



Cell Proliferation and Viability



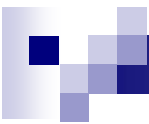
Objectives

- Compare cell proliferation in different serum concentrations over seven days
- Compare number of cells in S-phase after two days in different serum concentrations
- Determine the relationship between cell concentration and absorbance at 570nm



Cell Proliferation Assay: Materials and Methods

- Seed cells at 5,000 cells/mL
 - 6 wells in 1% serum for day 0
 - 3 wells of 1%, 5%, and 10% each for days 2, 5, and 7 each (27 wells total)
- Use Coulter counter to determine cell concentration on designated day
- Replenish media with appropriate serum concentration on days cells are not counted



Anti-Proliferating Cell Nuclear Antigen (PCNA) Assay: Preparation

- Seed 1 mL of cells at 20,000 cells/mL
 - One well each of 1%, 5%, and 10% serum
 - Three control wells in 10% serum
- Incubate for two days
- Let all wells stand in formalin
- Let all wells stand in methanol with 3%
 H_2O_2



Anti-PCNA Assay: Staining

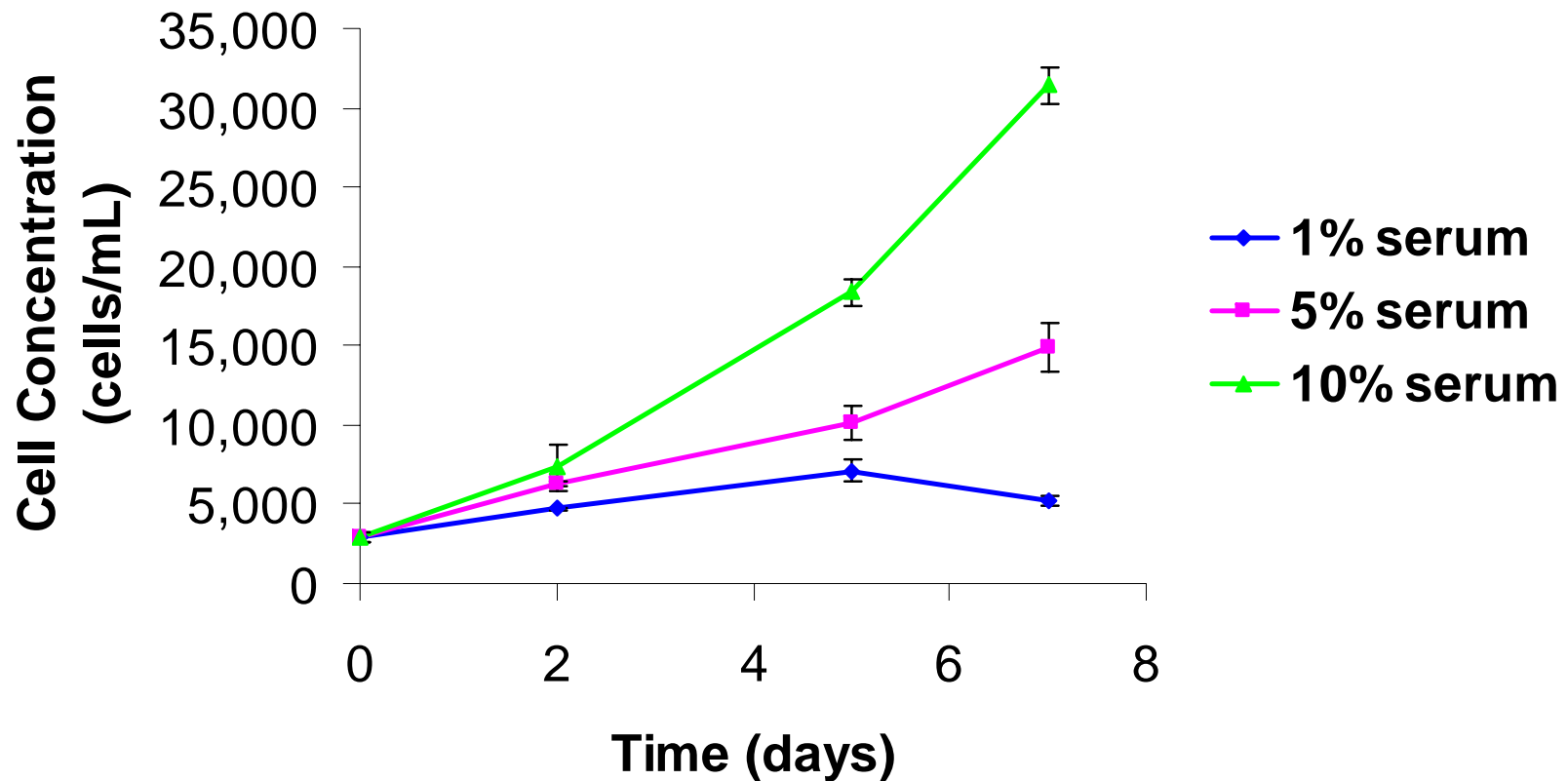
- Add anti-PCNA primary antibody to all wells except controls #2 and #3
- Add Anti-mouse IgG secondary antibody to all wells except controls #1 and #3
- Let all wells stand in AEC solution for 10 min
- Let all wells stand in hematoxylin for 2-4 min
- Add 37mM NH_4OH to wash all wells of excess dye
- View cells using light microscope with no filter
 - Count number of red nuclei (cells in S-phase) and number of blue nuclei (cells not in S-phase)



