A Thesis Entitled

Physical and Chemical Modifications of Free Radical Scavengers to Reduce their Radioprotective Potentials for Bacterial Agents

by

Mark D. Shuster

Submitted as partial fulfillment of the requirements for

The Master of Science degree in Bioengineering

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Adviser: Ozan Akkus, PhD

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Graduate School

The University of Toledo

December 2005

The University of Toledo

College of Engineering

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY *Mark D. Shuster*

ENTITLED *Physical and Chemical Modifications of Free Radical Scavengers to Reduce their Radioprotective Potentials for Bacterial Agents*

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Bioengineering

Thesis Advisor: *Advisor's Name*

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Committee member 2

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An Abstract of

Physical and Chemical Modifications of Free Radical Scavengers to Reduce their Radioprotective Potentials for Bacterial Agents

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Annually, an estimated 1.2 million allografts are transplanted in the United States for repair or reconstruction of skeletal defects caused by disease, illness, or injury. Sterilization of these allografts must be performed to prevent disease transmission and reduce the inherent risk of infection. Currently, there is *no single* accepted sterilization technique in the bone and tissue banking industry. Gamma irradiation is the most popular and the safest form of allograft sterilization. However, to attain that level of sterility assurance, the biochemical and biomechanical integrity of the allograft is

compromised, which is a serious concern since bone allografts are used in load bearing applications.

Damage to allografts results in the radiolysis of water molecules during gamma irradiation. The water molecules bound to the tissue are essentially split into highly reactive, damaging free radical molecules. These free radicals cleave the collagen molecules in bone allograft tissues. One method to control the formation of these free radicals is to add a free radical scavenger to the bone allograft before gamma irradiation sterilization. However, while the free radical scavenger is protecting the collagen, is there the unintended consequence that the free radical scavenger is also protecting the pathogenetic organisms that should be eradicated? It was hypothesized that small, positively charged, globularly shaped free radical scavengers will protect bacteria more efficiently because the scavenger will be able to penetrate the intracellular space of the cell and thus scavenger for the free radicals that should be killing the bacteria.

To test this hypothesis, viability tests were preformed with *E. coli*. Free radical scavengers were selected based on their charge, size, and shape. Solutions of these scavengers were added to *E. coli* suspended in media and incubated at time points of 0, 10, 20, and 40 hours and then subsequently irradiated to a dose of 500Gy. Results showed that positively charged scavengers protected *E. coli* from the harmful effects of irradiation, $p<0.05$. Results also indicated that a globular shaped scavenger protects *E. coli*, $p<0.05$. Additionally, a medium sized molecular weight molecule protected the *E. coli*, however, it may be possible that this protection was based more on chemical specificity than actual size of the molecule.

The global conclusion of this study is: the addition of a scavenger has proven to alleviate biochemical and biomechanical stress to a bone allograft. However, selection of the proper scavenger is essential. From the results of this study, it would be advantageous to select a scavenger with a molecular weight greater than 250Da, but smaller than 350Da to penetrate the fabric of bone, additionally, to select a scavenger that is linear in shape and has an overall net positive charge. Allograft tissues gamma irradiated in the presence of such a scavenger could be treated with excess doses of irradiation for an elevated level of sterility assurance without worry of biochemical and biomechanical degradation.

Acknowledgements

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Chapter One

Introduction

An allograft is human tissue which is removed from a cadaver and implanted into another person. In 2003, an estimated 1.2 million bone allograft procedures were performed in the United States, up from an estimated 800,000 procedures in 2000 [1]. Bone allograft transplants are preformed for repair or reconstruction of skeletal defects caused by disease, illness, or injury. Bone allograft popularity has been increasing because of their availability and biocompatibility. However, the risk of infection and disease transmission is an eminent risk; therefore terminal sterilization must be performed.

As with any surgical procedure, the risk of disease transmission and infection are inescapable. Traditionally, bone allograft procedures have not carried any additional trepidation due to detailed patient and serological screening. However, a few recent isolated cases have spawned a new look into the safety of allograft processing and handling. As of March 11, 2002, the Center for Disease Control (CDC) has received 26 reports of bacterial infections associated with musculoskeletal tissue allografts [2]. Thirteen of the 26 patients were infected with a *Clostridium* strain bacterium [2]. In 11 of the 13 cases, additional evidence implicated the allograft itself as the source of the infection [2]. In November 2001, one fatal case came out of Minnesota where a 23-year-old man underwent reconstructive knee surgery using a fresh femoral condyle allograft. A few days after surgery, the patient developed pain in the knee that rapidly progressed to shock; the patient died the following day [3]. A near-fatal case came out of Illinois, where a 17-year-old man underwent reconstructive knee surgery receiving a meniscus and fresh femoral condyle. The next day, the patient developed a fever which did not respond to the first-round of antibiotics. Eight days after surgery, he was admitted to the hospital with a fever of 103.5ºF. The patient received strong antibiotics and the fever subsided; the patient is recovering [2]. In each case, all allografts were processed aseptically but did not undergo terminal sterilization.

Bone Allograft Sterilization Overview

There is no current single standard bone allograft tissue processing technique used by tissue banks. Many techniques have been developed and are used by various allograft tissue processing centers around the country. All methods performed must be prepared, validated and in written protocol form to comply with regulations designed to prevent infections disease transmission or cross-contamination during tissue processing [4]. A few of the most popular sterilization techniques are outlined in Table 1.01.

Table 1.01 Comparison of current processing methods for allograft tissue sterilization [4].

	Aseptic Processing	**Ethylene Oxide**	**Gamma Irradiation**	**Chemical Treatment**
Kills Bacteria	No	√	√	√
Kills Fungi	No	√	√	√
Kills Spores	No	√	√	√
Kills Enveloped / Non-enveloped Viruses	No	No	Dose Dependent	No
Removes Blood and Lipids	Surface Only	No	No	Surface Only
Preserves Strength	√	√	Decreases – Dose Dependent	√
Preserves Biocompatibility	√	dose dependent	√	√
Penetrates into Tissue	Surface only	Thickness dependent	Full penetration	Surface Only

A handful companies have been started on the technology of chemical treatment sterilization. Regeneration Technologies, Inc. of Alachua, Florida has developed the patented BioCleanse^{TM} tissue sterilization process that operates on the basis of chemical sterilants and pressure/vacuum treatments to remove blood, lipids, and marrow [5]. NovaSterilis of Lansing, New York has developed the Nova2200 sterilization chamber that operates using supercritical carbon dioxide [6]. Clearant, Inc. of Los Angeles, California has developed the patented Clearant Process® that utilizes a combination of gamma-irradiation and chemical treatments to attain sterile assurance while maintaining mechanical integrity [7]. Each of these companies is in their infancy and their technologies safety and reliance has a limited track record.

