

Extended Essay

Biology

Research Questions:

To what extent do 5.00 mgL^{-1} , 2.50 mgL^{-1} , and 1.25 mgL^{-1} of the commonly used glycopeptide antibiotic, Vancomycin negatively affect the growth curves of the gram-positive bacterium *Staphylococcus aureus*?

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Abstract:

The purpose of this paper was to examine **to what extent 5.00 mgL⁻¹, 2.50mgL⁻¹, and 1.25 mgL⁻¹ of the commonly used glycopeptide antibiotic, Vancomycin negatively affects the growth curves of the gram-positive bacterium *Staphylococcus aureus*.** I chose to study the negative effects that differing concentrations of Vancomycin have on *Staphylococcus aureus* because I was interested in the increasing resistance that *Staphylococcus aureus* has shown to Vancomycin. Also, I wanted to see how effective Vancomycin was against laboratory strains of *Staphylococcus aureus* here in India. I was mainly interested as to how large the difference in detrimental effect would be between increasing concentrations of Vancomycin. I assumed that as we doubled the concentration, the negative effect upon the population growth curve would also double. Yet, what I discovered was that lower concentrations of Vancomycin were more effective than the higher concentrations.

In my experiment, I took % transmittance and absorbance readings, using a spectrophotometer, of five different Tryptone broths that had a population of *Staphylococcus aureus*. Three of the broths were inoculated with differing concentrations of Vancomycin; 1.25mgL⁻¹, 2.50mgL⁻¹ and 5.00mgL⁻¹. The other two broths were left to grow naturally. This allowed the construction of population growth curves that showed how large a detrimental effect the Vancomycin had on the *Staphylococcus aureus* populations. Surprisingly, the concentration of Vancomycin that had the greatest detrimental effect was the 1.25mgL⁻¹ inoculation which greatly inhibited the growth of the *Staphylococcus aureus* by delaying the log phase of growth. The 2.50mgL⁻¹ and the 5.00mgL⁻¹ inoculation were not as effective at delaying the log phase yet they did reduce the population size when the populations were inoculated.

Word count: 275

Contents:

- **Title Page..... (i)**
- **Abstract.....(ii)**
- **Contents page.....(iii)**
- **Acknowledgements(iv)**
- **Introductionpg.1**
 - **Background Information**
 - **Vancomycinpg.2**
 - ***Staphylococcus Aureus*pg.4**
 - **Bacterial population growth curvespg.6**
- **Procedure.....pg.7**
 - **Preparation of Tryptone Brothpg.8**
 - **Preparation of Sub-culturespg. 10**
 - **Inoculationpg. 12**
- **Data Collectionpg.13**
- **Data Processingpg. 17**
- **Graphs and Graph Analysis pg.20**
- **Evaluation pg. 28**
- **Conclusions pg. 30**
- **Bibliography pg. 33**
- **Appendix A (original graphs and data collection)..... pg. 40**
- **Appendix B (Vancomycin and Red man’s syndrome)..... pg. 47**
- **Appendix C (*Staphylococcus aureus*)..... pg. 50**

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Introduction:

Introduction:

The purpose of my study is to answer the question; **to what extent do 5.00 mgL⁻¹, 2.50 mgL⁻¹, and 1.25 mgL⁻¹ of the commonly used glycopeptide antibiotic, Vancomycin negatively affect the growth curves of the gram-positive bacterium *Staphylococcus aureus*?** The reason I chose to study *Staphylococcus aureus* and Vancomycin specifically is because until recently Vancomycin has been used solely as a ‘drug of last resort’ because of its high toxicity level¹. However, the emergence of glycopeptide resistant species of *Staphylococcus aureus* (called Vancomycin-resistant *Staphylococcus aureus*) has caused doubt to be shed on the value of Vancomycin when dealing with *Staphylococcus aureus* infections. Thus, I wanted to study how effective Vancomycin was on *Staphylococcus aureus* here in India.

Background Information:

Vancomycin:

Vancomycin is a glycopeptide antibiotic that works specifically against infections that have been caused by Gram-positive bacteria. It has historically been used solely as a ‘drug of last resort’ as it has a high toxicity and has been known to cause phlebitis (inflammation of a vein²) and necrosis (death of a portion of living tissue differentially affected by local injury (as loss of blood supply, corrosion, burning, or the local lesion of a disease³)). Vancomycin is usually administered over sixty minutes as a dilute solution. Intravenous use of Vancomycin can sometimes cause an infusion reaction known as Red Man’s Syndrome when given too rapidly. (Information on Red Man’s Syndrome can be found in Appendix B.)

¹ "Glycopeptide antibiotic." Wikipedia, the free encyclopedia. 09 Jan. 2009 <http://en.wikipedia.org/wiki/Glycopeptide_antibiotics>.

² Taken from “Phlebitis” Webster’s Medical Desk Dictionary. 1986. 539.

³ Taken from "Necrosis." Webster’s Medical Desk Dictionary. 1986. 466.



Figure 1.1
Vancomycin Powder

In a gram-positive bacterium, a peptidoglycan matrix forms a compound called Murein through the cross-linking of long polysaccharide chains and short amino acid chains⁴. Thus the bacterial cell wall is a single molecule that forms a sac-like protection around the cell. As a glycopeptide antibiotic, Vancomycin is effective only against gram-positive bacteria as it works by inhibiting the combining of N-acetylmuramic acid and N-acetylglucosamine into the peptidoglycan matrix. The large hydrophilic Vancomycin molecule is able to form hydrogen bonds with the terminal D-alanyl-D-alanine ends of N-acetylmuramic acid and N-acetylglucosamine. The hydrogen bonds formed here make these peptides unable to join the peptidoglycan matrix and thus inhibit Murein formation. When this molecule is prevented from formation by Vancomycin the cell is unable to function. As it is inhibiting the formation of the cell wall Vancomycin is effective against gram-positive bacteria yet cannot be used against gram-negative bacteria because their outer membranes are impermeable to the Vancomycin molecules.

There are bacteria that are inherently resistant to Vancomycin, including all gram-negative bacteria (with the exception of some non-gonococcal *Neisseria* forms), most *Lactobacillus* species and both the *Leuconostoc* and *Pediococcus* species⁵. The first case of an acquired resistance was in Vancomycin-resistant enterococcus in 1987 and

⁴ Soper, R., ed. *Biological Science 1 and 2*. 3rd ed. New York: Cambridge UP.

⁵ "Glycopeptide antibiotic." *Wikipedia, the free encyclopedia*. 09 Jan. 2009
<http://en.wikipedia.org/wiki/Glycopeptide_antibiotics>.

vancomycin-resistant *Staphylococcus aureus* and vancomycin-resistant *Clostridium difficile* have all been found in the last two decades⁶. One of the believed causes of this increase in resistance is the altering of the terminal of the peptide chains from D-alanyl-D-alanine to other variations such as, D-alanyl-D-lactate and D-alanyl-D-serine which allow less hydrogen bonding and thus can cause a thousand-fold increase in affinity to the polysaccharides.⁷

Staphylococcus aureus:

Staphylococcus aureus is a gram-positive coccus that usually groups together to look like a bunch of grapes⁸ (see figure 1.3). Thus the literal translation of *Staphylococcus aureus* is 'golden seed'.⁹ *Staphylococcus aureus* can be found predominantly in the nose and on the skin, although it is sometimes in the throat¹⁰ with about 20 % of the world's population being long-term carriers of the coccus.¹¹ It is usually found in the anterior nares as they are used as a colonizing point from which the disease can spread. When topical treatment is given to these regions the bacteria has been found to disappear from other parts of the body.¹² *Staphylococcus aureus* is a facultative anaerobic coccus as it can aerobically respire effectively when oxygen is present but is also able to perform fermentation when there is no oxygen available.¹³

⁶ "Emergence of vancomycin resistance in Staphylococc...[N Engl J Med. 1999] - PubMed Result." [NCBI HomePage](http://www.ncbi.nlm.nih.gov/pubmed/10021469). 18 Feb. 1999. 11 Jan. 2009 <<http://www.ncbi.nlm.nih.gov/pubmed/10021469>>.

⁷ "Active-site mutants of the VanC2 D-alanyl-D-serine...[Chem Biol. 1998] - PubMed Result." [NCBI HomePage](http://www.ncbi.nlm.nih.gov/pubmed/9545431). Apr. 1998. 11 Jan. 2009 <<http://www.ncbi.nlm.nih.gov/pubmed/9545431>>.

⁸ Soper, R., ed. *Biological Science 1 and 2*. New York: Cambridge UP.

⁹ "Staphylococcus aureus." [Wikipedia, the free encyclopedia](http://en.wikipedia.org/wiki/Staphylococcus_aureus). 13 Jan. 2009 <http://en.wikipedia.org/wiki/Staphylococcus_aureus>.

¹⁰ "Nasal Carriage as a Source of Staphylococcus aureus Bacteremia." [EBSCOhost](http://web.ebscohost.com/ehost/detail?vid=1&hid=113&sid=a540a08f-5a21-48fb-905e-b22c9da175b5%40sessionmgr109&bdata=JnNpdGU9ZWhvc3QtG12ZQ%3d%3d#db=afh&AN=24914973). 13 Jan. 2009 <<http://web.ebscohost.com/ehost/detail?vid=1&hid=113&sid=a540a08f-5a21-48fb-905e-b22c9da175b5%40sessionmgr109&bdata=JnNpdGU9ZWhvc3QtG12ZQ%3d%3d#db=afh&AN=24914973>>.

