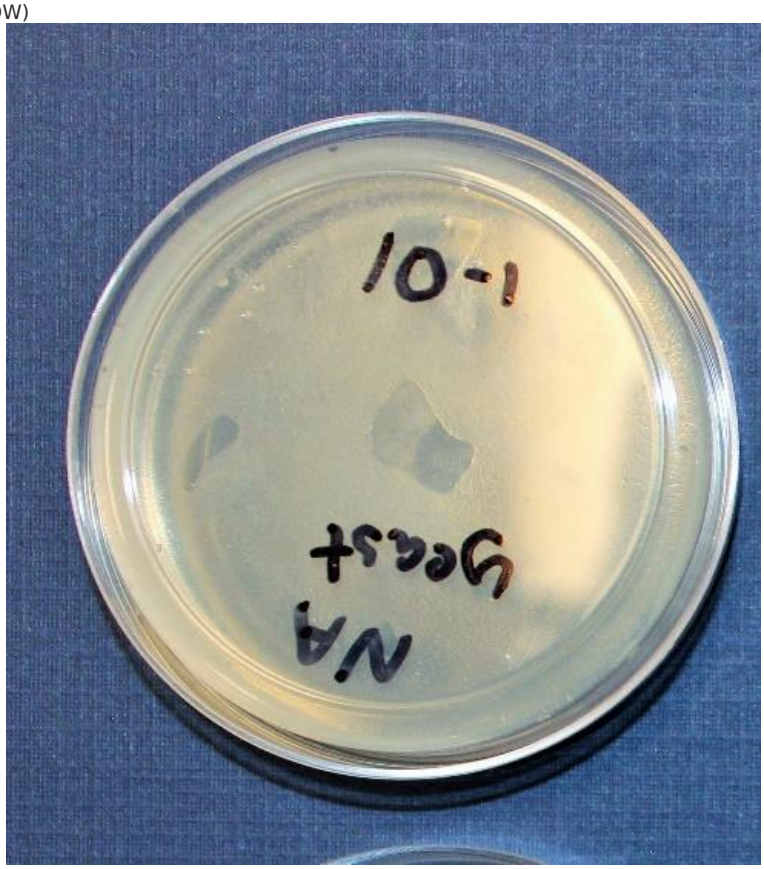


Photo 2: 10-2 Dilution
(SAMPLE ANSWER BELOW)

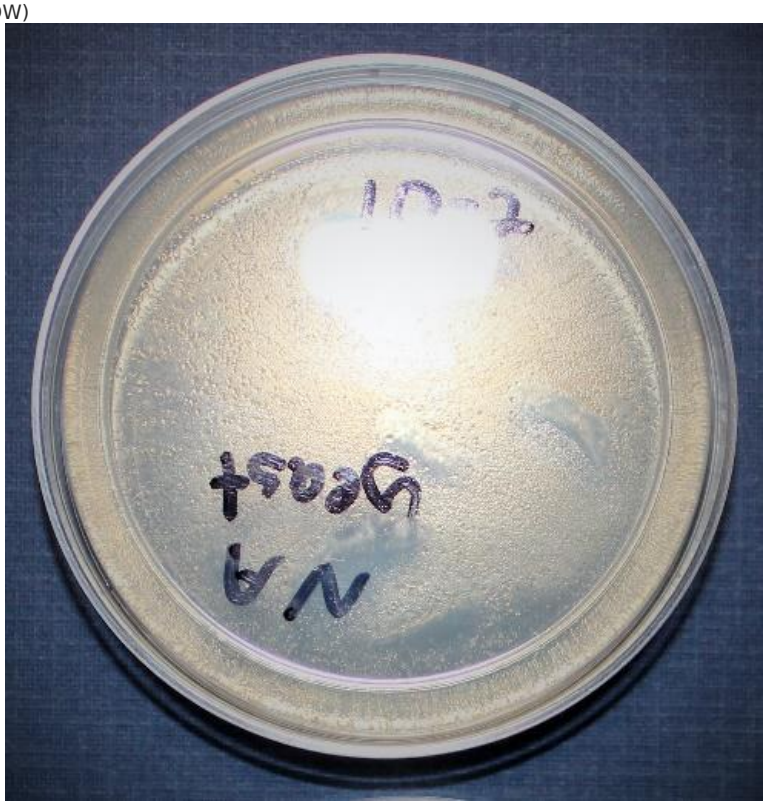


Photo 3: 10-3 Dilution
(SAMPLE ANSWER BELOW)