Ethylene oxide and gamma irradiation are the two classic sterilization methods. Both are proven methods of eliminating bacteria, viruses, and spores. However, ethylene oxide gas deposits residuals that cannot be fully evacuated from the tissues. These residuals cause an inflammatory response to host tissue making this method unsuitable for allograft tissues [8]. Gamma irradiation is the prime choice because of its well-established sterility assurance and it is the only method that penetrates the full thickness of an allograft. However, gamma irradiation will degrade the mechanical properties of bone allograft tissues in a dose dependent manner [9, 10]. Degradation of bone allograft tissues is a serious concern since bone allografts are used in load bearing applications [11].

Sterilization of Bone Allografts by Gamma Irradiation

Gamma irradiation offers superior sterility assurance over any of the competing techniques. For that purpose, many groups have been focused on developing techniques to alleviate the biomechanical damage to bone allograft tissue caused by gamma irradiation sterilization. There is a way to harness the destructive powers of gamma irradiation while maintaining sterility assurance. First, an investigation to how gamma irradiation causes damage to allograft tissues is required.

Gamma irradiation is a highly energetic wave (Figure 1.01) of photons produced from the radioactive decay of cesium-137, cobalt-60, technetium-99m, or americium-241 [12, 13]. Gamma photons are the most energetic photons in the electromagnetic spectrum, traveling at the speed of light; yet, have no mass and no electrical charge [13]. During radioactive decay, a neutron transforms to a proton and a beta particle. The nucleus ejects the beta particle; however, the nucleus still has too much energy and ejects the photon (gamma radiation) to become more stable [13].

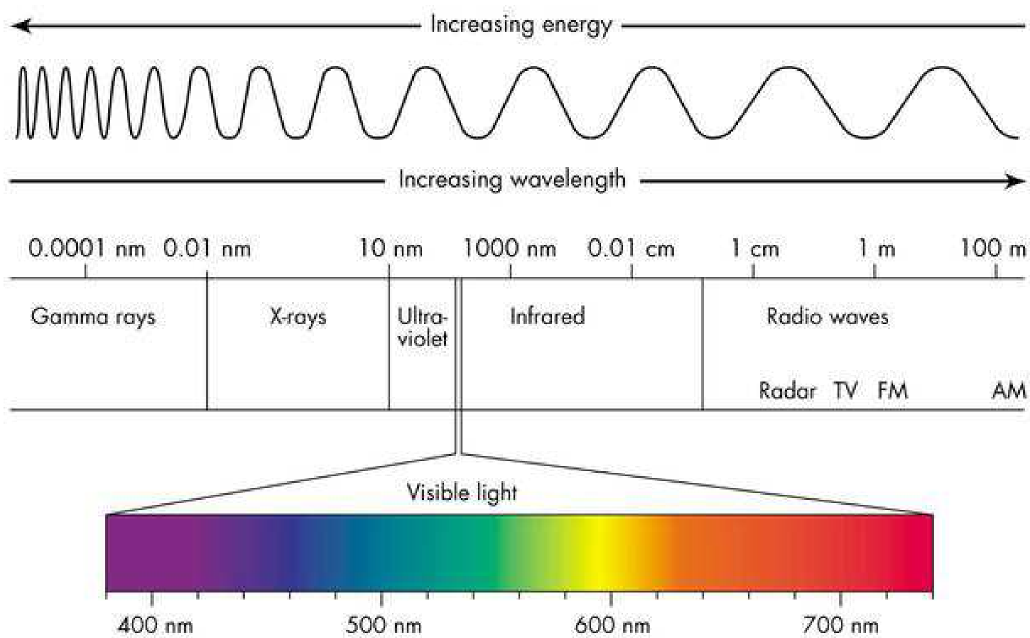


Figure 1.01 Electromagnetic wave spectrum [14].

Sterilization with gamma irradiation occurs when a pathogenetic organism is exposed to gamma rays. Bacteria can be rendered nonviable at a gamma radiation dose of approximately 1.5 – 4.5kGy, bacterial spores require approximately 10 – 45kGy, and viruses require a dose greater than 30kGy [15, 16]. The gamma rays destroy crucial intracellular structures at the molecular level, specifically causing irreversible damage to DNA, rendering the pathogen nonviable. This method works fine for sterilizing metallic surgical equipment, the gamma rays pass directly through the equipment without impairing the performance of the tool. However, when a bone allograft is exposed to gamma irradiation, the gamma photons penetrate the tissue and cause molecular damage compromising the biomechanical integrity of the tissue. The damage induced (by gamma irradiation) to bone allograft tissue occurs because of the inherent tightly bound water within the tissue. When the energy of the gamma photons act upon the water molecules the water molecules becomes ionized; essentially an electron is removed and what is left is an extremely reactive hydroxyl free radical with a lifetime on the order of 10^{-9} to 10^{-11} seconds [15, 17]. The oxidation effects of the ionized water molecules (free radicals) with collagen cause the depolymerization of the triple helix and the impaired ability of the triple helices to assemble into fibrils [15, 18].

This inability of the collagen molecules to reassemble into their native triple helix form after sustaining damage is what weakens the overall biomechanical integrity of the bone allograft. It has been shown that post-yield (plastic deformation) properties are impaired by sterilization while elastic modulus is largely unaffected[15, 19, 20]. Burstein et al. has shown that the elastic deformation of bone is governed by the mineral, while the plastic deformation of bone is governed by the elastic behavior of the collagen matrix [15, 21]. Therefore, it is understood that gamma irradiation impairs the overall integrity of the allograft tissue by deterioration of the collagen structure via a free radical attack [15]. For the purposes of this study, any mention of a free radical will be assumed to be the hydroxyl free radical unless otherwise noted.