¹¹ "Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks -- Kluytmans et al. 10 (3): 505 --." [Clinical Microbiology Reviews](http://cmr.asm.org/cgi/reprint/10/3/505?view=long&pmid=9227864). 13 Jan. 2009 <<http://cmr.asm.org/cgi/reprint/10/3/505?view=long&pmid=9227864>>.

¹² "Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks -- Kluytmans et al. 10 (3): 505 --." [Clinical Microbiology Reviews](http://cmr.asm.org/cgi/reprint/10/3/505?view=long&pmid=9227864). 13 Jan. 2009 <<http://cmr.asm.org/cgi/reprint/10/3/505?view=long&pmid=9227864>>.

¹³ "Staphylococcus aureus." [Online Textbook of Bacteriology](http://www.textbookofbacteriology.net/staph.html). 2008. 13 Jan. 2009 <<http://www.textbookofbacteriology.net/staph.html>>.

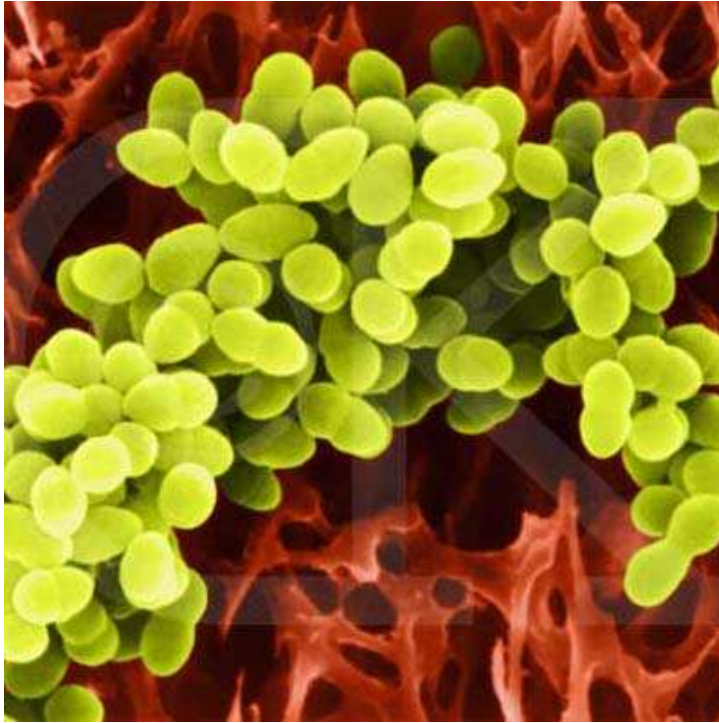


Figure 1.2. Golden Seed clusters



1.3 *Staphylococcus aureus*
Petri dish culture

Bacterial population growth curves:

Bacterial population growth curves usually go through four phases. They start off in the lag phase of growth, where the bacteria are adjusting to the new environment. Then there is the log phase, where population growth is at its highest rate. Then follows the stationary phase where growth is stopped which is immediately followed by the death phase where the bacteria die out due to lack of nutrients. Most bacteria follow an s-shaped population growth curve.

To complete my aim I will be taking a series of spectrophotometric readings of *Staphylococcus aureus* cultures that have been inoculated with 5.00 mgL^{-1} , 2.50 mgL^{-1} and 1.25 mgL^{-1} of vancomycin. Thus, I will be able to construct population growth curves that show to what extent the differing doses of vancomycin has detrimentally affected the growth of *Staphylococcus aureus*.

The *Staphylococcus aureus* was a laboratory strain and not the pathogenic strain and was obtained from the Bose Clinic, Madurai, Tamil Nadu and the Vancomycin was bought from Valliappa Medicals, Kodaikanal.

I think that as we increase the concentrations of the Vancomycin inoculations the growth of the *Staphylococcus aureus* will be slowed proportionally. Thus, as we add 1.25 mgL^{-1} of Vancomycin the negative effect that it has upon the population of *Staphylococcus aureus* will be half of that of 2.50 mgL^{-1} which will have half the effect of 5.00 mgL^{-1} . I think this because there will be twice as many Vancomycin molecules in the later inoculations and thus one assumes that they will be able to degrade the murein cell wall of twice as many bacteria. This assumption is based on two premises; firstly, that one Vancomycin molecule will only be effective on one Murein cell wall and secondly that there are enough bacteria for all the Vancomycin to be used.

Procedure:

Procedure:

Preparation of Tryptone broth:

Material	Quantity required
Tryptone	2.5 ± 0.1 g
Yeast	1.3 ± 0.1 g
NaCl	2.5 ± 0.1 g
Distilled water, H ₂ O	250 ± 1.5 ml
1.0 M NaOH	0.5 ± 0.01 ml
100 ml Measuring Cylinder	1
500 ml conical flask	1
Incubator set at 37°C	1
Digital Weighing scale	1
Glass Rod	1
Aluminum foil	1 roll
Bunsen Burner	1
Matches	1 Box
Tripod	1
Digital Pipette (accurate to 0.05 ml)	1
Spatula	3
Inoculation Rod	1
Cloth	1
<i>Staphylococcus aureus</i>	1 Petri dish culture
Spectrophotometer	1
Cuvette	2
Pipette	1

N.B. Before you start the experiment it is advisable to sterilize all of the equipment used using an autoclave.

N.B. Before using the spectrophotometer one should make sure it is properly calibrated with the help of a cuvette containing a distilled water or blank sample. For this

experiment, the wave length of the spectrophotometer was set to 550nm and the transmittance was 100%.

1. Measure out 2.5 g of Tryptone, 1.3 g of yeast and 2.5 g of NaCl into a 500 ml conical flask using the digital weighing scale.
2. Pour 250ml of distilled water using the 100ml measuring cylinder into the beaker.
3. Mix the ingredients thoroughly with the glass rod.
4. Add 0.5 ml of 1.0 M NaOH to the broth using the digital micropipette.
5. Place the conical flask upon a tripod above a lit Bunsen burner and heat the broth until all of the small particles have dissolved making sure that you stir it as it heats up.
6. Remove the conical flask from the heat and sterilize the rim of the flask by applying the flame to it.
7. Scrape a small amount of the lab strain of *Staphylococcus aureus* off the Petri dish containing the culture with the inoculation rod making sure not to lift the lid of the Petri dish too high.
8. Place the inoculation rod into the conical flask containing the broth and swirl so as to thoroughly mix the *Staphylococcus aureus* into the broth.
9. Take a sample of the broth, enough to fill the cuvette, and take the % Transmission and absorbance readings.
10. Cover the conical flask with aluminum foil and place it in the incubator.
11. Take the spectrophotometric readings regularly; I would suggest every three hours, also take qualitative readings. Leave the culture for twenty-four hours.

Preparation of sub-cultures:

Material	Quantity required
Tryptone	1.0 ± 0.1 g
Yeast	0.5 ± 0.1 g
NaCl	1 ± 0.1 g
Distilled water, H ₂ O	100 ± 0.5 ml
1.0 M NaOH	0.2 ± 0.01 ml
500 ml Measuring Cylinder	1
100 ml conical flask	5
Incubator set at 37°C	1
Digital Weighing scale	1
Glass Rod	1
Aluminum foil	1 roll
Bunsen Burner	1
Matches	1 Box
Tripod	1
Digital Pipette (accurate to 0.05 ml)	1
Spatula	3
Inoculation Rod	1
Cloth	1
Shaker Platforms (set to 100 rpm)	1
Spectrophotometer	1
Cuvette	2
Pipette	1

N.B. This section of the procedure should be started so that the sub-broths are ready by the time the twenty-four hour culture reaches the twenty-four hour mark. i.e., Step Seven should take place at the twenty-four hour mark.

1. Measure out 1 g of Tryptone, 0.5 g of yeast and 1 g of NaCl into 5 X 500 ml conical flasks using the digital weighing scale.
2. Pour 100ml of distilled water using the 500ml measuring cylinder into each of the flasks.
3. Mix the ingredients thoroughly with the glass rod.
4. Measure out 0.2 ml of 1.0 M NaOH using the digital micropipette and add that to each broth.
5. Place the conical flasks upon tripods above lit Bunsen burners and heat the broths until all of the small particles have dissolved making sure that you stir it as it heats up.
6. Remove the conical flasks from the heat and sterilize the rims of the flasks by applying the flame to them.
7. Take a 1 ml sample of the twenty –four hour (mother) culture using a digital micropipette and add it to the each of the conical flasks.
8. Take a sample of each broth using a pipette, enough to fill the cuvette, and take the % Transmission and absorbance readings.
9. Cover the conical flasks with aluminum foil and place them, in the shaker platform, in the incubator.
10. Take the spectrophotometric readings of absorbance and % transmission hourly, also take qualitative readings.

Inoculation:

Material	Quantity required
Incubator set at 37 ⁰ C	1
Digital Pipette (accurate to 0.05 ml)	1
Digital pH meter	1
Spectrophotometer	1
Cuvette	2
Pipette	1
Vancomycin Powder	500 mg
Distilled Water	100ml
Measuring Cylinder 25 ml	1
Permanent Marker	1
Spectrophotometer	1
Shaker Platforms (set to 100 rpm)	1

N.B. Step five of this procedure should occur just before the third hour absorbance readings are taken.