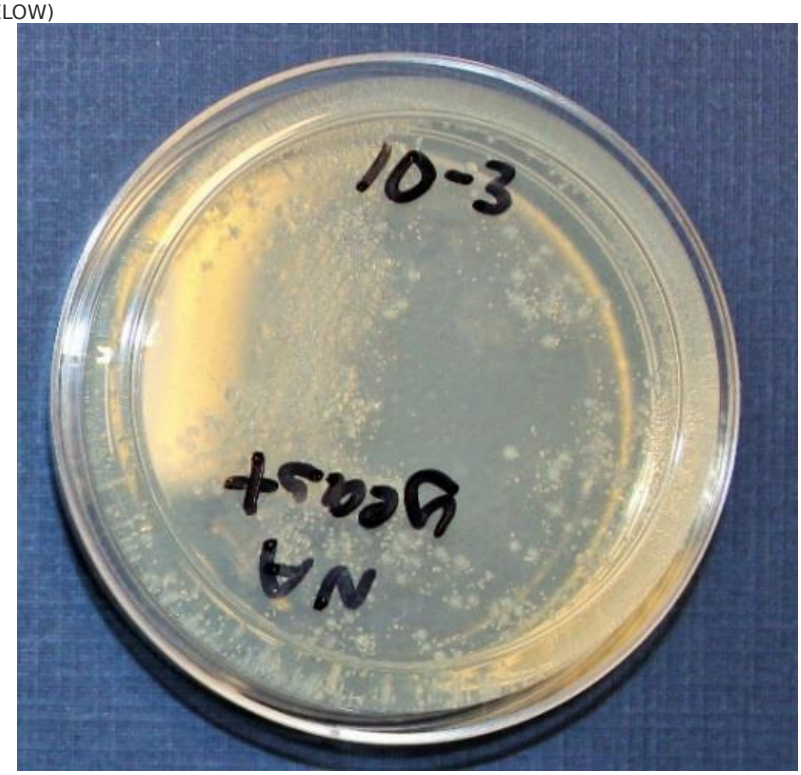


Photo 4: 10-4 Dilution
(SAMPLE ANSWER BELOW)

Photo 5: 10-5 Dilution
(SAMPLE ANSWER BELOW)

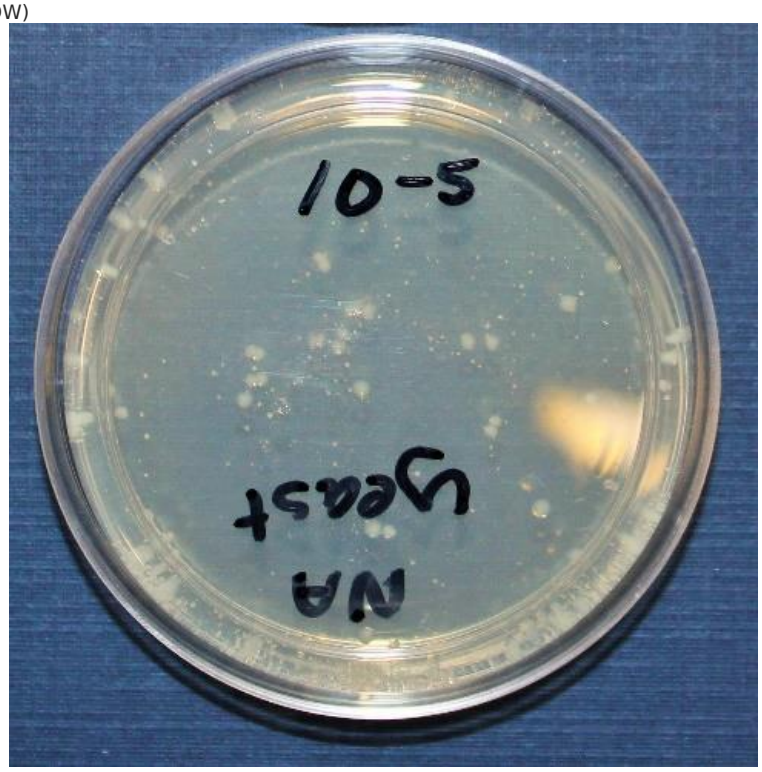


Photo 6: 10-6 Dilution
(SAMPLE ANSWER BELOW)

