Measurement of Cell Growth

I. Cell mass
   A. Direct methods—measuring both live (viable) and dead cells, a total measurement
      1. Take dry weight of all cells. Must wash the cells well to eliminate media.
   B. Indirect methods—also a total measurement, for both viable and dead cells are considered.
      1. Nitrogen content—Use biochemical assays to measure total amounts of nitrogen in the cell culture. Knowing the percentage of the cell mass that is generally nitrogen, one can now calculate to the total cell mass.
      2. Turbidometric assays—Bacterial cells will absorb and/or scatter light. The amount of light that is absorbed or scattered is proportional to the mass of cells in the culture. A colorimeter (monochromatic or uses just one wavelength) or a more versatile instrument known as a spectrophotometer (can vary the wavelength) can be used to measure turbidity (cloudiness).

II. Cell numbers
   A. Direct methods—measuring both viable and dead cells, a total measurement
      1. Microscopy—using a special slide known as a Petroff-Hausser counting chamber one can count the cells of a given volume that are placed on ruled grid. Knowing the number of cells in this given volume, one can then calculate the number of cells in the original culture tube. This can also be useful for visualizing cell arrangements, such as bacteria in chains, as the cells are not treated with stains or heat fixed. Drawbacks include visualization of unstained cells, and counting cells that are motile and can literally swim on and off of the counting grid.
      2. Particle or Coulter (inventor) Counter—uses a high tech instrument that counts bacteria that are suspended in an electrolyte. A probe is placed into this suspension, and a known volume of this bacterial suspension is drawn into the probe. An electrical circuit is interrupted each time an organism enters the probe. A digital counter records the numbers. The probes can be altered or changed to vary the hole size. With appropriate probes, viruses and larger cells can be counted. Also, the length of time it requires for each interruption can be used to distinguish large and small cells for purposes of differential counts.
   B. Indirect methods—measuring live cells only (viable counts)
      1. Plate counts—only viable cells will grow into colonies. The number of colonies is proportional to the number of cells. Plates should have between 30 and 300 colonies to be statistically significant. Dense populations should be diluted using serial dilution techniques.
         a. Pour plate technique
         b. Spread plate technique
2. Membrane filter technique—samples (good for air samples) can be drawn through a millipore filter (grid size can be 0.2 micrometers) using a vacuum. Bacteria are collected on the filter and are then transferred (by aseptically pressing and then removing the filter) onto a plate for growth. The plates are incubated and the colonies are counted.

3. Tube counts using MPN (Most probable number)—Samples are inoculated into a number of culture tubes using a precise protocol. The tubes are incubated and grown. The number of positive tubes is recorded. Using previously collected data and statistical probabilities, the most probable number of organisms in the original sample is determined.

III. Cell Activity—The activities of living cells are measured. All measurements are indirect, and all measurements are based upon the activities of live (viable) cells only.

A. Chemical methods or assays—any chemical changes in the media will be proportional to the activity of the cells. One can monitor the rate at which glucose amounts in the media decline. The faster glucose amounts decline, the more active the organisms. The same can be said of oxygen usage and decline. Carbon dioxide (waste product of metabolism) will increase at a greater rate if the organisms are active. Rates of increases in acidity (shown by a decline in pH) are also used to determine cell activity.

B. Physical methods or assays—these are sensitive and fast methods

1. Microcalorimetry—minute temperature changes over time can be monitored to assess cell activity using sensitive thermometers called thermistors. Antibiotic sensitivity can be tested using microcalorimetry.

2. Radioactive isotopes—radioactively labeled carbon released from glucose during metabolic activities in the form of carbon dioxide, can be detected and counted using a scintillation counter. The greater the count, the more active the culture. Antibiotic sensitivity can also be measured using radioactive isotopes.

End of Lecture Unit I