Salahaddin University- Erbil
College of Agriculture
Plant Protection Department
second Class

Isolation microorganisms from soil

Serial dilution

- Serial dilution is the stepwise dilution of a substance in solution. Usually, the dilution factor at each step is constant. Serial dilution may also be used to reduce the concentration of microorganisms or cells in a sample.

Isolation of microorganisms from soil by serial dilution

procedure

1-collect soil samples at random, a minimum of five, from a field, and mix thoroughly to make a composite sample for microbiological analysis.
2- Lable 90 ml sterile water and +10 grams of soil.
3- Add a 10 g sample of finely pulverised, air-dried soil into numbered water blank to make a 1:10 dilution.
4- shake the dilution to obtain a uniform suspension of microorganisms.
5- transfer 10 ml of suspension from flask number 1 into tube number 2 with a sterile pipette to make a 1:100 (10^2) dilution.
4- prepare another dilution 1: 1000 (10^3)by pipetting 10ml of the suspension into tube 3.

7- make further dilutions 10^3 to 10 10^7
8- transfer 1 ml aliquots each from 10^2 dilution tube into sterile petri dishes,

9- Add approximately 15 ml of the cooled medium to each petri dish. the two media are to be added to various dilution as follow :
A- For fungi- PDA Supplemented with streptomycin to PDA.
B- for bacteria – nutrient agar.
Incubate the plates inverted for 2 to 7 days.
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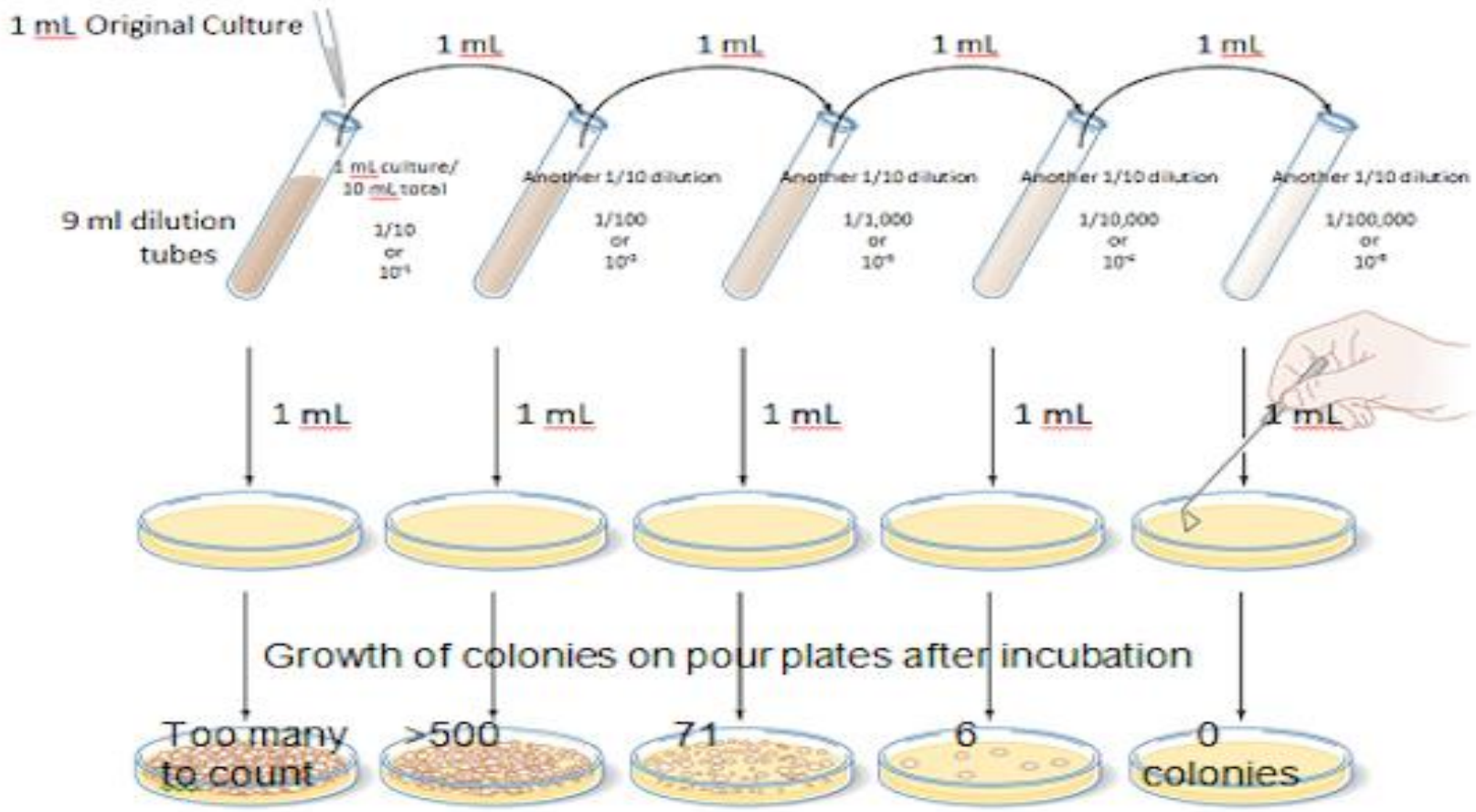
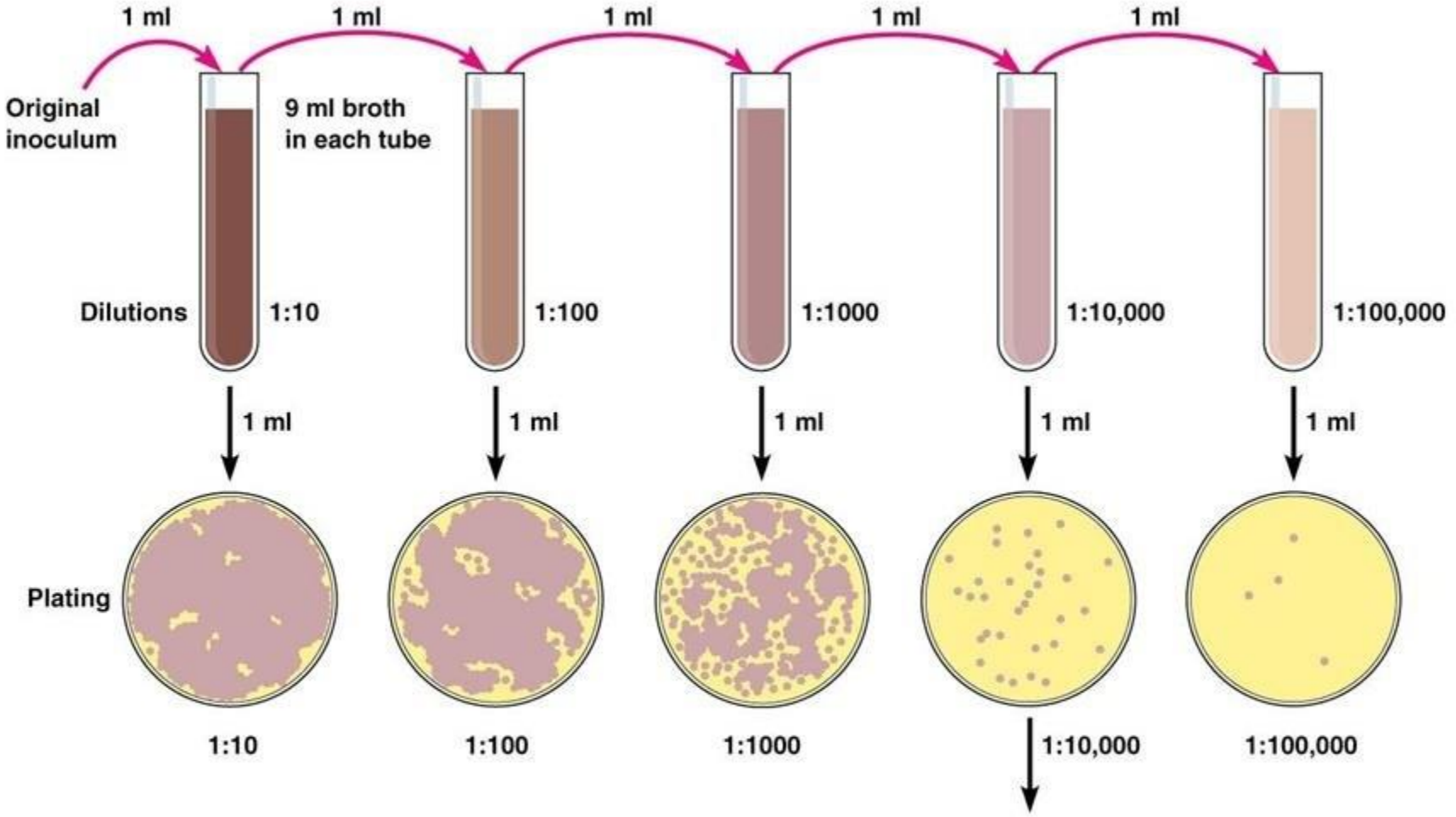
Serial dilution





Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

- No. of bacterial cells /1gm moist soil =No. of colonies × inverted dilution .
- No. of bacterial cells /1gm dry soil = No. of colonies ×inverted dilution
- Dry weight of 1gm soil sample
- The unit of measurement here (CFU) Colony forming unit where the colony may be the yields of the growth and multiplication of a single cell or more.