1. Take the 500 mg sample of Vancomycin and add 10 ml of distilled water so as to make it into 5.00 mgL⁻¹.
2. Take 5ml of the 5.00 mgL⁻¹ concentration and add 5 ml of distilled water so as to make it 2.50 mgL⁻¹.
3. Take 5ml of the 2.50 mgL⁻¹ concentration and add 5 ml of distilled water so as to make it 1.25 mgL⁻¹.
4. Label the flasks one through five with a permanent marker.
5. After the sub-cultures have reached the three hour mark of bacterial growth, take 1ml of the 5.00 mgL⁻¹ sample and put it in your third growth flask.
6. Then take 1ml of the 2.50 mgL⁻¹ sample and put it in your fourth growth flask.
7. Then take 1ml of the 1.25 mgL⁻¹ sample and put it in your fifth growth flask.
8. The first two conical flasks are controls for the experiment.
9. Replace the flasks in the shaker platform in the incubator and take spectrophotometric readings hourly.

Data Collection:

Data Collection:

Below is the data that was collected through the course of my first trial. I had to complete my first trial twice as there were some complications pertaining to the calibration of the spectrophotometer used.

Table showing the percentage transmission and Absorbance readings that were collected from the Original Culture over a twenty-four hour period

Time (\pm 0.00833 hours)	Percentage transmission (%)	Absorbance (AU)	Colour of the twenty four hour culture	Any other observations
0	35.9	0.446	Light yellowish brown, translucent	None
1	37.5	0.429	Slightly darker yellow, still translucent	A whitish substance can be seen forming on the surface of the culture
2	36.5	0.439	Still darker, almost brown, slightly less translucent	More whitish substance formed.
3	35.5	0.452	Remaining the same as hour 2.	Remaining the same as hour 2.
9	34.0	0.469	Deep yellowish, green.	A lot of white substance, foamy in appearance.
24	11.8	0.936	Culture has turned a dark murky green,	The white substance now resembles egg

			completely opaque.	white in appearance.
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As shown by the above measurements, as the *Staphylococcus aureus* culture turns the Tryptone broth a dark murky green and after a twenty four hour period it becomes almost opaque as opposed to being translucent initially. This is because the *Staphylococcus aureus* has, at the twenty four hour mark, had around 32 generations of growth (working on the basis that at 37⁰c *Staphylococcus aureus* takes 45 minutes per generation¹⁴). Thus, from our original culture one can safely assume that as the sub-cultures grow they will have decreased transmission (the fraction of incident light at a specified wavelength that passes through a sample¹⁵) and increased absorbance.

Data table showing the light absorbance for 0 mgL⁻¹, 1.25 mgL⁻¹, 2.50 mgL⁻¹, 5.00 mgL⁻¹ inoculations of Vancomycin in trial one

Time (hours)	Control (light absorbance) (AU)	0.00 mgL ⁻¹ (light absorbance) (AU)	1.25 ± 0.05mgL ⁻¹ (light absorbance) (AU)	2.50 ± 0.05mgL ⁻¹ (light absorbance) (AU)	5.00± 0.05mgL ⁻¹ (light absorbance) (AU)
1	0.317	0.356	0.245	0.337	0.303
2	0.316	0.360	0.244	0.333	0.299
3	0.327	0.351	0.244	0.246	0.310
4	0.400	0.398	0.257	0.359	0.280
5	0.561	0.421	0.286	0.364	0.330
6	0.908	0.710	0.353	0.406	0.385
7	0.987	0.890	0.562	0.789	0.652
8	1.042	1.030	0.751	0.900	0.857
9	1.056	1.047	0.807	0.948	0.917

The above table shows that the broths followed a similar pattern over the first two hours, with a slight decrease in light absorbance being the norm. From third hour, when I

¹⁴ "Wiley InterScience :: Session Cookies." 19 Jan. 2009
<<http://www3.interscience.wiley.com/journal/119730968/abstract?CRETRY=1&SRETRY=0>>.

¹⁵ "Transmittance." Wikipedia, the free encyclopedia. 19 Jan. 2009
<<http://en.wikipedia.org/wiki/Transmittance>>.

inoculated, there is a large decrease in the absorbance readings of the inoculated samples with the decrease being proportional to the amount of Vancomycin that was used. i.e., the 1.25 mgL^{-1} has no decrease in light absorbance yet it does slow down to some degree compared to the control, while the 5.00 mgL^{-1} inoculation has a decrease of 0.03 units between the third and fourth hours.

Data Processing:

Data Processing:

As all three of my trials followed the same pattern I decided to take the averages of the three trials so that I had a more conclusive set of graphs and data.

I used the windows computer program EXCEL to calculate the mean absorbance for the samples for each hour. The equation that I put in the cell was:

$$fx = \frac{SUM(\text{cell number 1, cell number 2, cell number 3})}{3}$$

I decided as the 0 mgL⁻¹ sample was only being used to allow me to make sure that it followed the same pattern as the control that I would take both of these samples as effectively one sample and I used this formula:

$$fx = \frac{SUM(\text{cell number 1, cell number 2, cell number 3....., cell number 6})}{6}$$

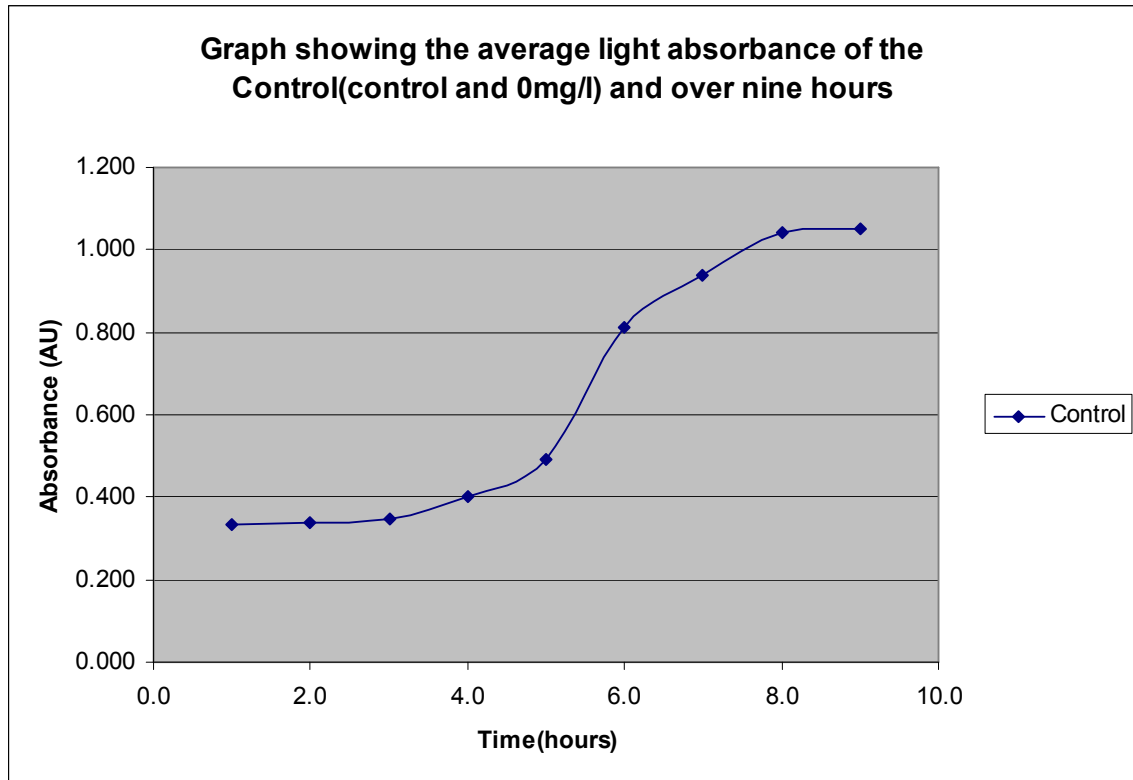
Thus I worked out a table containing all of the mean data for the three trials taking the control and the 0 mgL⁻¹ sample as the control.

Table showing the mean absorbance for all three trials for each of the samples

Time (hours)	Control(light absorbance) (AU)	1.25 ± 0.05mgL ⁻¹ (light absorbance) (AU)	2.50± 0.05mgL ⁻¹ (light absorbance) (AU)	5.00± 0.05mgL ⁻¹ (light absorbance) (AU)
1.000	0.332	0.258	0.333	0.308
2.000	0.336	0.248	0.333	0.300
3.000	0.347	0.255	0.264	0.336
4.000	0.403	0.267	0.371	0.280
5.000	0.492	0.281	0.381	0.329
6.000	0.814	0.376	0.407	0.393
7.000	0.940	0.575	0.823	0.672
8.000	1.041	0.758	0.898	0.861
9.000	1.051	0.840	0.924	0.914