One attempt to control the formation of free radicals from water molecules is to simply remove the water. However, there is no technique that can remove all traces of molecular water from allograft tissues. Several studies have been conducted that included lyophilizing the allograft tissue and then irradiating the tissue while it is frozen [22, 23]. The idea being that most of the water was removed by lyophilization and the remaining water, which is frozen, is immobilized from forming free radicals. The results of these attempts showed that mechanical burden was somewhat lessened, but not to an acceptable level [23].

Free Radical Scavenger

Another method to protect the collagen of allograft tissues from the free radical attack of ionized water would be to capture the free radicals before they have a chance to react with the collagen (Figure 1.02). These agents that are capable of capturing free radicals are known as free radical scavengers (FRS). In biological terms, any agent that is capable of scavenging for free radicals is known as an antioxidant. The human body naturally creates antioxidants to combat the oxidative stress created by the natural processes of cellular metabolism.

Work by Belaney et al. [15] has shown that cortical bone irradiated in the presence of the free radical scavenger thiourea has superior biochemical and biomechanical properties than cortical bone irradiated without the presence of such a scavenger. However, recent work has shown that thiourea is a possible carcinogen, which would not make it an ideal candidate. Therefore, it is necessary to identify potential biocompatible free radical scavengers; ideally those that are naturally occurring in the body or dietary intake.

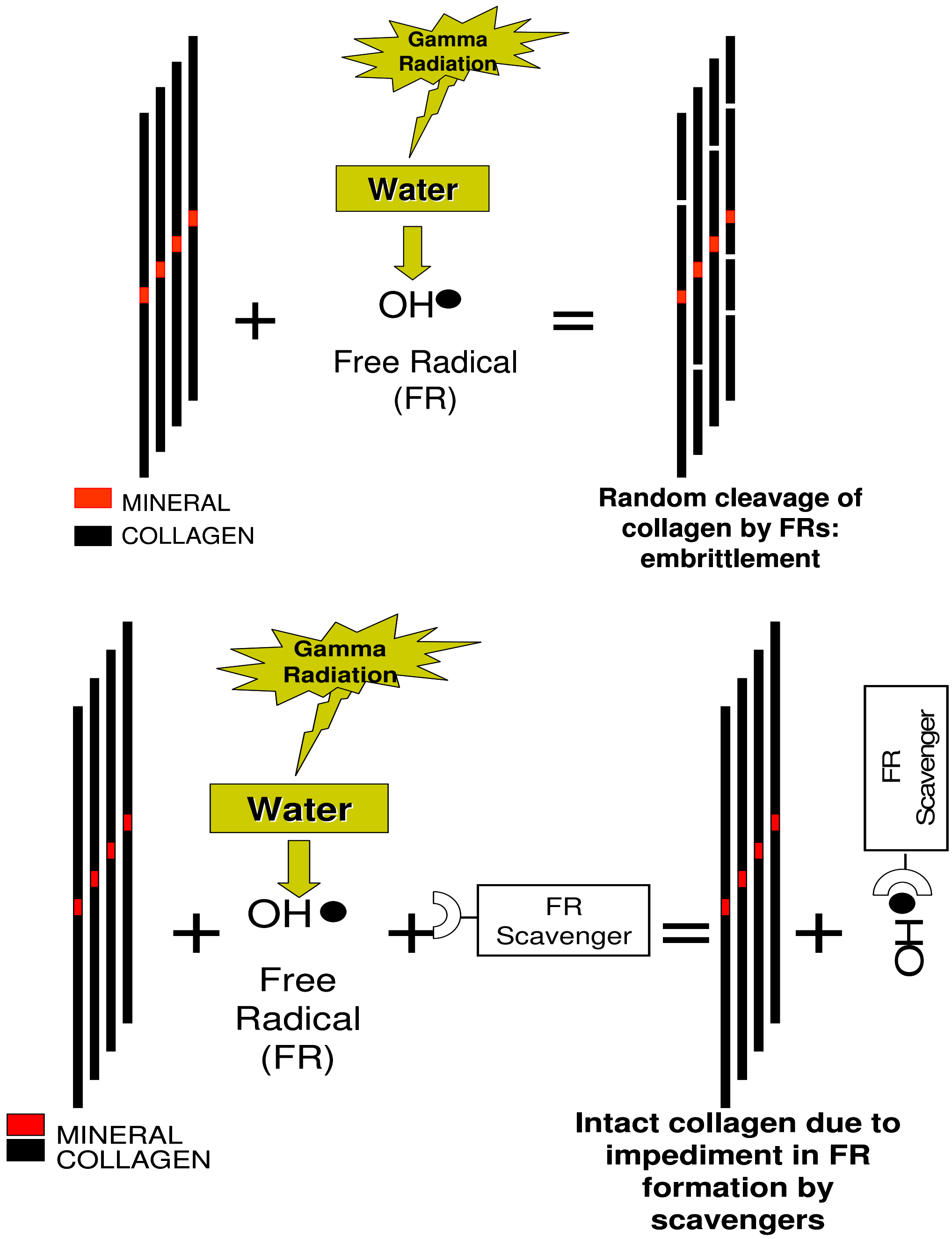


Figure 1.02 Free radical scavenger proposed mechanism [15].

One potential advantage of the free radical scavenger would be that bone allograft tissues could be irradiated at a much greater dose for greater sterility assurance. Since the collagen composition of the bone would be protected by the FRS, there is the potential to apply a dose of 50kGy or greater, which would safely deactivate the most radio-resistant spores and viruses.

Problem Statement and Hypotheses

In addition to the FRS thiourea being a potential carcinogen, previous work has not addressed another very serious issue. Since the free radical scavenger is protecting the collagen molecules from free radical attack, is there the unintended consequence that the free radical scavenger is protecting the pathogenetic organisms that should be eradicated?

To investigate this problem, bacterial cultures will be treated with various free radical scavengers and subsequently gamma irradiated. The free radical scavengers will be analyzed based on their charge, size, and shape. Viability will be assessed to determine the bacteria log-reduction. It is hypothesized that free radical scavengers that can penetrate the cell membrane will protect the bacteria from gamma irradiation, resulting in a low log-reduction. Scavengers that are positively charged will be attracted to the negatively charged bacteria and have an easier chance of penetrating the bacteria and protecting it from gamma irradiation. Further, scavengers that are small in size will penetrate bacteria easier than large sized scavengers. Scavengers that have an overall globular shape will penetrate bacteria easier than scavengers that are linear or planar.

