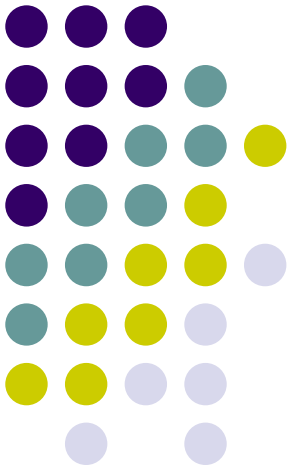
Cell Viability Assays:
Microtitration (MTT) Viability Test
Live/Dead Fluorescence Assay

Proliferation Assay:
Anti-PCNA Staining

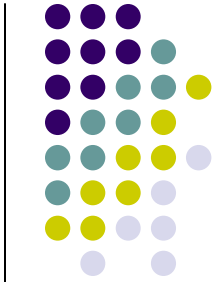


Spring 2008

Objectives

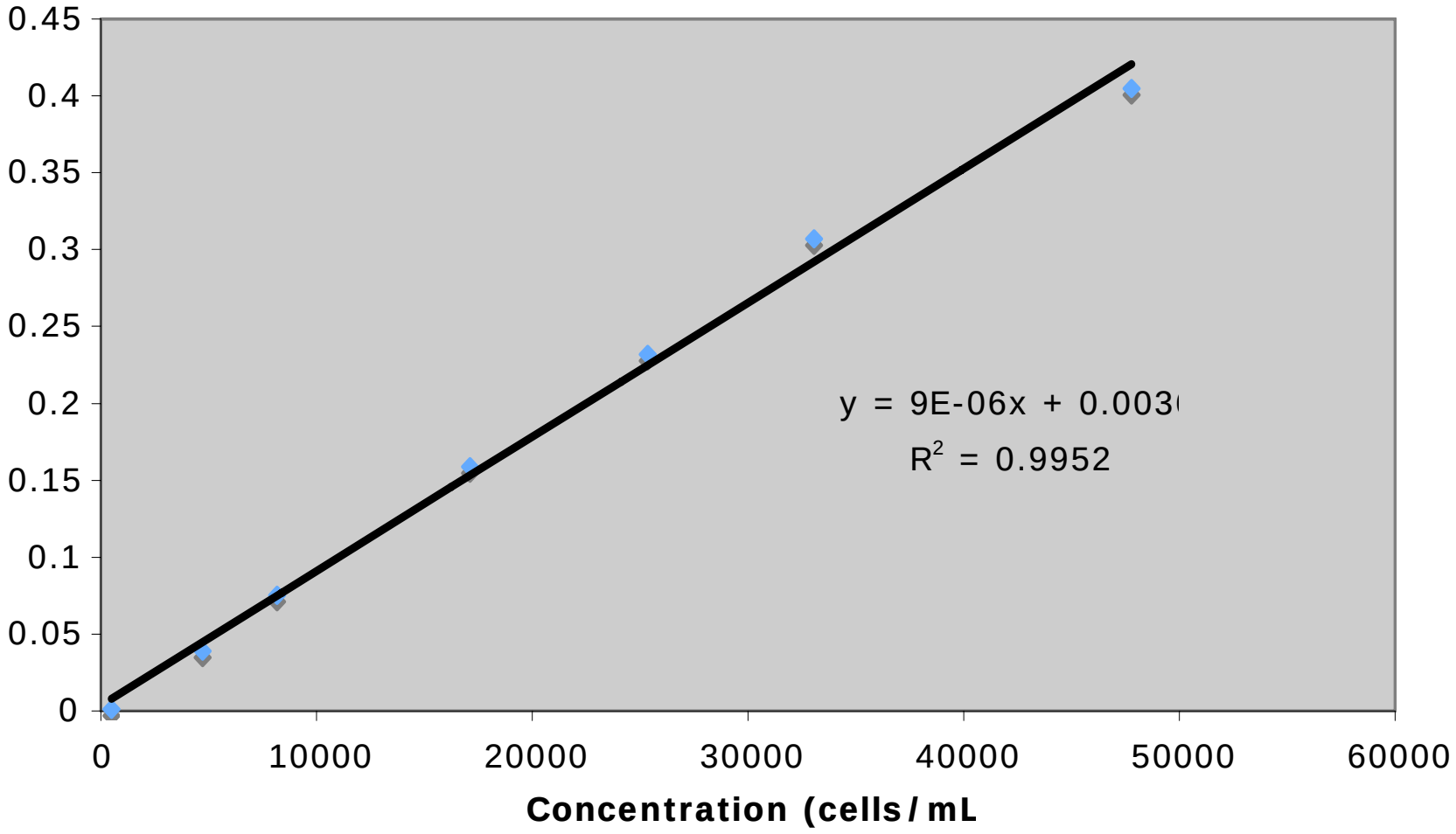
- To determine the viability of cells under different conditions
- To develop a relationship between absorbance and concentration of metabolically active cells
- To track live and dead cells with different chemicals
- To assess the impact of toxins on cell viability
- To determine the connection between cell cycle proliferation and media conditions