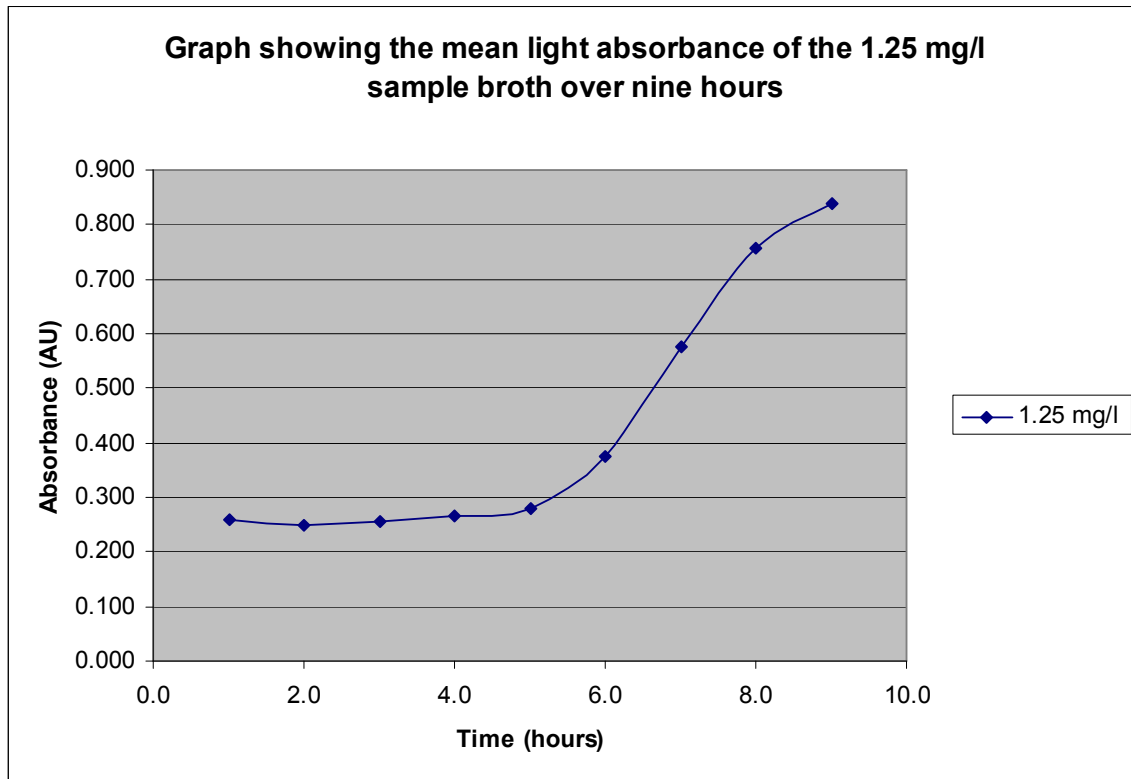
Graphs and Analysis:

Graphical representation of the mean light absorbance of the Control (control and 0mgL⁻¹) sample broth over a course of nine hours for the three trials



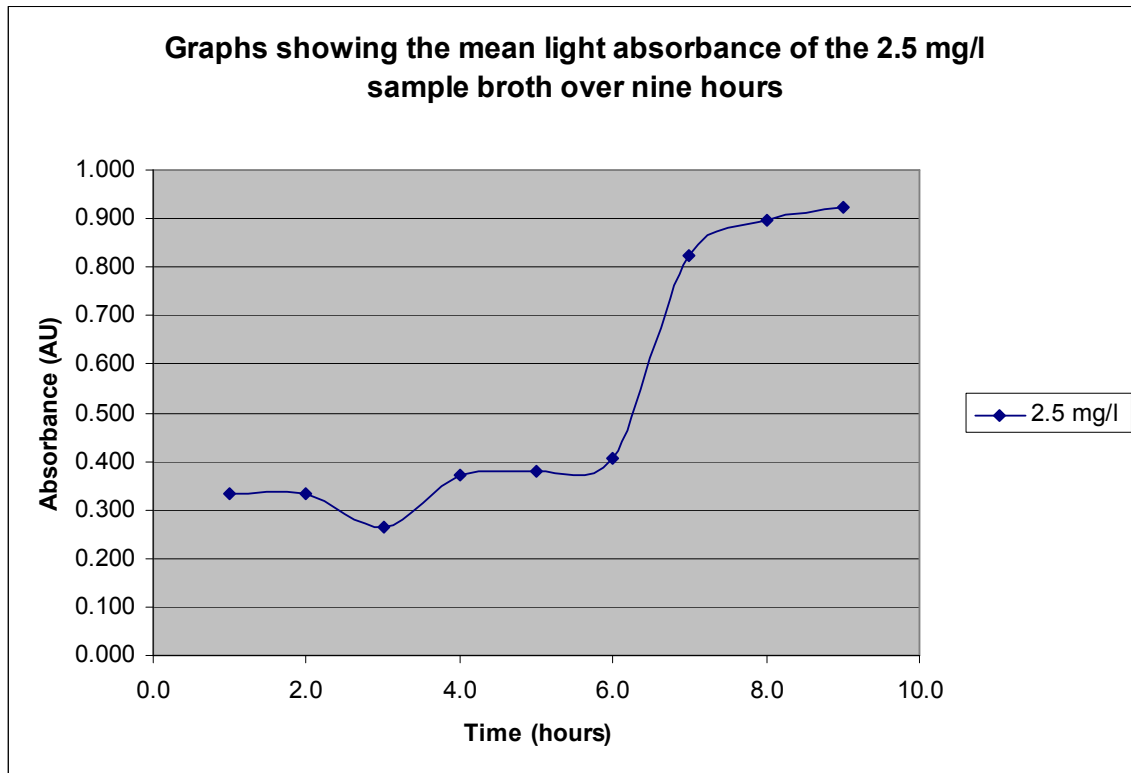
The above graph plots out the population growth curve for the control sample population. It enters the log phase around the third hour and enters the stationary phase around the eighth hour.

Graphical representation of the average light absorbance of the 1.25 mg/L sample broth over a course of nine hours for the three trials



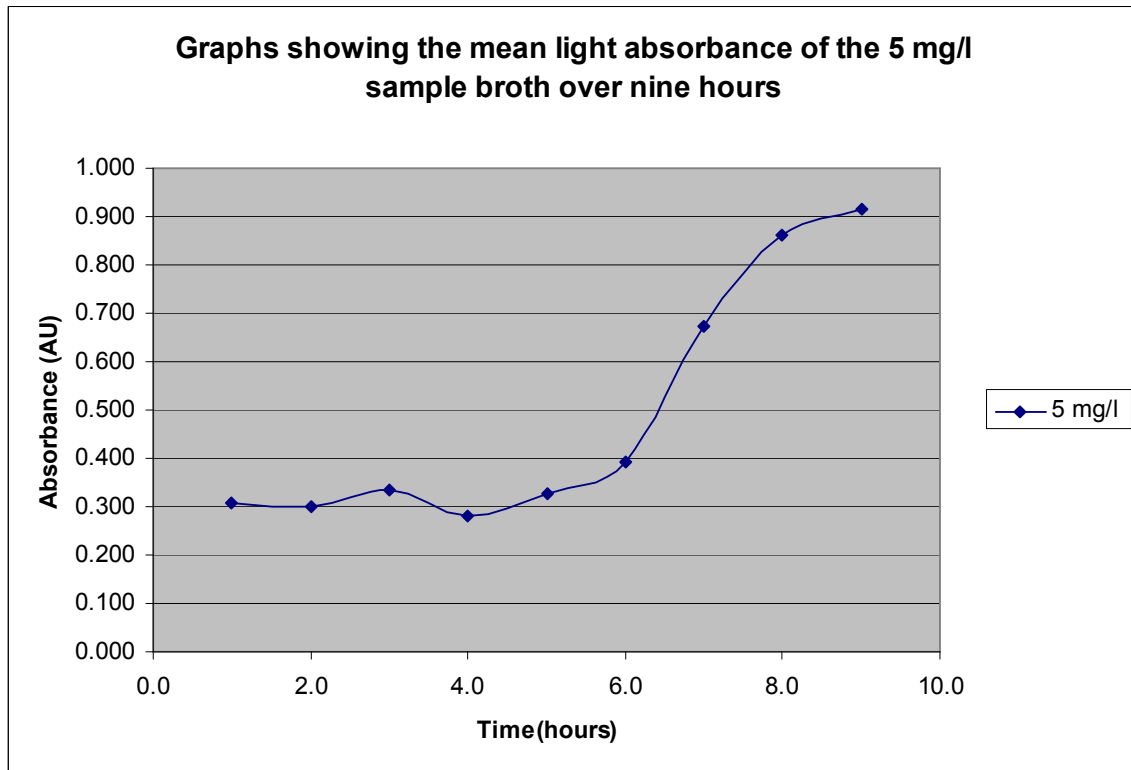
This graph shows the broth in which we added 1.25 mg/L of Vancomycin. This led to a delaying of the log phase of growth until around the fifth hour.

