Tutorial N°2: Microbiological Control Techniques

Exercise I :

We want to determine the number of live bacteria in the urine of a patient with a bacterial infection who is undergoing antibiotic treatment. Counts taken before treatment showed that the patient's urine contains a concentration of 10^6 live bacterial cells per ml of urine. After one day of treatment with Bactrim (trimethoprim + sulphamethoxazole), the pharmacist biologist deposits a volume of 0.01 ml on the surface of a Thoma cell. After 30 minutes of rest to allow the cells to settle, the slide is examined under a microscope at high magnification.

Calculate the concentration of cells per ml in this urine, knowing that 350 cells were counted in 10 small squares in a solution 10 times more concentrated than the original urine.

After staining with methylene blue, the percentage of blue cells was 80%. What does this result mean for cell counting?

Quel est la signification de ce résultat pour le malade ? Le traitement est-il efficace ?

Reminder: The Thoma cell (or hemocytometer) is a hollow slide with a grid of approximately 400 small squares in the centre. Each small square has an area of 1/400 mm². The distance between the grid and the cover slip is 1/10 mm.

Exercise II :

Consider a yeast suspension diluted 1000 times and counted in Malassez cells. Counting 4 rectangles gives the following results: 17, 22, 15, and 20 cells for each counting unit (rectangle). The volume of a sub-unit is 0.01 mm3.
Calculate the concentration of cells per ml in this suspension.

Exercise III :

You are counting cells in a cell suspension using a Malassez chamber. You diluted your cell sample 10 times before placing it in the Malassez chamber. After filling the chamber, you count 250 cells in 10 randomly selected squares.

Calculez la concentration cellulaire de votre échantillon initial (en cellules/mL).

If you need 1 million cells for an experiment, how much of your initial cell suspension should you take?

Exercise IV :

You are working with a cell suspension and using a Malassez chamber to determine the cell concentration. The Malassez chamber is divided into 100 squares, each measuring 1 mm^2, and the depth of the chamber is 0.1 mm. You diluted your cell sample 20 times before placing it in the Malassez chamber. After filling the chamber, you count 180 cells in 5 randomly selected squares.

1. Calculate the cell concentration of your initial sample (in cells/mL).
2. If you need 500,000 cells for an experiment, how much of your initial cell suspension should you take?