

## COUNTING CELLS WITH AN ELECTRONIC COUNTER

### Principle

The Coulter Counter is an electronic cell counter suitable for counting mammalian cells in suspension (e.g. blood cells, cells grown in culture). A measured volume of fluid containing the suspended cells is drawn automatically through a fine aperture approximately 100 microns in diameter. The aperture is immersed in saline solution between two large platinum electrodes between which an electric current flows. Since cells are relatively poor conductors, passage of a cell through the aperture increases the resistivity and causes a drop in current which is amplified and recorded on a decade scaler. The background count may be eliminated by adjusting the threshold so that all cells in the population are just included. Obviously, it is MOST IMPORTANT. THAT CELLS BE SUSPENDED SINGLY (not clumped) AND UNIFORMLY DISTRIBUTED IN THE SOLUTION.

### OPERATION:

1. Observe the orifice to make sure it is clear.
  2. Suspend cells and place sample, in shell vial, on platform in position for counting.
  3. Turn the upper stopcock switch to the open position. Watch the mercury column and make sure it drops between the electrode and the leveling bulb.
  4. Clear the electronic circuit by depressing the re-set button.
  5. Turn upper stopcock switch to the OFF position.
  6. Record your reading. To repeat readings, open upper stopcock, clear the electronic circuit, close upper stopcock.
  7. Observe the aperture occasionally during the count. Any blockage of the orifice will result in a slower movement of fluid (visible as the rate of movement of the mercury column) and a lowered rate of counting (or change in the rhythm of clicks). Faulty counts will be obtained under these conditions. To clear aperture, remove the vial and wipe the surface of the orifice with the finger or brush provided. Replace vial and recount. Never attempt to clear aperture with a wire or pin. A new one costs \$100.00.
  8. Dilutions should be made to obtain counts between 20- 30 x 10<sup>3</sup>. Counts over 10<sup>4</sup> need to be corrected from the table provided.
  9. For spleen cells, the following settings are made:
    - lower threshold - 15
    - higher threshold - 100
    - I/amp = 1/2
    - I/apert = 1/2
    - inside vernier - 20
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1. Turn on power switch and let warm up.
  2. If want total count, set I/amp threshold to 15 and upper to 100 .
  3. 20 ml (2 squirts) saline + 0.2 saponin (in vial) + 10<sup>6</sup> cells (spleen). Allow 1 min to lyse RBC.
  4. Wash out orifice and tube with saline.
  5. Count cells by placing probe in sample, open stopcock 50 Hg falls, reset and close stopcock. Read and record counts twice per sample.
  6. Rinse aperture tube 3 times with filtered saline and check background.

**Materials:** 10 mg/ml Saponin in 1% formal saline (use 10 $\lambda$  per ml counting solution).  
1% formal saline – 10mL formaldehyde/liter saline  
1 spleen in 1mL is diluted 1:2000 (10 $\lambda$  spleen in 20mL F-Saline  
with .2mL Saponin)