Graphical representation of the light absorbance of the 2.5 mg/L sample broth over a course of nine hours for the three trials



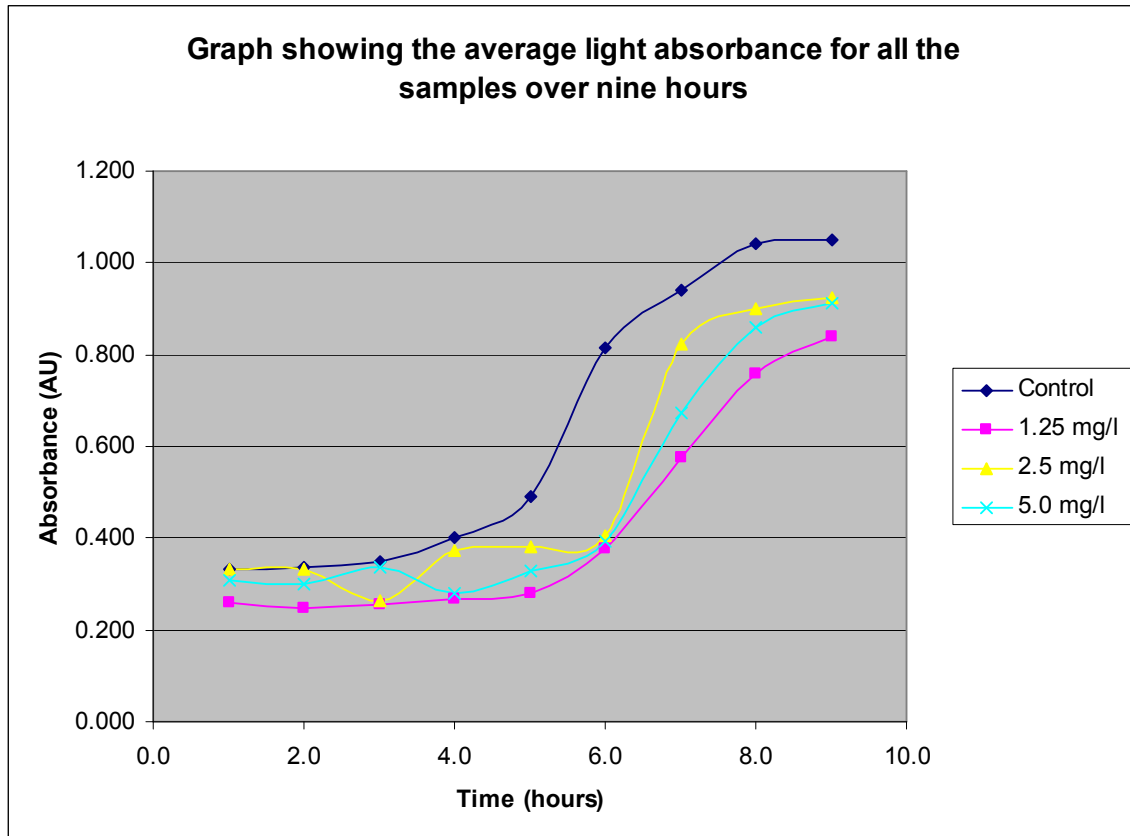
This graph shows the broth in which we added 2.5 mg/L of Vancomycin. This led to a delaying of the log phase of growth until around the seventh hour and it also led to a dip in the population at the third hour.

Graphical representation of the light absorbance of the 5 mg/L sample broth over a course of nine hours for the three trials



This graph shows the broth in which we added 5 mg/L of Vancomycin. This led to a delaying of the log phase of growth until the sixth hour and also a dip in the fourth hour.

Graphical representation of the average light absorbance of all of the sample broths over a course of nine hours



The above graph shows all of the average population growth curves. It allows one to compare between the differing curves in relation to the amount of Vancomycin added.

Graph Analysis:

One notices that *Staphylococcus aureus* follows an s-shaped population growth curve, as evident from the control sample. The culture starts in the lag phase, where growth is limited as the *Staphylococcus aureus* are adjusting to the conditions of the broth and are growing as individual organisms. During this stage the bacteria are synthesising new enzymes to maximise the digestion of the nutrients in the Tryptone broth.¹⁶ Around hour three, the *Staphylococcus aureus* enter the log phase, or exponential growth phase, of population growth curve. Thus this is the correct time to add the Vancomycin inoculations as they inhibit the log phase of growth. This is the time where bacterial growth is proceeding at its maximum rate, close to the ideal logarithmic rate where the growth rate would be a vertical line. This is why it is called the log phase of population growth. During this time the bacteria have fully adapted to the Tryptone broth and are maximizing the rich source of nutrients that it supplies.¹⁷ This causes the absorbance level to dramatically rise as there are more bacteria absorbing the light. The third phase of population growth is the stationary phase, which can be observed in the control graph. As the population of *Staphylococcus aureus* increases there is greater competition for resources and the growth rate slows to almost 0. Here, the mortality rate is usually equal to the natality rate and thus the population does not increase in size.¹⁸ Also, an increase in the amount of toxins that exist in the broth leads to a high mortality rate. The final phase of population growth that only occurs when the population is being cultured in a closed system with no addition of new nutrients is called the phase of decline, or the death phase. During this phase the population declines rapidly as there is a scarcity of nutrients, a high level of toxins in the broth and this leads to the stopping of multiplication of the bacteria. Thus the bacteria die out. One can assume as the growth rate of the *Staphylococcus aureus* has followed all of the previous phases that it will also enter a decline phase after a short stationary period.

The above graphs show that, the results from this laboratory came out almost as expected yet what I did notice was that the broth in which I added 1.25 mgL^{-1} of

¹⁶ Soper, R., ed. Biological Science 1 and 2. New York: Cambridge UP.

¹⁷ "Bacterial growth -." Wikipedia, the free encyclopedia. 19 Jan. 2009
<http://en.wikipedia.org/wiki/Bacterial_growth>.

¹⁸ Soper, R., ed. Biological Science 1 and 2. New York: Cambridge UP.

Vancomycin had a log phase that either started slower than the rest of the samples or had a log phase that did not show the normal level of increase in population size. Comparing between the fifth to seventh hours of the trials, all of the samples except for the 1.25 mgL⁻¹ sample showed rapid population growth. This is contrary to what was hypothesized as I expected the inoculated samples to have a very brief log period before a rapid decline. Also, in the third hour, when inoculation occurred, the 2.50 mgL⁻¹ and 5.00 mgL⁻¹ samples both showed a drop in their population sizes but the 1.25 mgL⁻¹ sample did not. The fact that the 1.25 mgL⁻¹ inoculation had a smaller effect on the population growth curves than the other inoculations was expected. However, the proportion of effect was not as hypothesized. My hypothesis was proven in so far as the 5.00 mgL⁻¹ inoculation inhibited the population growth rate of *Staphylococcus aureus* more than the 2.50 mgL⁻¹ inoculation. Yet it was disproved as the 5.00 mgL⁻¹ inoculation did not inhibit the growth rate twice as much as the 2.50 mgL⁻¹ sample.

Evaluation:

Evaluation:

From the above analysis, I can assume that the Vancomycin inoculation was not mixed properly in the 1.25 mgL^{-1} sample and thus it did not affect the *Staphylococcus aureus* as rapidly as with the 2.50 mgL^{-1} and the 5.00 mgL^{-1} . The 2.50 mgL^{-1} and 5.00 mgL^{-1} showed a larger difference in third and seventh hours of growth than at any other point, with the 2.50 mgL^{-1} dropping below the 5.00 mgL^{-1} broth only at the three hour point. This discrepancy can also be blamed on the way in which the inoculations were mixed as the 5.00 mgL^{-1} sample experienced a population drop in the fourth hour similar to the 2.50 mgL^{-1} in the third hour. I think that the 2.50 mgL^{-1} inoculation was completely used up by the seventh hour and that is why we see that rapid growth pattern at that point. Whereas, the 1.25 mgL^{-1} and 5.00 mgL^{-1} inoculations remained, if only in small amounts, in the broths until the end. Another discrepancy that is noticed is the slight drop that appeared during the second hour of nearly all the samples in the trials. This can perhaps be attributed to the spectrophotometer which required half an hour to properly calibrate and did give some erroneous results in the first attempt at trial one. Also, because there are only three variable with three trials the results of this experiment can never be conclusive as there is always scope for error.

This procedure worked well yet did lead to a lot of results that did not have a large scope for processing and much of the work that I did was purely research. Also, the quantities required to be mixed for the Tryptone broth came dangerously close to the lower limit of the digital weighing scale that I used. This meant that my measurements cannot have been completely accurate leading to some cultures having a larger supply of nutrients than others. This could have affected their growth curves and thus lead to errors in results.

To improve this experiment I think that taking the readings over a longer course, perhaps two days, would allow one to see the full s-shaped growth curve including the decline or death phase which I did not get to see in any of my results. Also, I think that taking different molarities of Vancomycin would allow one to get a more accurate reading of the degree of negative effect a small change in the Vancomycin concentrations gives. Also, this would allow a more accurate reading on the concentrations of vancomycin that is most effective against *Staphylococcus aureus*.

Conclusions:

Conclusion:

The aim of this paper was to examine, through the use of spectrophotometric absorbance readings, the detrimental effects that 5.00 mgL⁻¹, 2.50 mgL⁻¹, and 1.25 mgL⁻¹ of the commonly used glycopeptide antibiotic, Vancomycin, had on the growth curves of the gram-positive bacterium *Staphylococcus aureus*. My logical conclusion after the experiments and the processing of the data is that 1.25 mgL⁻¹ of Vancomycin is the most effective growth inhibitor to *Staphylococcus aureus* as it delays the log phase thereby reducing rapid population growth. This does not support my hypothesis yet as all three of my trials have confirmed it, I must conclude that 1.25 mgL⁻¹ is more effective than both 5.00 mgL⁻¹ and 2.50 mgL⁻¹ vancomycin inoculations. This conclusion leads to a series of questions that must be answered.

One of the main unresolved questions that emerges from this experiment is the question of why 1.25 mg/L of Vancomycin inhibited the growth of the *Staphylococcus aureus* population more than the higher doses. Usually it could be put down to mistakes made in the administering of the inoculations yet the result was consistent throughout the three trials, and lends itself to the idea that there is an underlying answer to the question. A question that comes out of the previous question is what the most effective dose of Vancomycin for humans is, i.e. whether a lower dose could be more effective. A final question that must be addressed is whether or not *Staphylococcus aureus* grows in a similar way in the human body as it does in the Tryptone broth that I used as this would affect the effective dose of vancomycin needed.

