Colby College BIOLOGY 225 Spring 2013
Prof. Hannum

Lab 4
Tissue Culture

Today you will culture leukocytes under conditions that artificially stimulate T cell activation. The biggest challenge will be using sterile technique, so that the cultures won't become contaminated with the bacteria and fungi that surround us.

Next week, we'll quantitate the IL-2 produced in these cultures by quantitative ELISA.

Notes on sterile technique

We'll also cover this in lab, as the best way to learn sterile technique is to see it done. Here are some things to remember:

a. Turn on the tissue culture hood blower and let it run a few minutes before using the hood.

b. before using the tissue culture hood, spray the work surface with 70% alcohol and wipe with a paper towel. Repeat when you are done.

c. Wear gloves – your skin is a great source of contaminants. Remember, though, that the exterior of the gloves are not sterile either.

d. Keep your head and body out of the hood; just work in there with your hands. (You'll be surprised how hard it is to remember this)

e. Don't let the end of the pipet or pipet tip you're working with touch ANYTHING except the inside of the sterile tube containing the liquid you're measuring. If it touches the work surface of the hood, the outside of a tube, your hand, etc. DISPOSE of it, and get another one.

f. When using sterile wrapped pipets, only unwrap the end to insert into the pipet-aid. Once the pipet-aid is in place, carefully slip the rest of the wrapper off.

g. Keep the box of pipet tips closed unless you are removing a tip. Dispose of pipet tips in container in the hood.

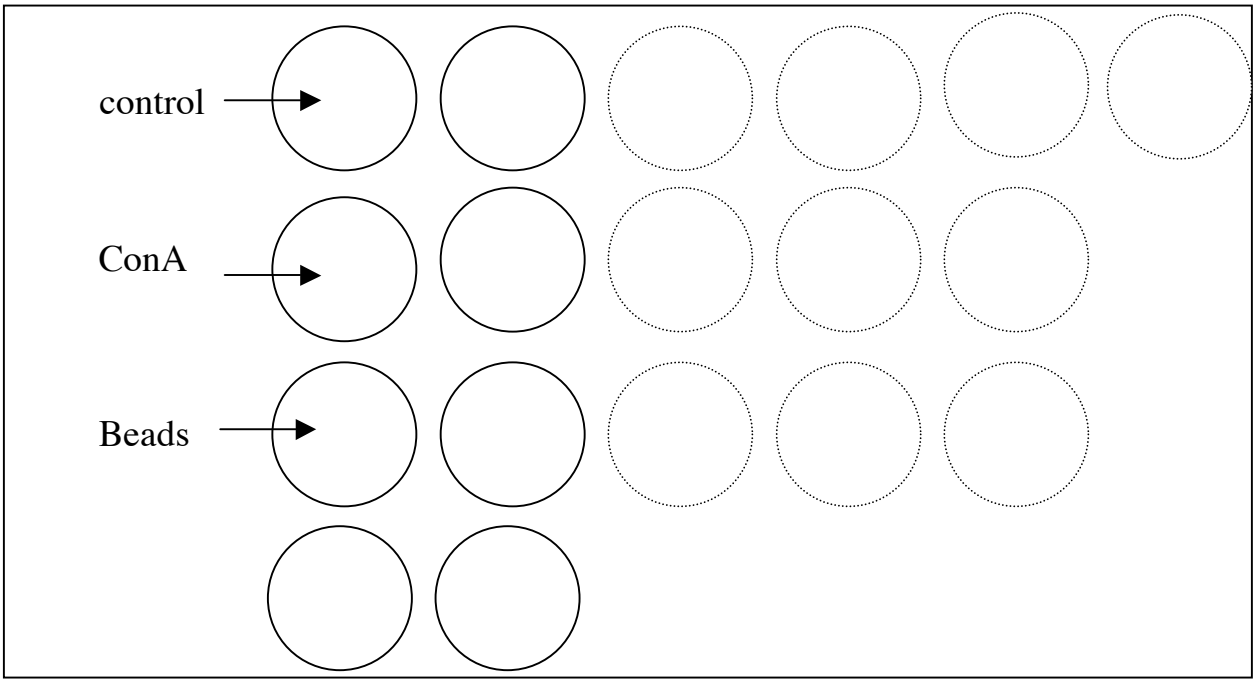
h. When you remove the screw-cap from a sterile tube, either hold it or place it clean side up, slightly outside the area where you'll be working.

Overview of plate setup.

You will culture spleen cells in 8 wells of a sterile 24-well tissue culture plate (see cartoon below).

The top row wells will be the negative controls (spleen cells in RPMI media alone).

The wells in the other 3 rows will contain cells in RPMI media plus conconavalin A (Con A, 14ug/ml) or CD3/CD28 beads to artificially stimulate T cell activation.



Cell Counts

You will receive spleen cell suspensions from which the red cells have already been lysed. Remove a sample to count live and dead leukocytes following the same procedure as last week in lab (1:10 dilution). Record cell counts, and calculate the live cell concentration and % viability.

Calculating dilutions

You will be adding 5 x10^5 leukocytes in a volume of 500 μl RPMI to each well. This means that the concentration of your cell solution must be adjusted to 1 x10^6 cells/ml before plating the cells.

The formula $C_1V_1 = C_2V_2$ will be helpful here. We will review its use in lab.
Briefly,

C_1 = the current concentration of your cells
V_1 = the current total volume of your cell suspension
C_2 = the desired concentration of cells
Solve for V_2 , which is the volume to which the cells must be diluted to achieve the desired concentration.

Plating the cells

1. Dilute your cells to 1 x10^6 cells/ml in RPMI.

2. Dispense 200 μl of RPMI or RPMI+ ConA or RPMI+beads into the appropriate wells.
   Note: these RPMI solutions *will be provided to you, already prepared.*
   Row 1 wells: add 200 μl RPMI (negative control)
   Row 2 wells: add 200 μl RPMI+ConA
   Row 3 wells: add 200 μl RPMI+beads (pipet to mix well first)

3. Dispense 500 μl of your cell suspension (at 1 x10^6 cells/ml) into the 6 wells. Refer to the plate map on the previous page.

4. Label your plate and place it in the 37˚C incubator.

5. **After 24 hours**:
   a. observe the cells using an inverted microscope. (Put the whole plate on the inverted scope, leaving the cover on). Note if there are differences in the appearance of the cells (clumpy vs dispersed) between the control, Con A and beads wells.

   b. *In the tissue culture hood*,
   remove 200 μl of supernatant (no cells, <u>just</u> the culture media from the top) from each of the 2 RPMI+ConA wells and
   each of the 2 RPMI+beads wells.

   For each treatment, pool the supernatants into a microcentrifuge tube labeled with your initials, plus "24 hrs" and the treatment. Put these **2** tubes in the freezer, in the box labeled with your lab day.

6. **After 48 hours**:
   a. observe the cells again using an inverted microscope. Note if there are differences in the appearance of the cells (clumpy vs dispersed) between the control, Con A and bead-treated wells.

   b. *In the tissue culture hood*, using sterile pipet tips:
   remove 200 μl of supernatant (no cells, <u>just</u> the culture media from the top) from each of the 2 control wells
   each of the 2 RPMI+ConA wells and
   each of the 2 RPMI+beads wells.

   For each treatment, pool the supernatants into a microcentrifuge tube labeled with your initials, plus "48 hrs" and the treatment. Put these **3** tubes in the freezer, in the box labeled with your lab day.

You will have a total of **5** frozen samples in the freezer when you're finished.