Chapter Two

Experimental Methods and Materials

The purpose of this study was to identify biocompatible free radical scavengers. Additionally, to determine if the selected free radical scavengers protect microbial organisms when exposed to gamma irradiation.

It is widely known that DNA is an easy target for radical attack because the nucleic bases that compose DNA are excellent antioxidants (free radical scavengers), Table 2.01. Thus, it can be assumed that DNA can be more easily damaged than collagen under free radical attack. Therefore, to allow the desired damage to bacterial DNA, the selected free radical scavenger should not penetrate the cellular membrane of a bacterium and enter the intracellular space to protect that DNA.

Table 2.01 Typical second-order rate constants for reactions of free radicals scavengers with the hydroxyl radical [24].

Compound	**Abbreviation**	**Rate Constant ($M^{-1}s^{-1}$)**
Ascorbic Acid	AA	7.2×10^{9}
L-Cysteine	LC	7.9×10^{9}
D-Cysteine	DC	7.9×10^{9}
N-Acetyl-L-Cysteine	NAC	7.9×10^{9}
L-Cysteine Ethyl Ester	LCEE	7.9×10^{9}
L-Cysteine Methyl Ester	LCME	7.9×10^{9}
Uracil	U	3.1×10^{9}
Epigallocatechin Gallate	EGCG	4.1×10^{9}
Trehalose	T	2.7×10^{9}
Glutathione	G	8.8×10^{9}
Thiourea	Th	4.7×10^{9}
Tryptophan	Trp	8.5×10^{9}
Trp-Trp-Trp	TTT	$>8.5 \times 10^{9}$
Adenine		3.0×10^{9}
Cytosine		2.9×10^{9}
Guanine		1.0×10^{10}
Thymine		3.1×10^{9}

It is known that scavengers with comparable affinities for the hydroxyl free radical will exhibit differing radioprotective effects. For example, glycerol and cysteamine are both potent free radical scavengers; however, glycerol can provide protection to *Salmonella* whereas cysteamine cannot [25-27]. This difference is the important implication that the permeation of a scavenger through the bacterial wall differs. Only those which can permeate the wall will be able to elicit protection to pathogens.

The permeability of a scavenger is a consequence of the diffusibility (kinetics) and solubility (thermodynamics). Diffusibility and solubility depend on the physical and chemical characteristics of the scavenger. Three of those characteristics were considered for this study: 1) molecular charge, 2) molecular size, and 3) relative molecular shape.

Selection of Free Radical Scavengers

The amino acid cysteine has no toxic effects as part of a daily diet, and it becomes toxic only after it is administered on a daily basis at high concentrations [28-30]. Cysteine was also selected because the **charge** structure can be easily modified by blocking or adding charged substituent groups. Therefore, cysteine was chosen to analyze how the charge of a scavenger affects penetration into a bacterial pathogen. The most common form of cysteine is L-cysteine (LC) and is a zwitterionic molecule. For the positive and negatively charged form of cysteine, N-acetyl-L-cysteine (NAC) and L-cysteine-ethyl-ester (LCEE) were chosen, respectively. All three, LC, NAC, and LCEE are approximately the same size (~150Da) and all are the same linear shape with a cysteine backbone.

For the **size analysis**, a small, medium, and large molecular weight compound was selected. The small size molecule was Uracil (U), 112Da. Uracil is a ribonucleic base found in RNA. The medium size molecule was ascorbic acid (AA), 176Da. Ascorbic acid is more commonly known as vitamin C, and its antioxidant effects are well studied. The large size molecule was epigallocatechin gallate (EGCG), 458Da. Epigallocatechin gallate is the naturally occurring antioxidant compound found in Chinese green tea. The antioxidant activity of EGCG is at last 100 more times more effective than vitamin C and 25 times more effective than vitamin E at protecting cells and DNA from damage [31]. All three, U, AA, and EGCG have a neutral charge at pH 7 and all have approximately the same planar overall shape.

For the **shape analysis**, a globular and linear shaped molecule was chosen. The globular shaped molecule was trehalose (T). Trehalose is a disaccharide which is composed of two glucose molecules. The linear shaped molecule was glutathione (G). Glutathione is a tripeptide composed of glutamate, cysteine, and glycine that has numerous important functions within cells [32]. Both molecules, T and G, have a neutral charge at pH 7 and both are approximately the same size (~320Da). A fourth group was also investigated, regarding what could be categorized as another size analysis. Tryptophan (Trp) is an essential amino acid which is readily absorbed via dietary intake. A tripeptide of tryptophan (TTT) is easily obtained and would be advantageous to see if monopeptides penetrate more easily than a tripeptide.

Selection of Bacterium

Previous data has indicated that bacterial pathogens existing in an allograft are of the more “durable” and “hardy” variety. The cases discussed previously involved infection caused by a *Clostridium* strain of bacteria. *Clostridia* are anaerobic, Gram-positive, spore-forming, rod-shaped bacteria [33]. The cell wall of Gram-positive bacteria is composed of a thick layer of murein (a peptidoglycan), compared to gram-negative bacteria that are which have a thin layer of murein, Figure 2.01 [34]. Therefore, it would be deduced that a free radical scavenger would already have limitations in crossing the cellular wall of a Gram-positive bacteria (a good thing). The outer cell wall of a Gram-negative bacteria, such as *E. coli*, is fairly permeable to smaller solutes below a molecular weight of approximately 400Da [35]. Such solutes can freely permeate via a concentration gradient [35]. However, under physiologic stress, the diffusion is slowed due to slowed bacterial activity [35].