Although the dosages that I took were far from the common human dose of 500 mg over six hours¹⁹, the results of this experiment showed that even in these small quantities Vancomycin can still be effective. From the results that I obtained I see two main areas where further research may benefit science. The first area is the idea of Vancomycin still having detrimental effects on *Staphylococcus aureus*' population

¹⁹ "Vancomycin Injection (Vancomycin Injection) Drug Information: Uses, Side Effects, Drug Interactions and Warnings at RxList." 27 Jan. 2009 <<http://www.rxlist.com/vancomycin-injection-drug.htm>>.

growth curve yet perhaps not to the degree that it had ten years ago. This increase in resistance could lead to the elimination of Vancomycin as an effective drug against *Staphylococcus aureus* infections. Thus we must research the degree to which *Staphylococcus aureus* is becoming resistant to Vancomycin. Secondly, we need to research whether or not Vancomycin's structure can be modified chemically so as to increase its effectiveness against *Staphylococcus aureus*. The main implication of my work is that the dosage of Vancomycin that we are commonly using in hospitals may not be the most effective dosage that can be given. As I saw, 1.25 mgL^{-1} of Vancomycin was the most effective dose while 2.50 mgL^{-1} and 5.00 mgL^{-1} were less effective. If this result is corroborated by other researchers then we must look at how *Staphylococcus aureus* is treated with Vancomycin in modern medical practice.

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Appendices:

Appendix A

Trial one, Trial Two and Trial three

Raw Data and Graphs showing all three trials

Trial One

Table showing Light absorbance for all the samples over nine hours

Hour	Control	0 mg/L	1.25 mg/L	2.5 mg/L	5 mg/L
1.000	0.317	0.356	0.245	0.337	0.303
2.000	0.316	0.360	0.244	0.333	0.299
3.000	0.327	0.351	0.244	0.246	0.310
4.000	0.400	0.398	0.257	0.359	0.280
5.000	0.561	0.421	0.286	0.364	0.330
6.000	0.908	0.710	0.353	0.406	0.385
7.000	0.987	0.890	0.562	0.789	0.652
8.000	1.042	1.030	0.751	0.900	0.857
9.000	1.056	1.047	0.807	0.948	0.917

Trial two

Table showing Light absorbance for all the samples over nine hours

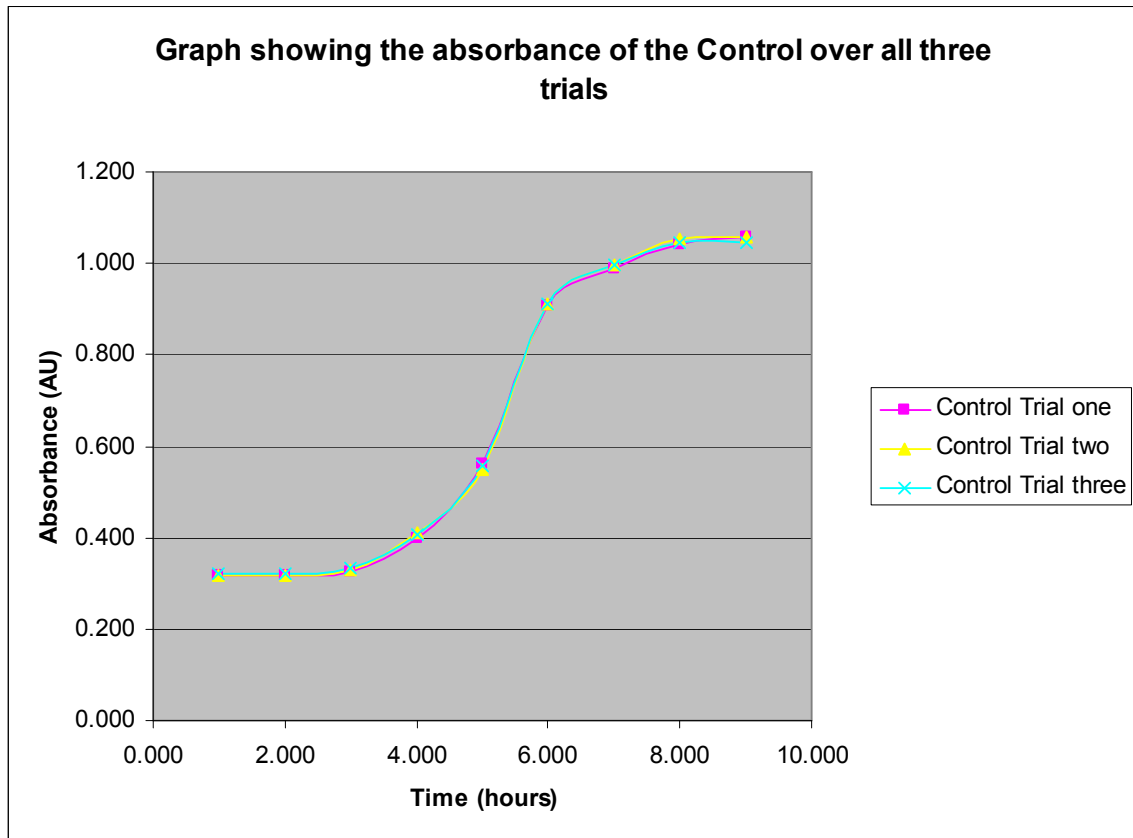
Hour	Control	0 mg/L	1.25 mg/L	2.5 mg/L	5 mg/L
1	0.317	0.356	0.276	0.330	0.310
2	0.316	0.360	0.244	0.333	0.300
3	0.330	0.362	0.257	0.267	0.308
4	0.412	0.402	0.267	0.378	0.279
5	0.551	0.431	0.278	0.389	0.326
6	0.910	0.723	0.374	0.406	0.398
7	0.996	0.891	0.582	0.890	0.684
8	1.053	1.034	0.761	0.905	0.857
9	1.056	1.057	0.845	0.923	0.924

Trial Three

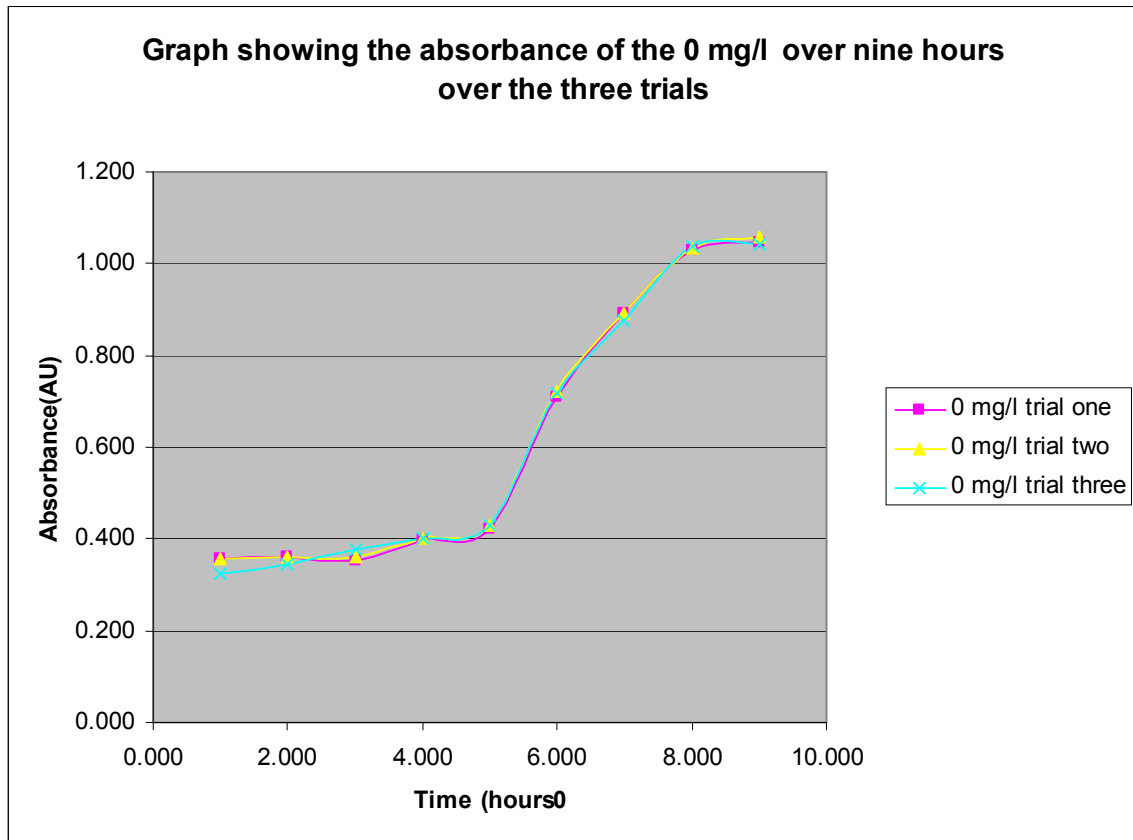
Table showing Light absorbance for all the samples over nine hours