MTT Viability Assay: Materials and Methods

- Seed cells in dilutions from 50,000 cells/mL
 - Two wells of each: 1:1, 1:1.5, 1:2, 1:3, 1:6, 1:12 dilutions and 2 controls with no cells (14 wells total)
- Incubate for two nights
- Count one well of each using Coulter Counter
- Add MTT dye to the other well of each
- Add Solubilization/Stop solution to each well containing MTT dye
- Record absorbance of each well and a control vial at 570 nm using spectrophotometer

Cells Proliferate Quicker in 10% Serum Than in 1% or 5% Serum

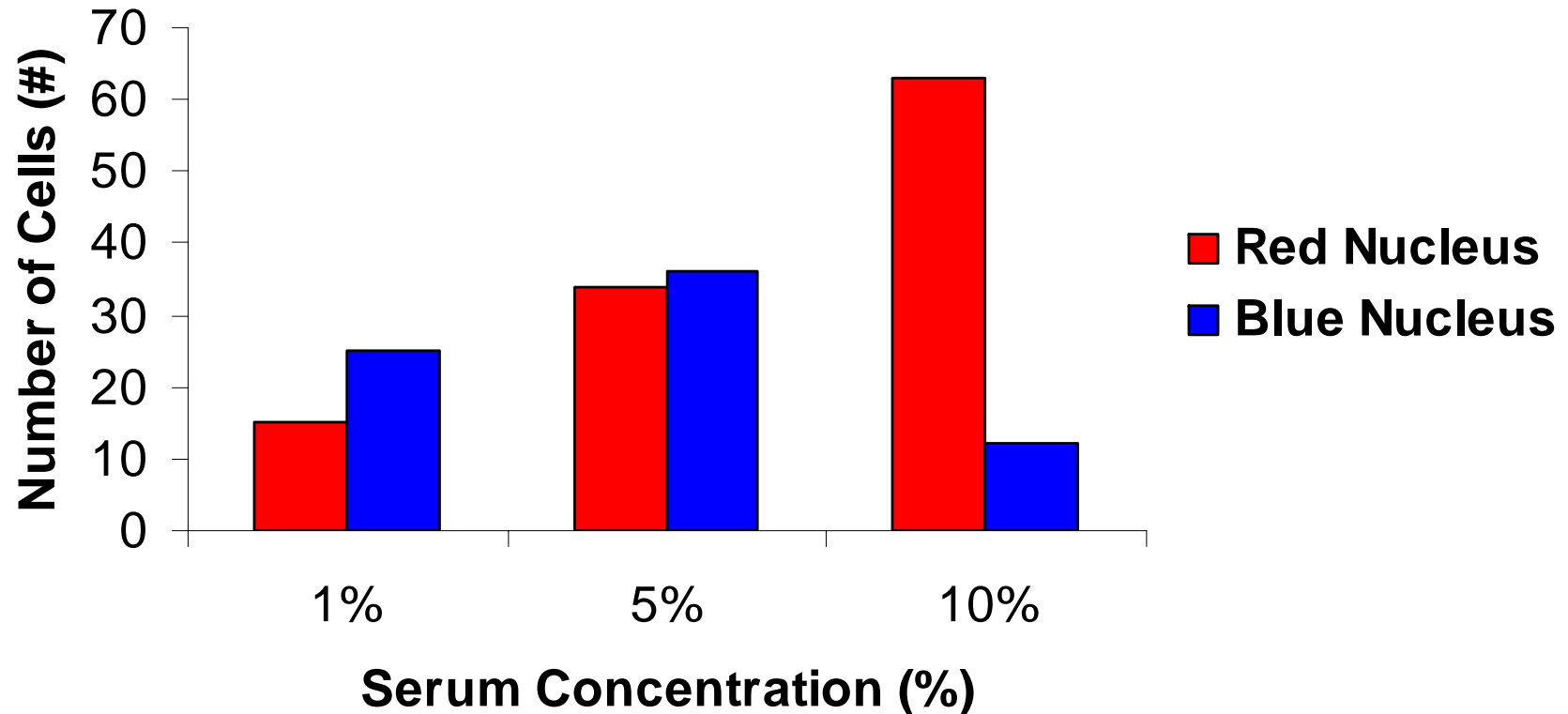




Cell Proliferation: Statistics and Exponential Growth Curves

- Differences found using ANOVA
 - Between groups
 $p < 0.25$ which shows uncertainty of the differences
 - Between 10% values
 $p < 0.00001$ so every value of 10% is different
- Exponential Growth Curves
 - 10% serum:
 $y = 3,700 * e^{0.21 * \text{time}}$
 - 5% serum:
 $y = 3,600 * e^{0.31 * \text{time}}$
 - 1% serum: data not appropriate for exponential curve

