**Study design and background.**

The increase in the cell size and cell mass during the development of an organism is termed growth. It is the unique characteristics of all organisms. The organism must require certain basic parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and nutritional factors. There are multiple methods by which growth can be measured, but the use of closed tissue culture tubes and a spectrophotometer to track increases in optical density (absorbance at 600 nm) over time offers several advantages: 1) it is less subject to technical error and contamination, 2) read out is fast and simple, 3) growth as measure by increased absorbance (turbidity) is directly proportional to increases in cell mass. There are four distinct phases of bacterial growth. Lag phase, log (exponential phase), stationary phase, death phase. From these data, bacterial generation times (doubling time) during the exponential growth phase can be calculated.

Doubling time =

The goal of this study was to compare growth yield and doubling time of *Mycobacterium tuberculosis* grown in rich medium under two assay conditions. One set of cultures were grown in tubes with a low culture volume relative to a large air head space to allow free oxygen exchange. A second set of cultures were grown in tubes filled to near capacity, resulting in limited air head space which has been shown elsewhere to limit oxygen availability over time. The caps on both sets of cultures were sealed to restrict air exchange during the study.

An excel-based workbook was created to allow the student performing the work to (1) calculate the amount of initial inoculum (cell culture) to add to each tube to begin the study, (2) record the raw data absorbance measurements, (3) graph the data on both a log and linear scale, and (4) calculate doubling time in two phases of growth using the equation listed above. Columns were added to allow the student to track the time (column A), the difference in time (hours) between each time point in which data were collected (column B), the date on which data were gathered (column C), and the time in hours for each data point from the start of the study for graphing purposes (column D). Absorbance data for each sampling timepoint were listed in Columns E-F (high oxygen conditions; VA001 A1, A3) or columns G-I (limited oxygen conditions; VA001 L1, L2, L3).

What the researchers found appealing about the format of this excel sheet was the ease with which the student could accomplish the study goals. They also cited transparency of the raw data and ease with which additional sampling data points could be added. The data being graphed in real time and the inclusion of a simple macro to calculate doubling time, allowed the student to see tangible differences between the two assay conditions. This was also somewhat problematic as the equation to calculate doubling time was based on anchored time points built into the original spreadsheet resulting in two different results that were not properly linked to the correct data time points.