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Counting Cells by Hemocytometer

Outline

Prepare a hemocytometer slide. Stain cells with trypan blue and add the mix to the counting chambers of a hemocytometer. Count the cells under a microscope, and calculate cell concentration and percent viability.

Materials

Sterile

- Cell in suspension, trypsonized cell monolayer or a suspension culture (approx. 10^6 cells/mL)
- Pipette, 1 mL

Nonsterile

- Gloves
- Pipette Aid
- Alcohol, 70% in spray bottle
- Hemocytometer, improved Neubauer
- Trypan Blue solution, 0.4% (Cat. No. 25-900-CI, Mediatech)
- Pipettor, 20 μL or adjustable 100 μL
- Yellow pipettor tips
- Microfuge tubes, 1.5 mL
- Lab counter with one or two counting units

Protocol

1. Clean surface of hemocytometer with 70% alcohol (take care not to scratch the semisilvered surface)
2. Clean the coverslip with 70% alcohol
3. Place coverslip on the middle of the hemocytometer
4. Transfer 20 μL of cell suspension + 20 μL of Trypan Blue solution to a microfuge tube
5. Mix by pipetting and collect 20 μL of the stained cell suspension
6. Expel a stoplet of the suspension and let it be drawn under the coverslip by capillary action (it is important: not to overfill or underfill the chamber)
7. Mix the stained cell suspension again, and fill the second chamber
8. Put the hemocytometer on the stage of an inverted microscope
9. Select 10× objective, and focus on the grid lines in the chamber
10. Move the slide so that the field you see is on central area of the grid (central grid area is 1 × 1 mm)
11. Count the cells in the central grid area (25 boxes) - viable cells are clear, nonviable cells are blue
12. Count between 100 and 300 cells
    - If there are very few cells, count one or more additional squares surrounding the central square (each 1 mm^2)
    - If there are too many cells, count only five small boxes (each 200 × 200 μm) across the diagonal of the 1 mm^2 square; or alternatively dilute the original cell suspension
13. Record the number of stained and unstained cells
14. Count the cells in the second chamber, and calculate the average of the two counts
15. Calculate percent viability = [1 – (no. stained cells) / (total cell count)] × 100%
16. Calculate cell concentration in cells/mL = avg. cell count × 20,000
    - If additional 1-mm^2 squares were counted, then divide by the total number of 1-mm^2 squares
    - If only five small boxes were counted across the diagonal, then multiply by 5
17. Wash the hemocytometer and coverslip with 70% alcohol, and return it to the box