Cultures in 10% Serum had More Cells Stained in S-Phase (Red Nucleus)



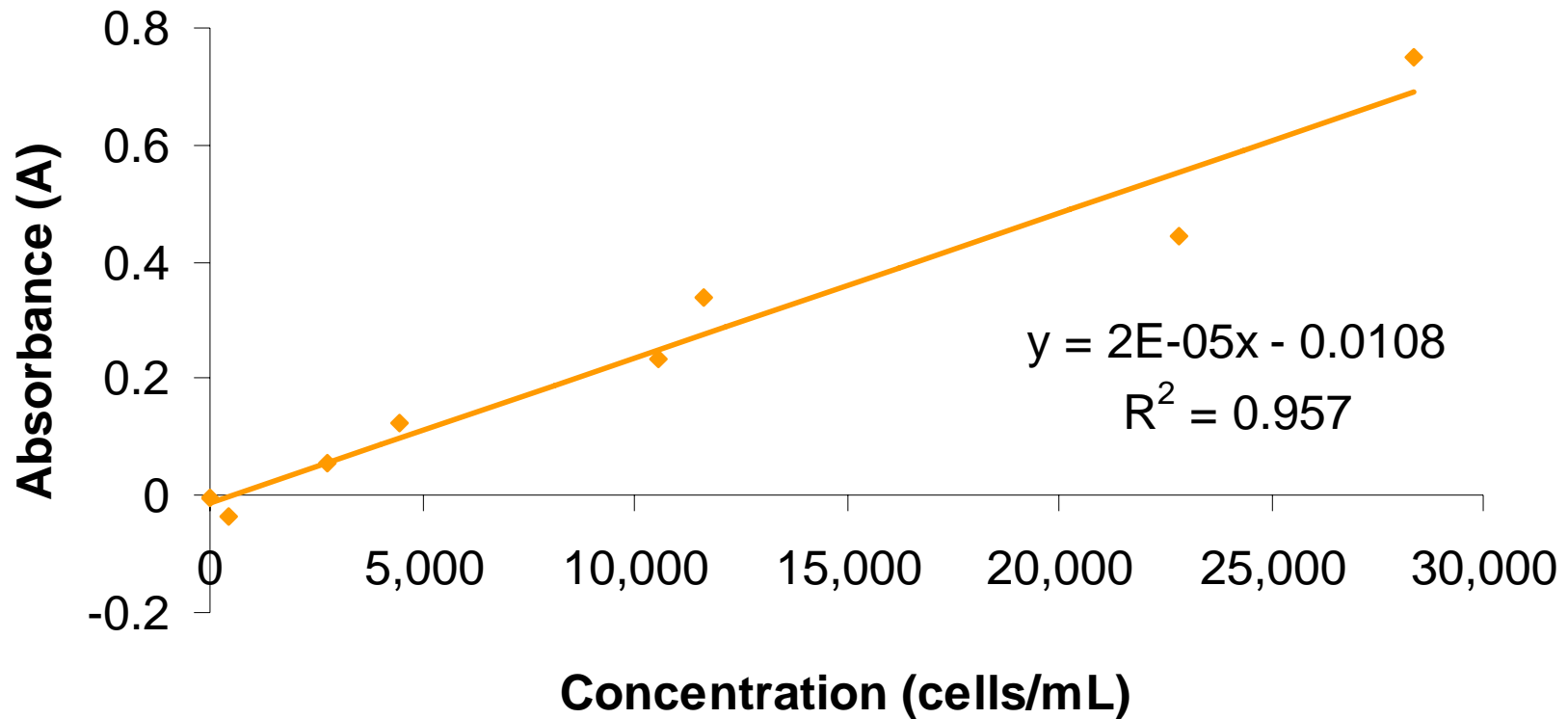
*no statistical data because only one value for each serum concentration



Cell Proliferation and Anti-PCNA Staining Assays

- More cells proliferating in 10% serum
 - Cell concentration in 10% serum showed greater growth
 - Anti-PCNA staining showed a greater number of cells in S-phase in 10% serum
- Poor proliferation in 1% serum
 - Cell concentration showed negative growth in 1% serum
 - Anti-PCNA staining showed more cells not in S-Phase in 1% serum

Cell Concentration and MTT Absorbance Show a Linear Relationship





Summary

- Cells proliferate more in 10% serum than in 1% or 5% serum over a seven day period
- More cells can be found in S-phase in 10% serum than in 1% or 5% serum after staining with antibodies, AEC, and hemotoxylin
- Cell concentration and absorbance of live cells stained with MTT dye were shown to have a linear relationship