Data Table 1: Dilution Plate Counts
(SAMPLE ANSWER BELOW)

Dilution	Count and Viability (Viable, TFTC, or TNTC)
10^{-1}	300+, TNTC
10^{-2}	300+, TNTC
10^{-3}	300+, TNTC
10^{-4}	95, Viable
10^{-5}	36, Viable
10^{-6}	18, TFTC

Data Table 2: Calculations of Sample CFU/mL
(SAMPLE ANSWER BELOW)

Calculation	Answer
Dilution Plate CFUs	$95\ CFU$
Dilution Factor of Viable Plate	$10^{-4}\ or\ 1:10,000$
Volume of Diluent added to Plate	$0.20\ \mathrm{mL}$
CFU/mL of Original Sample: (CFU x Dilution Factor)/Volume	$4,750,000\ \mathrm{CFU/mL}$

Competency Review

Dilutions are necessary because most undiluted cultures contain more organisms than can be directly counted.

- [x] True
- [ ] False

CFU numbers less than _____ reduce accuracy when calculating the number of cells in the original sample.

- 10
- 30 ✔
- 200
- 400

Diluents are solutions, such as _____, used to dilute a sample that will neither harm the microbe nor support its growth.

- isopropyl alcohol
- bleach
- nutrient broth
- distilled water ✔

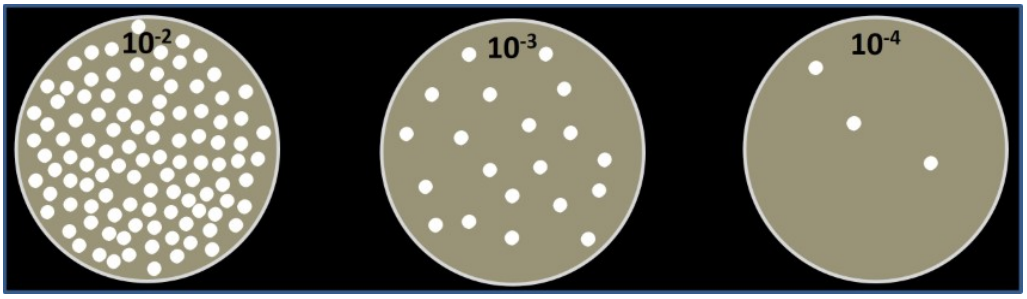
The first dilution of the culture is calculated by _____ the volume of the sample added to the tube by the total volume of solution in the tube.

- dividing ✔
- multiplying
- adding
- subtracting

The *S. cerevisiae* solution should be pipetted up and down several times to ensure all organisms are rinsed from the pipet into the test tube when creating a serial dilution.

- True ✔
- False

The ___ dilution plate in the series below is considered viable for use in estimating cell numbers in an original sample.



- [x] 10^{-2}
- [ ] 10^{-3}
- [ ] 10^{-4}
- [ ] All of the above

A viable plate has a count of 95 colonies produced from 0.20 mL of a 1:10,000 dilution of the original sample. The original sample contains ____ CFU/mL.

$$\text{Concentration of Original Tube (CFU/mL)} = \frac{\text{Viable plate count (CFU) x Dilution factor}}{\text{Volume plated (mL)}}$$

- [ ] 760
- [ ] 12,000
- [ ] 7,600,000
- [x] 4,750,000

Extension Questions

A microbiology student used nutrient broth as a diluent, instead of distilled water, when creating a serial dilution of *Saccharomyces cerevisiae* and waited 12 hours before plating the samples. Apply your knowledge of serial dilution and viable plate counts to predict how the student's estimates of CFU/mL in the original sample would be affected. (SAMPLE ANSWER BELOW)

If the dilution series was created using nutrient broth and allowed to incubate for 12 hours before plating, *S. cerevisiae* CFUs would have multiplied in the dilution tubes resulting in more CFUs than were added to each tube originally. The dilutions would no longer represent the first principle of serial dilutions and plate counts: The number of cells in the original solution will always exceed the numbers in a diluted sample. The student's estimates would be higher than the number of actual CFU/mL in the original sample.