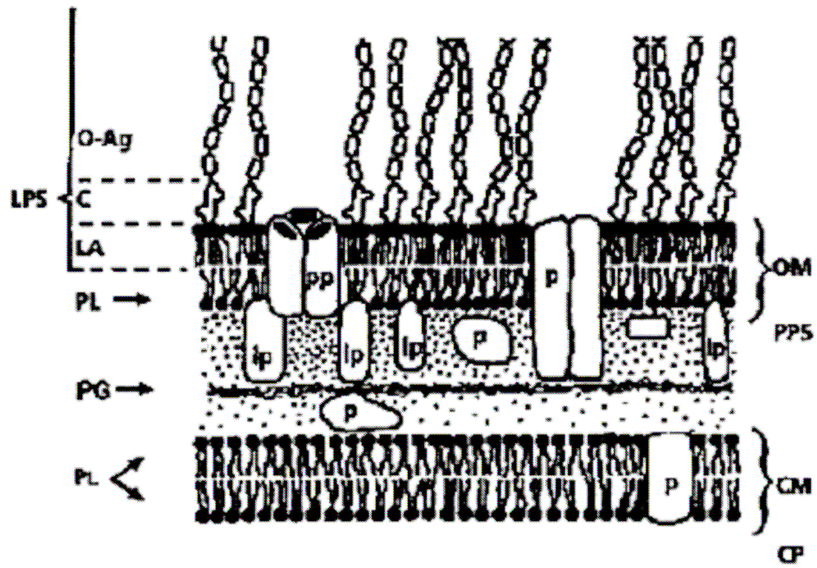


Figure 2.01 Scheme of the cell wall of a gram-negative bacterium. LPS: lipopolysaccharide; PL: phospholipids; PG: peptidoglycan; OM: outer membrane; CM: cell membrane; PPS: periplasmic space; CP: cytoplasm [36].

Therefore, a worst-case-scenario is desired; such that a pathogen is highly susceptible to being protected. The optimum conditions for a scavenger to cross the cellular wall would occur when the cell is: 1) metabolically active, 2) aerobic, and 2) contains a gram-negative wall. The bacterial model selected for this study was *Escherichia Coli*. *E. coli* is a gram-negative, aerobic, non-spore forming bacterial model that is safe and reliable. Additionally, a dose of approximately 170Gy is sufficient for a

1-log inactivation of *E. coli* [37]. This allows for sufficient kill at a relatively low dose of gamma irradiation which means the samples spent less time in the irradiator.

Preparation of Free Radical Scavengers

Free radical scavengers were ordered from the appropriate vendors (Appendix K). Scavengers were prepared to the concentration as indicated in Table 2.02. All scavengers were in powder form, appropriate amount of scavenger was weighed (AB204, Mettler Toledo, Columbus, OH, USA) and added to a 25mm x 95mm vial with a screw cap (Fisher Scientific, St. Louis, MO, USA). Distilled deionized water was added to a volume of 20ml and the vial was shaken until the scavenger was completely in solution. The pH was measured (UB-10, Denver Instrument, Arvada, CO, USA) and adjusted to approximately 7.0 using concentrated sodium hydroxide and concentrated hydrochloric acid. The scavenger was then filter sterilized into a sterile vial with screw cap using a 0.20μm filter (Whatman, Clifton, NJ, USA) and 20ml syringe (Becton-Dickinson, Franklin Lakes, NJ, USA).

Table 2.02 Concentrations of free radical scavengers tested.

	Compound	Code	Concentration	Size	Charge	Shape
Charge	Water (No Scavenger)	NS	-	-	-	-
	L-Cysteine	LC	0.1M	121Da	Neutral	Planar
	N-Acetyl-L-Cysteine	NAC	0.1M	163Da	Negative	Planar
	L-Cysteine-Ethyl-Ester	LCEE	0.1M	185Da	Positive	Planar
Size	Water (No Scavenger)	NS	-	-	-	-
	Uracil	U	0.01M	112Da	Neutral	Planar
	Ascorbic Acid	AA	0.01M	176Da	Neutral	Planar
	Epigallocatechin Gallate	EGCG	0.001M	458Da	Neutral	Planar
Shape	Water (No Scavenger)	NS	-	-	-	-
	Trehalose	T	0.1M	342Da	Neutral	Globular
	Glutathione	G	0.1M	307Da	Neutral	Planar
Tripeptide	Water (No Scavenger)	NS	-	-	-	-
	Tryptophan	Trp	25mM	204Da	Neutral	Planar
	Trp-Trp-Trp	TTT	8.5mM	612Da	Neutral	Planar

Preparation of Bacteria Cultures

Wild type *E. coli* was obtained from the undergraduate Bioprocessing Laboratory at The University of Toledo (Toledo, OH, USA). The *E. coli* was stored at -80ºC in 10% glycerol. Each vial contained 1.0ml at a concentration of approximately 1 x 10^6CFU/ml. The vials were allowed to warm to room temperature on the bench. In a sterile 150ml flask, 40ml of nutrient broth media (Appendix P) was added and brought to 37ºC in a shaker incubator (G24, New Brunswick, Edison, NJ, USA). Thawed stock *E. coli* was transferred to the flask and the cells were allowed to proliferate for 24 hours in the shaker incubator at 37ºC and 150rpm before being used to test scavengers.

Treatment of Bacteria with Scavengers

For the charge analysis, there were 32 treatment groups: 2 factors of irradiation (irradiated and non-irradiated), 4 factors of scavenger (no scavenger, LC, NAC, and LCEE), and 4 time points (0, 10, 20, and 40 hours).

For the size analysis, there were also 32 treatment groups: 2 factors of irradiation (irradiated and non-irradiated), 4 factors of scavenger (no scavenger, U, AA, and EGCG), and 4 time points (0, 10, 20, and 40 hours).

For the shape analysis, there were 24 treatment groups: 2 factors of irradiation (irradiated and non-irradiated), 3 factors of scavenger (no scavenger, T, and G), and 4 time points (0, 10, 20, and 40 hours).

For the tripeptide analysis, there were also 24 treatment groups: 2 factors of irradiation (irradiated and non-irradiated), 3 factors of scavenger (no scavenger, Trp, and TTT), and 4 time points (0, 10, 20, and 40 hours).

Using aseptic techniques, 2ml of each scavenger was added to its own individual vial with screw cap. Sterile deionized water (no scavenger, NS) was used in place of scavenger for the control samples. Continuing aseptic processing, 2ml of *E. coli* culture (grown for 24 hours) was added to each individual vial. Zero hours of incubation samples were immediately irradiated. The 10, 20, and 40 hour samples remained on the bench at ambient temperature for the duration of their incubation period, and then were irradiated. Samples containing scavenger and *E. coli* were irradiated to a dose of 500Gy (Isotopes, Inc., Westwood, NJ, USA) at room temperature. Controls remained on the bench at ambient temperature until the irradiated samples returned.

Picture 2.01 Gamma irradiation machine.