Experimental Methods: MTT assay

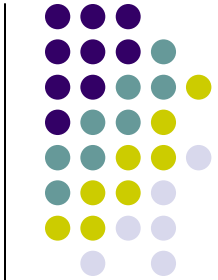


- Prepared two plates with different dilutions for:
  1. Cells Treated with MTT Dye
  2. Cells Counted on Coulter Counter
- Dilutions included: (1:1, 1:1.5, 1:2, 1:3, 1:6, 1:12, and control).
- Plates placed in incubator for 2 nights.
- Plate 1:
  - Added MTT dye to each well; incubated for 2 hrs.
  - Added Solubilization/Stop solution to each well; left for 45 min.
  - Measured absorbance at 570 nm on Genesys 10 UV Spectrophotometer.
- Plate 2:
  - Measured cell concentration on Coulter Counter by trypsinizing cells.

MTT Assay:
Linear relationship between Absorbance and Concentrat



Results: MTT Assay



- Graph shows how concentration and absorbance are linearly related
- As the concentration determined by the Coulter Counter increases, the absorbance indicative of the metabolic activity increases proportionately.
- Discrepancies can be accounted for by the fact that the MTT dye (trerazolium) is only reduced by live cells, while the Coulter Counter does not address viability.

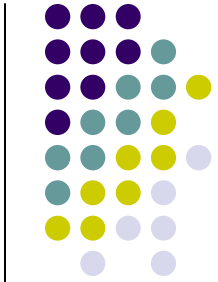
Experimental Methods: Live-Dead Assay

- Seeded 1:3 diluted cells into 9 wells (3 wells/condition)
- Placed in incubator for 2 nights.
- Added the following for condition A, B, C:
  - Condition A: 250 µL PBS, 100 µL dye
  - Condition B: 250 µL ethanol, 100 µL dye
  - Condition C: 250 µL PBS, 2 drop of ethanol, 100 µL dye
- Incubated 30 min. at room temperature
- Observed cells under Nikon Fluorescent Microscope through TRITC and FITC filters.

Results: Live/Dead Assay*

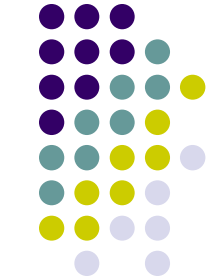
Condition	Observations:
A	-Cells fluoresce green (indicative of live cells) -70% are elongated with pseudopodia extended -cells in clusters throughout well
B	-Cells fluoresce red (indicative of dead cells) -Nuclei are brighter -Cell morphology same as condition A
C	-patches/clusters of both green and red -30% green, 70% red -Cell morphology same as condition A

Compare/Contrast results



- Both assays are indicative of live cells
- Both use a dye that under goes a reaction in the presence of live cells
- Both showed that it is difficult to distinguish between live and dead cells (morphology is often the same, cannot distinguish on light microscope).
- MTT is more quantitative, while the Live/Dead assay is more qualitative
- Live/Dead showed that ethanol is toxic to cells, resulting in cell death.

Experimental Methods: Anti-PCNA Staining



- Seeded cells at different conditions:
  - 1%, 5%, and 10% serum
  - 3 controls at 10% serum
- Incubated for 2 days
- Followed standard staining procedure with Anti-PCNA primary antibody
- Viewed cells using light microscope

Results: PCNA

Conditions	Observations
10% serum	-Cytoplasm is stained blue (for all conditions) -60% of cells have red nuclei -50% confluency -Cells are elongated and robust
5% serum	-30% of cells have red nuclei -40% confluency -Cell morphology same as 10%
1% serum	-20% of cells have red nuclei -20% confluency -Cell morphology same as 5 and 10%
controls	-All nuclei are blue -60% confluency -Cells are elongated and healthy

Results: PCNA

- Uses Proliferating Cell Nuclear Antigen that is indicative of cells in S-phase
- When cell nuclei are red, the cell is replicating its DNA.
- The higher the % serum, the higher the % of cells in S-phase.
- Thus, higher serum levels have higher cell cycle proliferation (up to 10%).

Conclusions

- MTT is indicative of metabolism and thus is a viable assay (can develop relationship between absorbance and concentration)
- Live/Dead assay distinguishes live and dead cells through staining (can see the effect of toxins on cells)
- Anti-PCNA stain is indicative of cell cycle proliferation by labeling cells in S-phase

*I compared my results to XXX's results.