Hour	Control	0 mg/L	1.25 mg/L	2.5 mg/L	5 mg/L
1	0.320	0.324	0.254	0.332	0.310
2	0.321	0.345	0.256	0.334	0.300
3	0.334	0.376	0.265	0.280	0.390
4	0.407	0.401	0.276	0.376	0.280
5	0.556	0.429	0.279	0.390	0.330
6	0.913	0.718	0.400	0.410	0.397
7	0.998	0.876	0.582	0.790	0.680
8	1.045	1.039	0.763	0.890	0.870
9	1.045	1.043	0.867	0.900	0.900

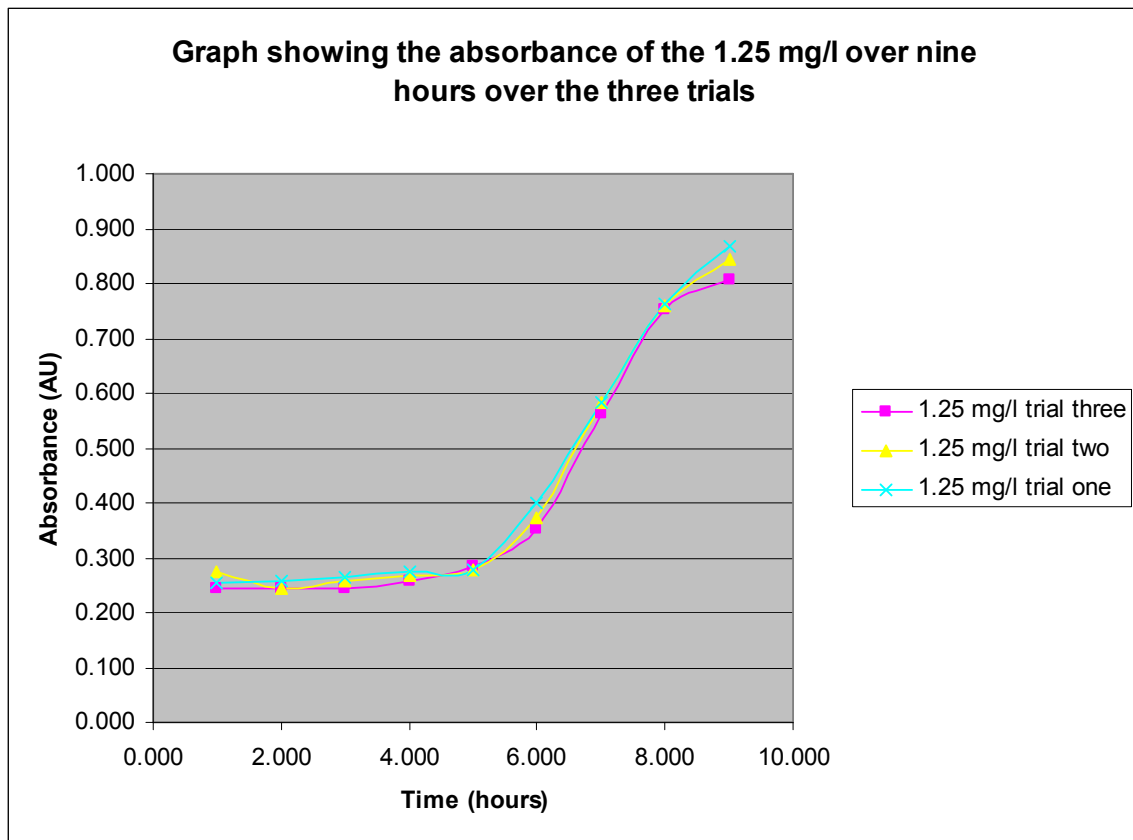
Graph showing the absorbance of the Control over nine hours over the three trials



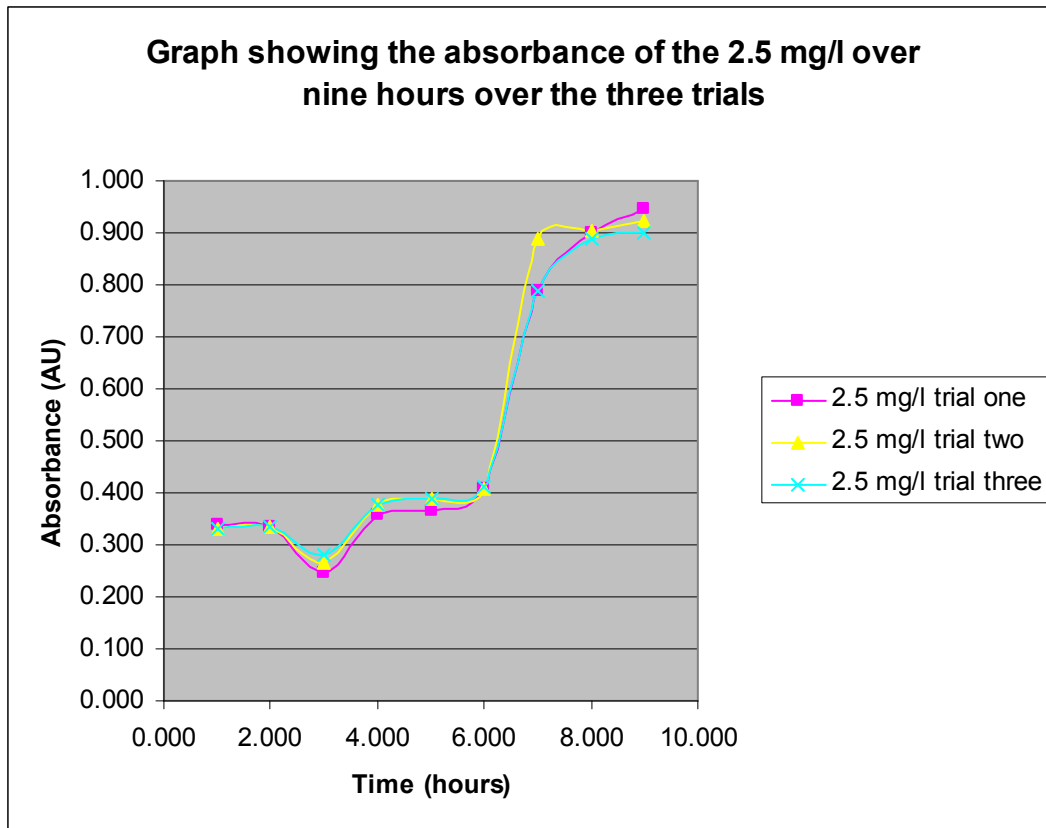
Graph showing the absorbance of the 0 mg/L over nine hours over the three trials



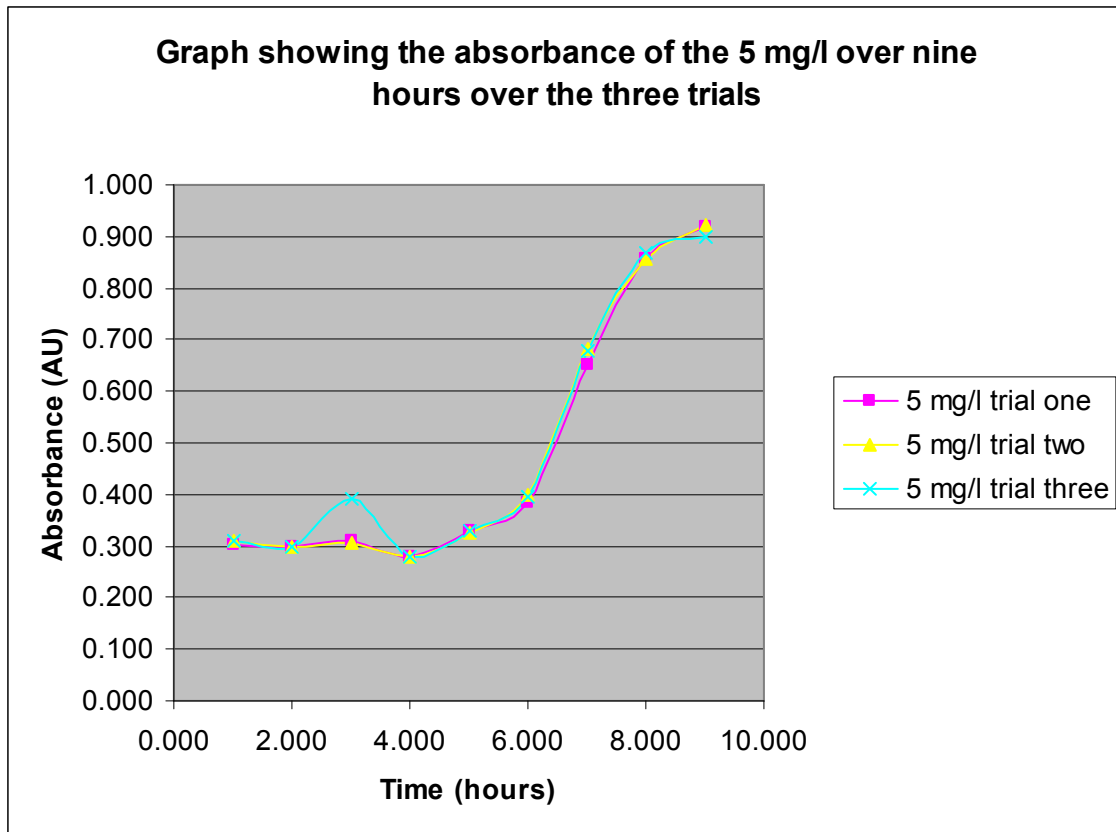
Graph showing the absorbance of the 1.25 mg/L over nine hours over the three trials