The irradiation machine, Picture 2.01, has a gamma irradiation delivery rate of 147Gy/hour. The desired dose of 500Gy was selected because it would allow for a greater than 2-log inactivation of *E. coli*. This required the samples to be in the irradiator for 3.4 hours. At the completion of receiving 500Gy of gamma irradiation, the samples were serial diluted nine times at a five-fold dilution and then three times at a two-fold dilution with nutrient broth media; 100µl of the diluted sample was plated (n=4) on 100mm x 15mm Petri dishes (Fisher Scientific, St. Louis, MO, USA) containing agar media (Appendix O). The agar plates were incubated overnight at 37ºC (Fisher Scientific, St. Louis, MO, USA) and a colony count was performed for each treatment group. Log concentration values were calculated by counting the colony forming units (CFU) on the plates and then equating the volume of cells plated with the dilution factor.

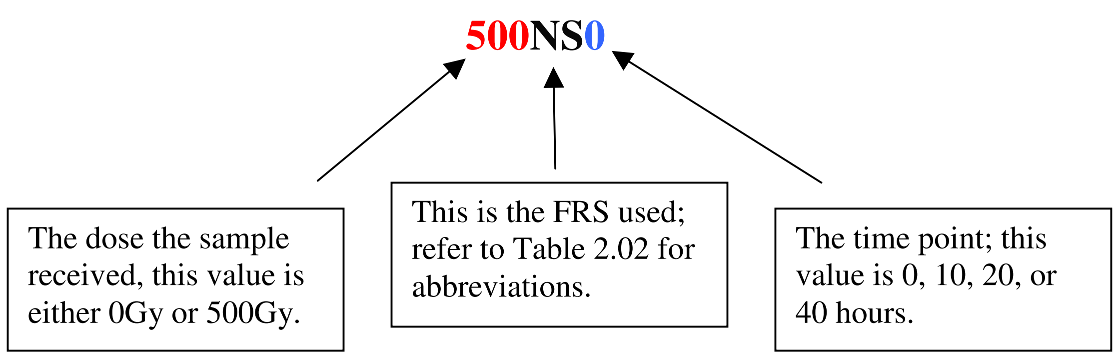
Statistical Analysis

A generalized multivariate ANOVA was performed to determine the significances of the effects of irradiation, duration, and scavenger. When significant differences existed among any two groups, the difference was tested by a Tukey's *post hoc* test. A difference at the level of $p<0.05$ was reported as significant and a difference at the level of $0.05<p<0.1$ was reported as borderline significant. Dixon's outlier test was performed to determine if any concentration value was to be omitted at 95% confidence level. Those failing the outlier test are represented in red in the raw data section of the appendices indicating that they were omitted from the data set.

Chapter Three

Results

All raw data can be found in Appendices A through J in this thesis. Values are reported in terms of log value of the concentration of the sample (log(concentration) CFU/ml). The labeling system of the samples was as follows, all data is presented in this form (the Appendix data also follows this convention):



E. coli Gamma Radiation Dose Response

A standard curve of *E. coli* log reduction (with no scavenger) was constructed to estimate log reduction at various doses of gamma irradiation, Figure 3.01. It was found that 1-log reduction occurred at a dose of approximately 300Gy.

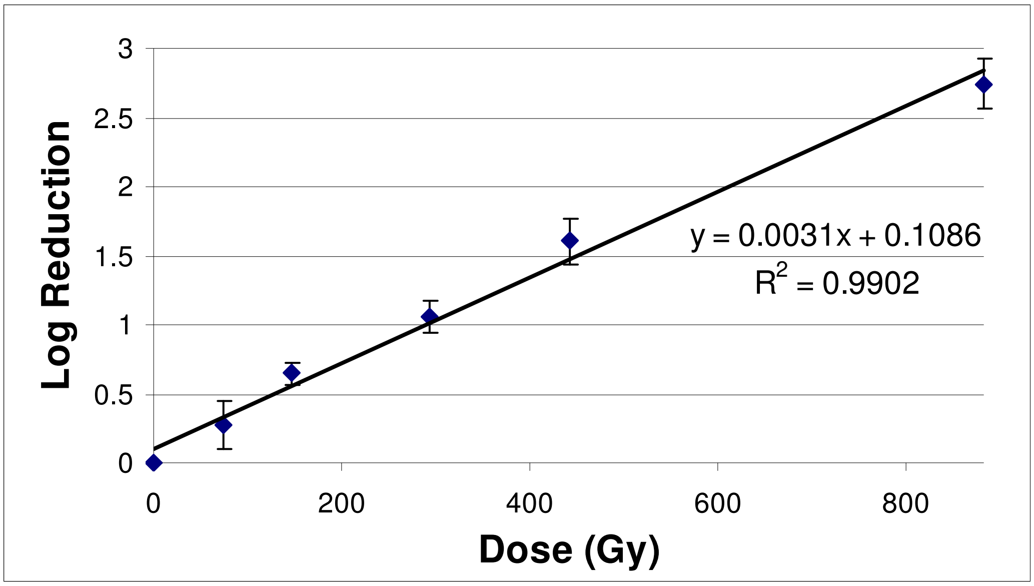


Figure 3.01 Dose response log-reduction of *E. coli*.

Cysteine FRS Pilot Study

To assess the radioprotective effects of the cysteine based molecules, a pilot study was developed. The five identified cysteine based scavengers: L-cysteine (zwitterionic, LC), D-cysteine (zwitterionic, DC), N-acetyl-L-cysteine (negative, NAC), L-cysteine-methyl-ester, (positive, LCME), and L-cysteine-ethyl-ester (positive, LCEE) all at 0.1M were irradiated with *E. coli* to a dose of 500Gy, Figure 3.02. There was no prolonged incubation time of the scavengers with the *E. coli*, so this would be defined as an incubation time of zero hours. Results are reported as log-concentration values. The higher the log value, the more *E. coli* survived. The sample 500NS is *E. coli* with no scavenger treatment, therefore any groups having a higher concentration that than that group experienced a protective effect from the scavenger.

