STATION #4

HEMOCYTOMETER CELL COUNT

PURPOSE

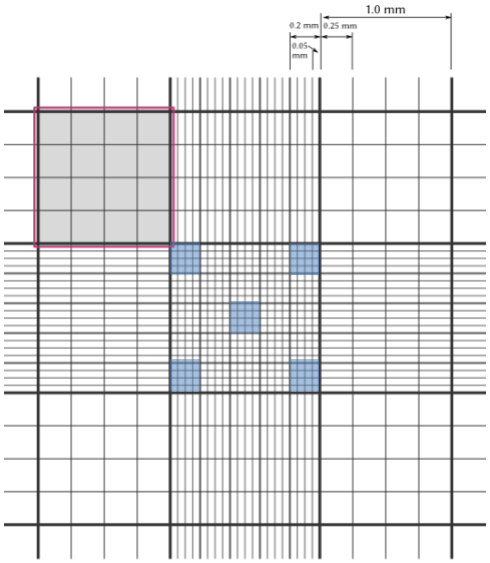
Experience counting yeast cells using a hemocytometer and microscope. The cells counted using this method are converted to cell density using a simple calculation. The data collected will be combined with the Packed Cell Volume (PCV) data to produce a standard curve that relates PCV to cell density.

MATERIALS

- 1 liter of Stock yeast slurry (this same stock slurry will be used for the entire group today)
- Diluted yeast slurry (your group will use this same diluted sample for this station **and** the PCV station)
    - Sample 1: 150 ml slurry + 50 ml water (3:4 dilution factor)
    - Sample 2: 100 ml slurry + 50 ml water (2:3 dilution factor)
    - Sample 3: 100 ml slurry + 100 ml water (1:2 dilution factor)
    - Sample 4: 50 ml slurry + 100 ml water (1:3 dilution factor)
    - **Note:** Ratios can be written as fractions, like ¾, or as a shorthand like 3:4. The two notations are the same. This is important to note when performing calculations.
- 100 ml volumetric flask
- 1 ml disposable pipette (used to prepare dilution)
- Hemocytometer
- Handheld counter
- Disposable glass pipette (used to load sample into hemocytometer)
- Microscope

BASIC STEPS

1. Record the dilution factor of sample
2. Transfer 1 ml of diluted slurry into 100 ml volumetric flask
3. Carefully add water to the 100 ml mark
4. Close top of flask, invert 5 times to mix sample
5. Carefully load hemocytometer
6. Transfer hemocytometer to microscope
7. Position slide under 40x objective on microscope, carefully adjust focus and slide position to permit counting
8. Count cells contained in the outer 5 squares of the counting chamber (see diagram). Cells may cross the lines on the counting chambers; when this happens, only count cells crossing the left and bottom grid lines. This counting rule helps for an accurate count.
9. Record the number of cells counted in these 5 squares



Numer of cells in a 1mm^2 square (red) x 10^4 = No. cells/ml.

CALCULATION

The combined calculation is simple and is shown by the following:

$$\text{Cell Density} = \frac{\text{Cells}}{\text{5 grids}} \times \frac{\text{25 grids}}{\text{chamber}} \times \frac{\text{1 chamber}}{\text{0.0001 ml sample}} \times \frac{\text{100 ml sample}}{\text{1 ml yeast slurry}}$$

$$\text{Cell Density} = \frac{\text{Cells}}{\text{5 ~~grids~~}} \times \frac{\text{25 ~~grids~~}}{\text{~~chamber~~}} \times \frac{\text{1 ~~chamber~~}}{\text{0.0001 ~~ml sample~~}} \times \frac{\text{100 ~~ml sample~~}}{\text{1 ml yeast slurry}}$$

Cell Density = cells / ml yeast slurry

SAMPLE CALCULATION

Given Data:

- 173 cells counted in 5 chambers
- Sample diluted 1:100 in volumetric flask
- Sample diluted 3:4 from stock (Group 1 slurry dilution)

1. Cell Density = $\frac{173 \text{ cells}}{5 \text{ grids}} \times \frac{25 \text{ grids}}{\text{chamber}} \times \frac{1 \text{ chamber}}{0.0001 \text{ ml sample}} \times \frac{100 \text{ ml sample}}{1 \text{ ml yeast slurry}}$

   Cell Density = $\frac{432{,}500 \text{ cells}}{0.0005 \text{ ml}}$

   Cell Density = $\frac{4.325 \times 10^{5} \text{ cells}}{5 \times 10^{-4} \text{ ml}}$

   (Scientific Notation Note: Subtract exponents when dividing and add when multiplying. In this example the exponent becomes 4-(-5) or 4+5)

   Cell Density = 0.865×10^{9}

   Cell Density of Sample = 8.65×10^{8} cells/ml = 865 million cells/ml

2. Cell Density of Stock Sample = Cell Density of Group's Diluted Sample ÷ Dilution Factor (3:4)

   Cell Density of Stock Sample = Cell Density of Group's Diluted Sample x Inverse of Dilution Factor (4:3)

   Cell Density of Stock Sample = (8.65×10^{8} cells/ml) x (4/3)

   Cell Density of Stock Sample = 1.15×10^{9} cells/ml = 1.15 billion cells/ml