Graph showing the absorbance of the 2.5 mg/L over nine hours over the three trials



Graph showing the absorbance of the 5 mg/L over nine hours over the three trials



Appendix B

Information on the Nephrotoxic and Ototoxic effects of Vancomycin and also Red Man's Syndrome and Other Side-effects of Vancomycin

Vancomycin has generally been considered to be both Nephrotoxic (a poisonous effect of some substances, both toxic chemicals and medication, on the kidney²⁰) and Ototoxic (producing, involving, or being adverse effects on organs or nerves involved in hearing or balance²¹). Yet recent studies have shown that Vancomycin has a renal dysfunction rate of around 5-7 % with only three cases being reported out of eighty-two in a 1994 study.²² This is a similar rate to that of many antibiotics that are considered to be non-nephrotoxic such as cefamandole (a semisynthetic second-generation cephalosporin antibiotic; used primarily as *c. nafate*, the sodium salt of the cefamandole formyl ester.)²³ and benzylpenicillin (also known as penicillin G). Vancomycin, when given orally, has a high excretion rate with more than 90 % being excreted through urine in the first twenty-four hours²⁴ and this high renal excretion rate has been known to cause renal dysfunction which may have been the reason for Vancomycin's classification as a nephrotoxic drug. The main reason that Vancomycin is commonly given intravenously as opposed to orally is that it is a large hydrophilic molecule and thus does not pass easily through the gastrointestinal mucosa. The only viable time when Vancomycin is administered orally is in the treatment of colonic infections such as pseudomembranous colitis, caused by the bacterium *Clostridium difficile*²⁵. This is because the infection is located in the colon and thus the hydrophilic Vancomycin molecules can reach it without

²⁰ Taken from "Nephrotoxicity." [Wikipedia, the free encyclopedia](http://en.wikipedia.org/wiki/Nephrotoxicity). 07 Jan. 2009
<http://en.wikipedia.org/wiki/Nephrotoxicity>.

²¹ Taken from "Ototoxic." [Webster's Medical Desk Dictionary](#). 1986. 503.

²² Statistics taken from Cantu TG, Yamanaka-Yuen NA, Lietman PS. Serum vancomycin concentrations: reappraisal of their clinical value. *Clin Infect Dis* 1994;19(6):1180-2.

²³ Taken from "Cefamandole - definition of cefamandole in the Medical dictionary - by the Free Online , Thesaurus and Encyclopedia." [Medical Dictionary](http://medical-dictionary.thefreedictionary.com/cefamandole). 08 Jan. 2009 <<http://medical-dictionary.thefreedictionary.com/cefamandole>>.

²⁴ "Glycopeptide antibiotic." [Wikipedia, the free encyclopedia](http://en.wikipedia.org/wiki/Glycopeptide_antibiotics). 09 Jan. 2009
<http://en.wikipedia.org/wiki/Glycopeptide_antibiotics>.

²⁵ "Pseudomembranous colitis: Treatments and drugs." [Mayo Clinic medical information and tools for healthy living - MayoClinic.com](http://www.mayoclinic.com/health/pseudomembranous-colitis/DS00797/DSECTION=treatments-and-drugs). 09 Jan. 2009 <<http://www.mayoclinic.com/health/pseudomembranous-colitis/DS00797/DSECTION=treatments-and-drugs>>.

being absorbed earlier in the digestive tract. Also, Vancomycin has been proved to have a faster reaction rate than the intravenous drug Metronidazole.

Signs of Red Man's syndrome usually occur within the first four to ten minutes of an infusion yet there have been cases of delayed reactions around the end of a ninety or one hundred and twenty minute infusion.²⁶ Until recently it was believed that Red Man's syndrome was caused by some impurities in the Vancomycin infusions but recently it has been shown that a certain proportion of the population may release large amounts of histamine when Vancomycin is added to the system.²⁷ These hypersensitivity reactions are believed to be caused by Vancomycin's effect on mast cells, specifically on peritoneal mast cells where Vancomycin has been shown to cause degranulation in rats.²⁸ Red Man's syndrome can present with an erythematous rash (a severe redness of the skin or mucous membrane associated with some local inflammation.²⁹) on the upper torso and neck. In some rare cases it has been shown to cause hypotension (abnormally low pressure of the blood³⁰) and an angioedema (an allergic skin disease characterized by patches of circumscribed swelling involving the skin and its subcutaneous layers, mucous membranes, and sometimes the viscera.)³¹. Red Man's Syndrome is usually treated with antihistamines as they counteract the effects of the histamine release by the body. Vancomycin has also been shown in rare cases to cause thrombocytopenia (a common bleeding disorder, resulting from a shortage of platelets in the blood³²) and bleeding, it can also cause florid petechial hemorrhages (a tiny pinpoint red mark that is an important sign of asphyxia caused by some external means of obstructing the airways.³³), ecchymoses (the escape of blood back into the tissue from ruptured blood

²⁶ "Red man syndrome." PubMed Central Homepage. 09 Jan. 2009
<<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=270616>>.

²⁷ "Red man syndrome." PubMed Central Homepage. 09 Jan. 2009
<<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=270616>>.

²⁸ "Vancomycin-induced release of histamine from rat p...[Agents Actions. 1991] - PubMed Result." NCBI Home Page. 09 Jan. 2009 <<http://www.ncbi.nlm.nih.gov/pubmed/1713735>>.

²⁹ Taken from "Erythema." The World Book - Medical Encyclopedia. 1994. 318.

³⁰ Taken from "Hypotension" Webster's Medical Desk Dictionary. 1986. 320.

³¹ Taken from "Angioedema" Webster's Medical Desk Dictionary. 1986. 33.

³² Taken from Larson, David E., ed. Mayo Clinic Family Health Book : The Ultimate Home Medical Reference. New York: HarperCollins, 1990.

³³ "Petechial Hemorrhage: World of Forensic Science." 10 Jan. 2009 <<http://www.enotes.com/forensic-science/petechial-hemorrhage>>.

vessels marked by a livid black-and-blue or purple spot or area³⁴), and wet purpura(any of several hemorrhagic states characterized by patches of purplish discolouration resulting from extravasation of blood into the skin and mucous membranes³⁵).³⁶

³⁴ Taken from "Ecchymosis" Webster's Medical Desk Dictionary. 1986. 198

³⁵ Taken from "Purpura" Webster's Medical Desk Dictionary. 1986. 593.

³⁶ "Vancomycin-induced immune thrombocytopenia. [N Engl J Med. 2007] - PubMed Result." NCBI HomePage. 10 Jan. 2009 <<http://www.ncbi.nlm.nih.gov/pubmed/17329697>>.

Appendix C:

Staphylococcus aureus from other bacteria

As *Staphylococcus aureus* has the ability to produce the enzyme catalase, a catalase – test is commonly used to separate Staphylococci from Enterococci and Streptococci as both of these genres are catalase-negative.^{37, 38} A coagulase-test is also used to distinguish between *Staphylococcus aureus* and its common counterpart *Staphylococcus epidermidis* as the later is coagulase-negative and may even have a protective role in humans³⁹.

Staphylococcus aureus skin infections can cause cellulitis (a spreading bacterial infection just below the skin surface. It is most commonly caused by *Streptococcus pyogenes* or *Staphylococcus aureus*⁴⁰) or impetigo (A bacterial skin infection caused by the *staphylococcus* or, more rarely, streptococcus bacteria. The first sign of impetigo is a patch of red, itchy skin. Pustules develop on this area, soon forming crusty, yellow-brown sores that can spread to cover entire areas of the face, arms, and other body parts⁴¹.) In some cases, scalded skin syndrome (A potentially serious side effect of infection with the Staph (Staphylococcus) bacteria that produces a specific protein which loosens the "cement" holding the various layers of the skin together. This allows blister formation and sloughing of the top layer of skin)⁴² can develop. Staphylococcal pneumonia is usually only found in people who have a previous or underlying lung problem, such as lung cancer or asthma. Staphylococcus is also known to cause endocarditis and osteomyelitis. Staphylococcal food poisoning can cause nausea, vomiting, diarrhea, and

³⁷ "Streptococci That Kill White Blood Cells." Home of CELLS alive! 13 Jan. 2009 <<http://www.cellsalive.com/strep.htm>>.

³⁸ "Enterococcal Infections: Overview - eMedicine." EMedicine The Continually Updated Clinical Reference. 13 Jan. 2009 <<http://emedicine.medscape.com/article/216993-overview>>.

³⁹ "Staphylococcus aureus." Online Textbook of Bacteriology. 13 Jan. 2009 <<http://www.textbookofbacteriology.net/staph.html>>.

⁴⁰ "Cellulitis: Definition from Answers.com." Answers.com - Online Dictionary, Encyclopedia and much more. 24 Jan. 2009 <<http://www.answers.com/topic/cellulitis>>.

⁴¹ "Impetigo definition - Medical Dictionary definitions of popular medical terms easily defined on MedTerms." 24 Jan. 2009 <<http://www.medterms.com/script/main/art.asp?articlekey=3948>>.

⁴² "Scalded skin syndrome definition - Medical Dictionary definitions of popular medical terms easily defined on MedTerms." 24 Jan. 2009 <<http://www.medterms.com/script/main/art.asp?articlekey=5412>>.

dehydration. It is caused by eating foods contaminated with toxins produced by *Staphylococcus aureus*.