Spreading of microbial sample
**a) Spreading of pure culture of bacteria (*Escherichia coli*) on LB Agar plates to count CFU/ml of the culture**
b) Spreading of environmental sample (soil) on LB Agar plates to recognize microbial diversity of the sample

Viable microbial count: The number of viable (live) microbes in a culture can be ascertained by determining the number of **colony forming units (CFU)** by counting the number of colonies on media plates. The theory behind the technique of CFU establishes that a single microbe can grow and become a colony via division. Sometimes microbes clump; in these cases a colony may grow from several microbes clustered together.

Preparing dilutions of sample: Between 30 and 300 CFU can be counted on a plate. Thus, microbial cultures of high density must be **diluted** before they are plated. This is done by making serial 10-fold dilutions of the sample. A known volume of dilutions of culture is then plated onto media plates.

Spreading sample on media plates: Generally 0.1 mL of each dilution is spotted on the center of an agar plate using a pipette. The sample is spread uniformly on the plate with the help of a **spreader** (a bent glass rod). Spreader is sterilized before use by first dipping it into alcohol solution and then passing it through the Bunsen burner flame. It is important to cool the spreader before using it to avoid killing microbes by heat. The plates are incubated at a required temperature for a specific time period, and the number of colonies on plates is counted.

Calculating CFU/ml: The number of viable microbes per ml of the initial culture (CFU/ml) can be calculated from the **number of colonies on the plate, volume of sample plated** and the known **dilution factor**. It is difficult to count plates with more than 300 colonies because in these plates colonies are so closely spaced that it does not allow accurate count. Plates with less than 30 colonies are also inappropriate for counting colonies because the number of colonies is too few to allow statistically accurate counts.

Microbial Life 2e, Figure 6.6
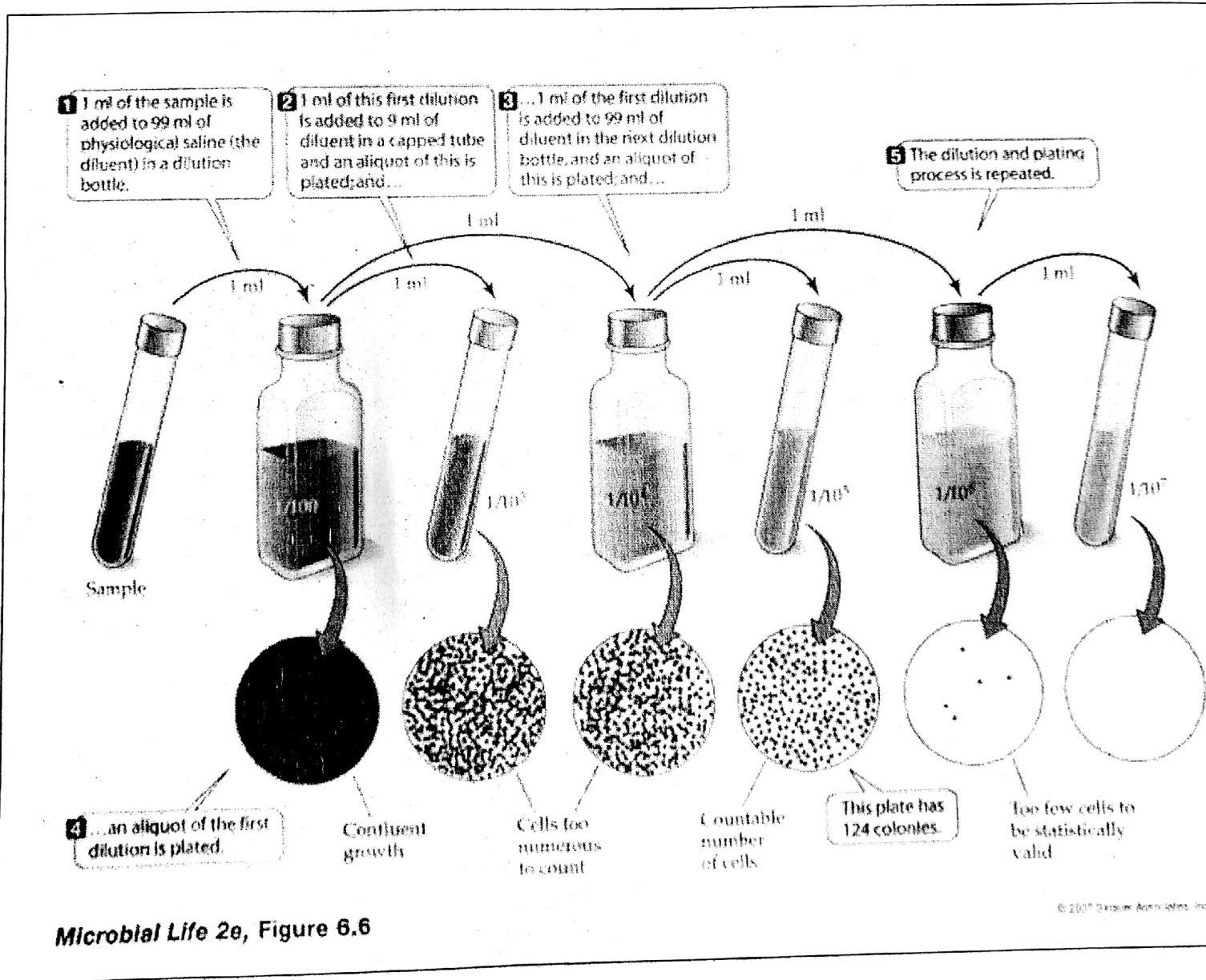
Schematic for Dilution and Spreading of samples