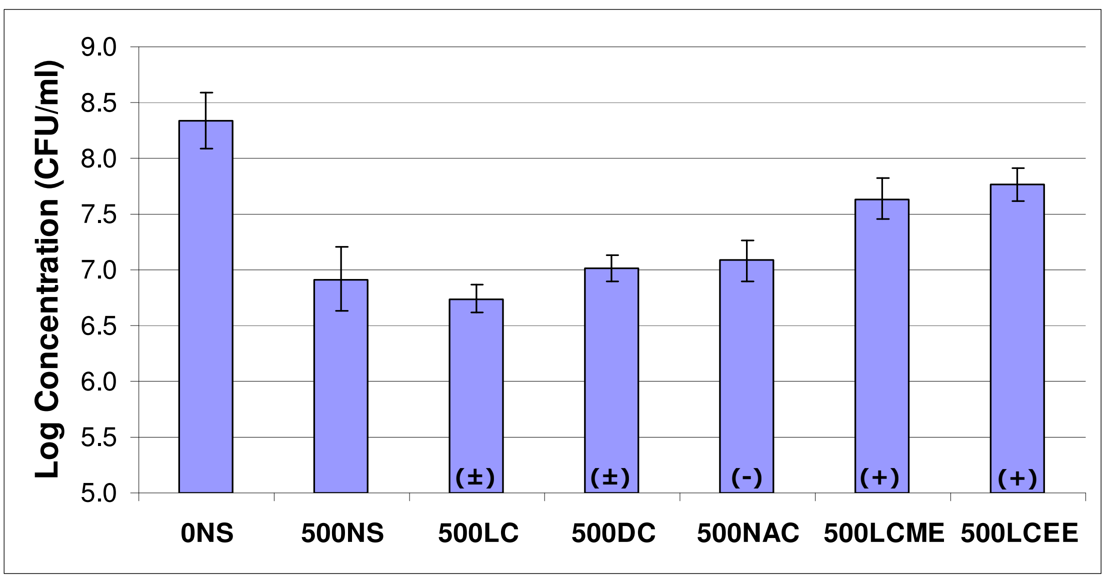


Figure 3.02 Cysteine pilot study; concentrations of *E. coli* after irradiation.

Charge Analysis

Figure 3.03 shows the effects of differently charged scavengers, Table 2.02, with *E. coli* irradiated to a dose of 500Gy. Log concentration values are reported with respect to the scavenger and time points 0, 10, 20, and 40 hours incubation. A scavenger treated group that has a higher log concentration value than its corresponding control groups indicates protection of the *E. coli* occurred. Those samples that attained a significant difference, $p<0.05$, than their corresponding control are indicated by a star. Shown in Table 3.01 are the reported p values for the respective parameters. Those p values below 0.05 indicate significant differences among the sample groups. Found in Appendix D is a pair-wise comparison chart between all samples, significance is reported as either significantly different (S) or not significantly different (NS) at a level of $p<0.05$.

Table 3.01 P values for parameters of charge analysis.

	Irradiation	**Scavenger**	**Time**	**Scavenger-Irradiation**	**Scavenger-Time**
Charge	0.000	0.000	0.000	0.000	0.000

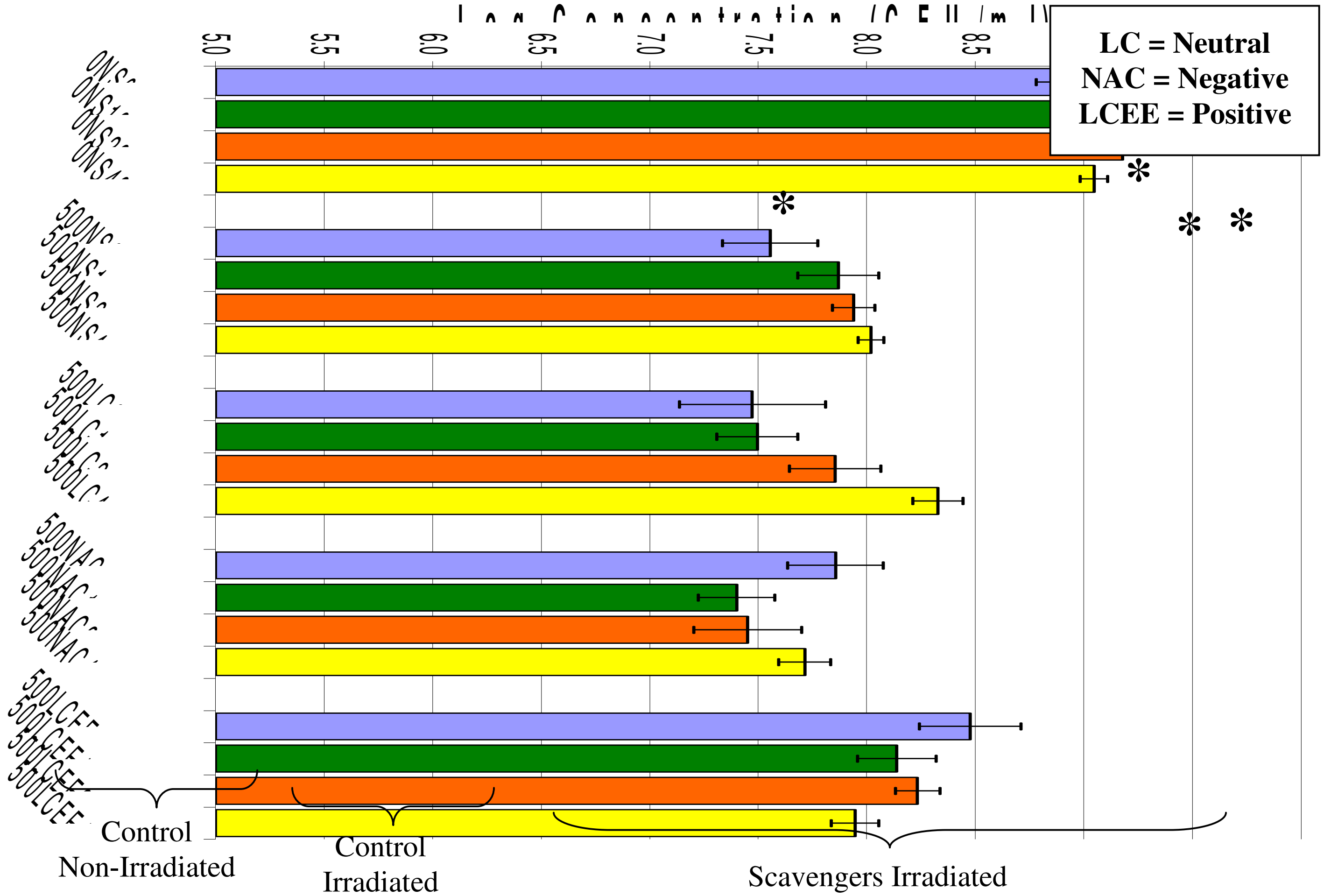


Figure 3.03 Charge analysis; *E. coli* concentration after irradiation to 500Gy. Blue = 0 hours incubation; Green = 10 hours incubation; Orange = 20 hours incubation; yellow = 40 hours incubation. A sample marked with ‘*’ indicates a significant difference from its control group (500NS__).

