Microbial Genetics

Third Step ***Assit. Prof. Alaa Naseer***

Lab.3

Isolation of Antibiotic Resistance

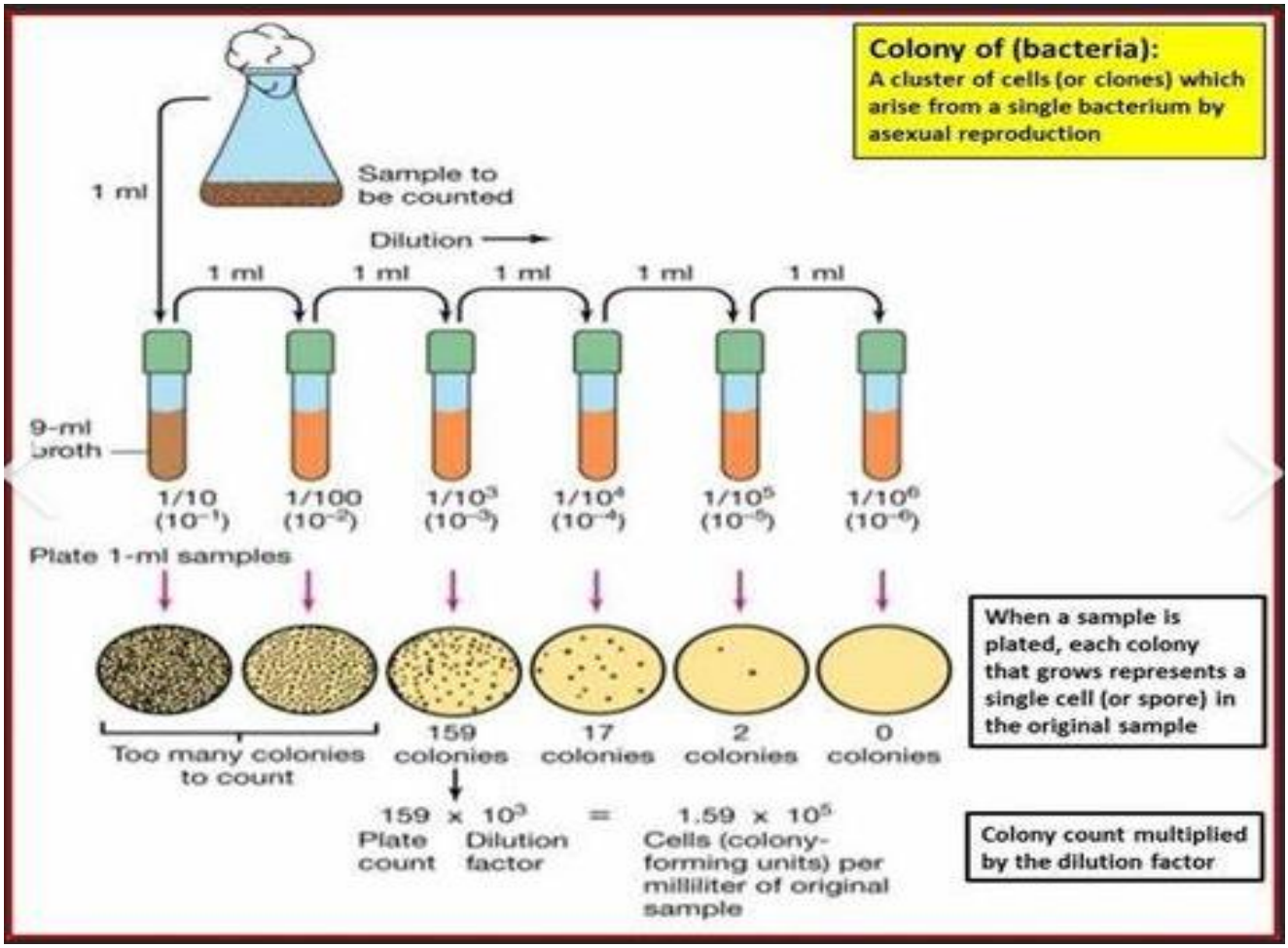
The rise of multidrug-resistant (MDR) microorganisms, which pose a grave threat, has made choosing antibiotics to treat bacterial infections incredibly challenging. The prescription antibiotics have to consistently be effective against the identified related infections. Thus the research team wanted to find the antibiotic sensitivity profiles and pathogenic bacterial isolates in various patient specimens. most important reasons of their occurrence :-

1- U.V. light (10-400)nm especially wave length (260)nm .
2- Chemical Mutagens.
3- Error in DNA replication.

If the mutation is more suitable to the environmental conditions so its growth will overcome the mother culture , so it will be dominant , Antibiotic resistance spontaneous mutations can be observed easily when the bacteria grow with presence of antibiotic concentration which due to inhibition of normal microbes . It should be noted that spontaneous mutations arise from inadvertently in the use of mutagens .

Materials and Methods :-

1- Prepare E.coli broth culture using nutrient broth incubated for (18-24) hrs.
2- Attend serial dilutions to 10^{-5} from broth culture by normal saline , take 0.1 ml from last dilution10^{-5} and cultured in plate contains nutrient agar only by spreader incubated in 37c° for 24hrs .
3- For viable count . Apply the following law :
Viable Cell count / ml = Dilution factor * No. of colonies in 1ml. = Average no. of colonies in the plate * 10 * 10^5
4- 0.1ml is taken from the stock culture and cultured by spreader in plate contains nutrient agar with 10µg/ml of antibiotic incubated in 37c° for 24hrs. (2 replicates) . read the results in the next day .
5- Apply the following law :
No. of Mutant bacteria = Average no.of mutant bacterial colonies in two plates * 10
6- Apply the following law for getting spontaneous mutation frequency :
No. of mutant bacteria= Average no.of mutant bacteria in 1ml / viable cell count.



Serial Dilution

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.

Serial Dilution Objectives

- 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.