

An Investigation in Serial Dilution and Streak Plate Isolation

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Objective:

Serial Dilution allows for a given quantity of a solution to be sufficiently diluted by several orders of magnitude over a succession of dilutions (in series, or serially.) It allows for a considerable amount of a sample to be stored in a concentrated form, and then sufficiently diluted in order to be properly investigated. Streak plate isolation allows for a sample to be grown across a succession of streaks across a plate of growth medium, commonly a blood of nutrient agar, for a particular organism. It allows a microbial culture to be grown so that it may be further investigated. For this experiment, a concentrated solution of the pathogenic bacterium *Staphylococcus aureus* underwent serial dilution, and was then run across a streak plate, in order to determine the approximate number of colonies in the original, undiluted sample. Due to the pathogenic nature of *S. aureus*, care was used in maintaining proper aseptic techniques during its handling.

Results:

I. Streak Plate Isolation Questions:

- i. Our streak plate began displaying isolated colonies after the third streak, and with adequate spacing after the fourth.
- ii. The isolated colonies consisted of small white dots.
- iii. Diagram:

II. Serial Dilution Techniques

- i. Yes, thanks to step 2B, the series of 1:10 dilutions allowed the sample to be isolated during the streak plate isolation.
- ii. Results of the streak are recorded in Table1:

Tube Label	Description of Sample	Number of colonies obtained. (CFU/500 µL)	Comments
4D	Dilution #4	≈700	The colonies appear as large clumps.
5D	Dilution #5	≈120	Colonies appear to be the same size.
6D	Dilution #6	≈16	Colonies appear to be the same size.

Table 1: Streak Plate Results

$$\begin{aligned} \text{iii. 4D: } & \frac{7.0 \cdot 10^2 \text{ CFU}}{500 \mu\text{L}} \cdot \frac{10^4}{1} = \frac{1.4 \cdot 10^6 \text{ CFU}}{100 \mu\text{L}} \\ \text{5D: } & \frac{1.2 \cdot 10^2 \text{ CFU}}{500 \mu\text{L}} \cdot \frac{10^5}{1} = \frac{2.4 \cdot 10^6 \text{ CFU}}{100 \mu\text{L}} \end{aligned}$$

$$6D: \frac{1.6 \cdot 10^1 CFU}{500 \mu L} \cdot \frac{10^6}{1} = \frac{3.2 \cdot 10^6 CFU}{100 \mu L}$$

$$\text{Average: } \frac{(1.4 + 2.4 + 3.2) \cdot 10^6 / 3}{100 \mu L} = \frac{2.4 \cdot 10^6 CFU}{100 \mu L}$$

$$iv. \frac{2.4 \cdot 10^6 CFU}{100 \mu L} \cdot \frac{1000 \mu L}{1 mL} \cdot \frac{1}{10^3} \cdot \frac{1 Colony}{1 CFU} = \frac{2.4 \cdot 10^4 Colonies}{1 mL}$$

$$v. \frac{1 \cdot 10^6 CFU}{1 mL} \cdot \frac{1 mL}{1000 \mu L} \cdot \frac{1}{2 \cdot 10^4} \cdot \frac{1 Colony}{1 CFU} = \frac{50 Colonies}{100 \mu L}$$