The scenario: Your PI wants you to determine the affect of lead (using lead acetate) on the growth of *Pseudomonas aeruginosa* when grown in LB media. Your task is to safely and efficiently design and carry out a publication-quality experiment to answer this question. This exam will take you through this process, your task is to answer the questions as you go along.

1. Before beginning, you remember that lead is toxic, and you correctly decide that it’s a good idea to look this up. Go to the NCSU MSDS sheet database at: [http://www.ncsu.edu/ehs/MSDS.htm](http://www.ncsu.edu/ehs/MSDS.htm) Either use the “MSDS Management System”, or if you’re off-campus use the “Fisher Scientific” link at the bottom of the page, to look up the MSDS sheet for lead acetate. What are the listed hazards of lead acetate? What should you do if exposed to lead acetate? (2 points)

2. The formula weight of lead acetate (lead (II) trihydrate, which is what you have on the shelf) is 379.3 g/mol. You won’t need a lot for these experiments, so you hope to make 10ml of a stock solution at a concentration of 1M. You check online for the solubility of lead acetate (which is a white powder) in water, and find it is 44.4 g/100ml at 20°C. Will a 1M stock be possible? If so, how would you make this solution? If not, what concentration could you make, and how would you make it? (2 points)
You realize that before you can design your final, definitive experiment, you need to do a preliminary experiment to determine approximately what concentration of lead is required to inhibit growth, i.e. to get you in the right ballpark. Design a simple “quick-and-dirty” experiment to give you an “order of magnitude” idea of what lead acetate concentration you should focus on in your future experiments. (5 points)
4. After setting up your experiment according to plan, you load up the Bioscreen plate but when you start up the Bioscreen instrument it gives you a “BLANK ERROR (F1-BZ)” fault message. What are the likely causes and solutions for this error? (1 point)

5. After correcting/fixing the machine, you run your experiment and when the results come in, your 1mM lead acetate sample gives you the following growth curve relative to LB without lead acetate. What specific aspect of the growth of \( P.\ aeruginosa \) is most affected by lead acetate at this concentration? What aspects of growth are not much affected? (2 points)

6. Given the result in question 5, design your definitive experiment to determine the concentration of lead required to inhibit growth (whatever aspect of growth in most affected above) by 50%; in other words, you want to determine the 50% Inhibitory Concentration (IC\(_{50}\)) of lead acetate for \( P.\ aeruginosa \) in LB. This is the experiment your PI asked you to do in the beginning, and your results will be used in a formal paper, so be sure to include all controls and whatever else you need to be convincing in your answer. Be sure to show how you would determine the ID\(_{50}\) from your results. Describe WHY each component of the experiment is needed. (8 points)