Typed 24 November 2009
Original 3/5/91

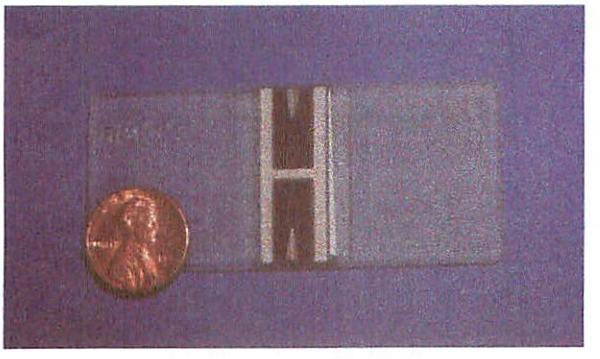
# COUNTING CHLAMYDOMAS CELLS

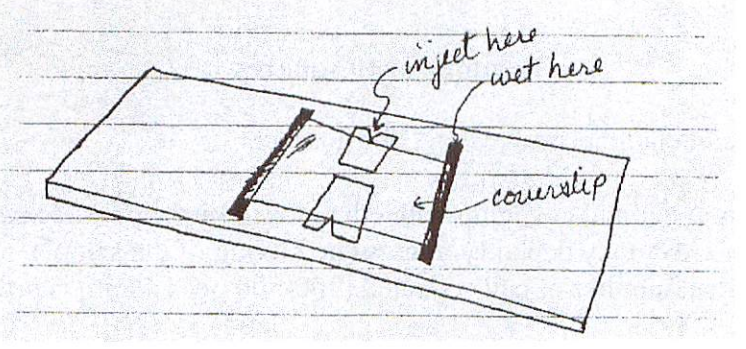
1. Take ~1 ml of culture in an eppendorf and add 10 λ of "cell stop" (.25% wescondyne in 95% ethanol).

2. Prepare hemocytometer slide:

    a. Clean hemocytometer and coverslip with ethanol and kimwipes. (NOTE: The coverslip is very fragile). After both pieces have dried, place coverslip on hemocytometer. Gently mix cells
    b. Wet the two ridges with some water so that coverslip adheres to slide.
    c. Place coverslip on slide. This is a special coverslip

3. Inject 10 λ of sample in chamber. Insert pipette tip into the grove between the hemocytometer and the coverslip at the edhe of the chamber and eject the cell suspension under the coverlip (see Freshney p. 130). Hold the micropipette at approximately 45° angle to the table. Let the cell suspension be drawn under the coverslip by capillary action.

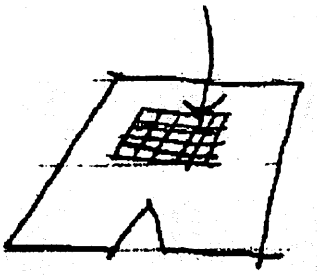





4. View under microscope 10x 25x or 40x objective

    NOTE: Turning light source knob counterclockwise (to reduce light intensity) before switching on, and then increase light intensity.

5. Move stage and find 1mm$^2$ grid in the center of one of the chambers.

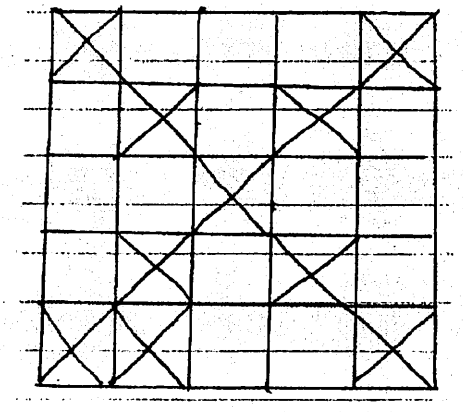


a. Count 10 squares and average them. Multiply this number by 2.5 X $10^5$ to geth the number of cells

OR

b. Count 25 squares and multiply by 1 X $10^4$ to get number of cells/ml.

NOTE: When counting, count cells that lie on the top and left-hand lines of each square but not those on the bottom or right-hand lines. This method should help you avoid counting the same cell twice. Be slow and deliberate when you begin counting cells so that you can establish a routine that ensures that each cell is counted once.



**Example of 10 squares**

6. Repeat for second chamber and average the numbers

7. Clean hemocytometer and coverslip with ethanol and kimwipes. See instructions for hemocytometer. Accuracy depends on accurate mixing of the sample, number of chambers counted, number of cells counted (200-500 per 1.0$mm^3$ is practical).