Size Analysis

Figure 3.04 shows the effects of different size scavengers, Table 2.02, with *E. coli* irradiated to a dose of 500Gy. Log concentration values are reported with respect to the scavenger and time points 0, 10, 20, and 40 hours incubation. A scavenger treated group that has a higher log concentration value than its corresponding control groups indicates protection of the *E. coli* occurred. Those samples that attained a significant difference, $p<0.05$, than their corresponding control are indicated by a star. Shown in Table 3.02 are the reported p values for the respective parameters. Those p values below 0.05 indicate significant differences among the sample groups. Found in Appendix F is a pair-wise comparison chart between all samples, significance is reported as either significantly different (S) or not significantly different (NS) at a level of $p<0.05$.

Table 3.02 P values for parameters of size analysis.

	Irradiation	Scavenger	Time	Scavenger-Irradiation	Scavenger-Time
Size	0.000	0.000	0.000	0.000	0.000

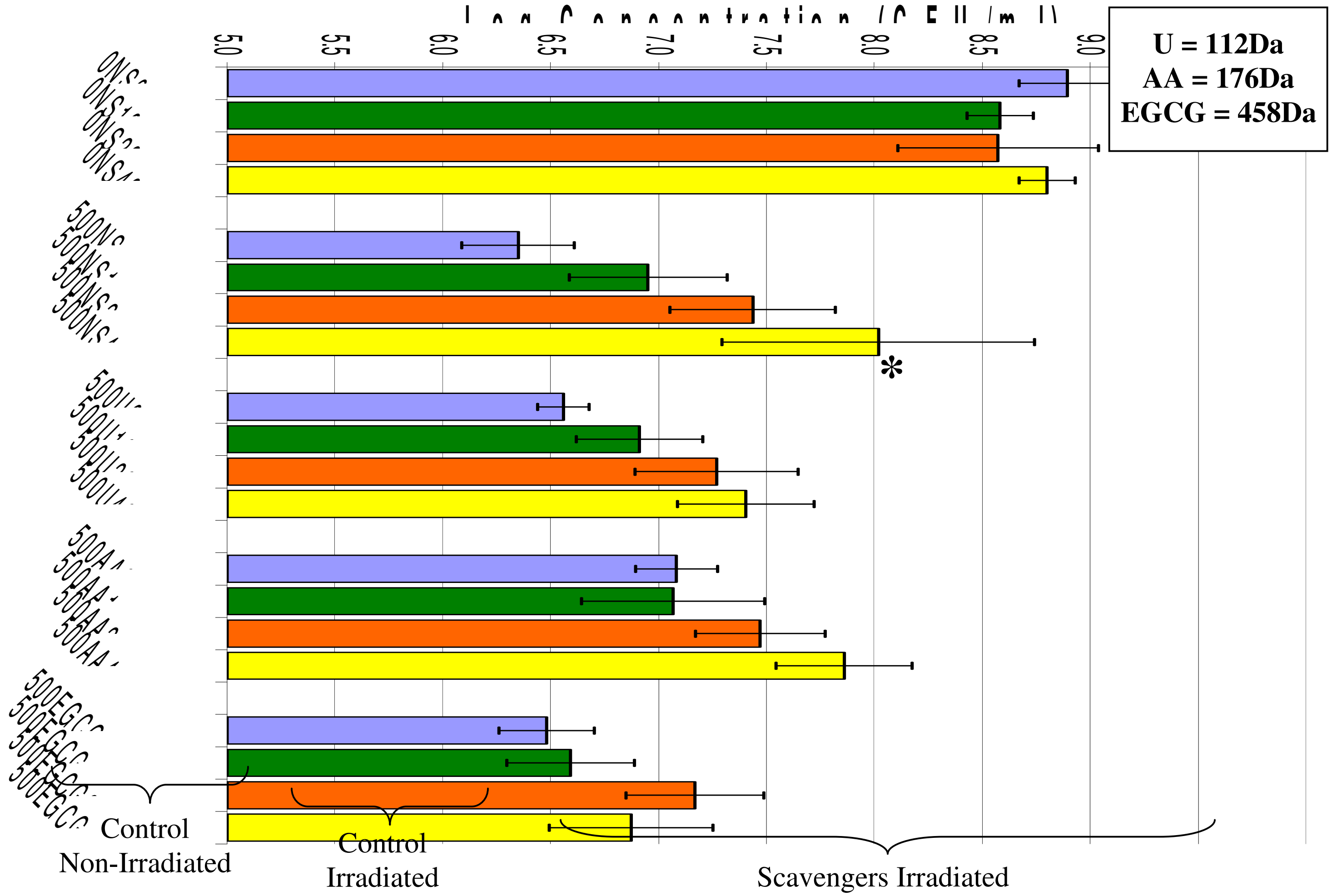


Figure 3.04 Size analysis; *E. coli* concentration after irradiation to 500Gy. Blue = 0 hours incubation; Green = 10 hours incubation; Orange = 20 hours incubation; yellow = 40 hours incubation. A sample marked with '*' indicates a significant difference from its control group (500NS__).

Shape Analysis

Figure 3.05 shows the effects of different shaped scavengers, Table 2.02, with *E. coli* irradiated to a dose of 500Gy. Log concentration values are reported with respect to the scavenger and time points 0, 10, 20, and 40 hours incubation. A scavenger treated group that has a higher log concentration value than its corresponding control groups indicates protection of the *E. coli* occurred. Those samples that attained a significant difference, $p<0.05$, than their corresponding control are indicated by a star. Shown in Table 3.03 are the reported p values for the respective parameters. Those p values below 0.05 indicate significant differences among the sample groups. Found in Appendix H is a pair-wise comparison chart between all samples, significance is reported as either significantly different (S) or not significantly different (NS) at a level of $p<0.05$.

Table 3.03 P values for parameters of shape analysis.

	Irradiation	**Scavenger**	**Time**	**Scavenger-Irradiation**	**Scavenger-Time**
Shape	0.000	0.000	0.000	0.000	0.000

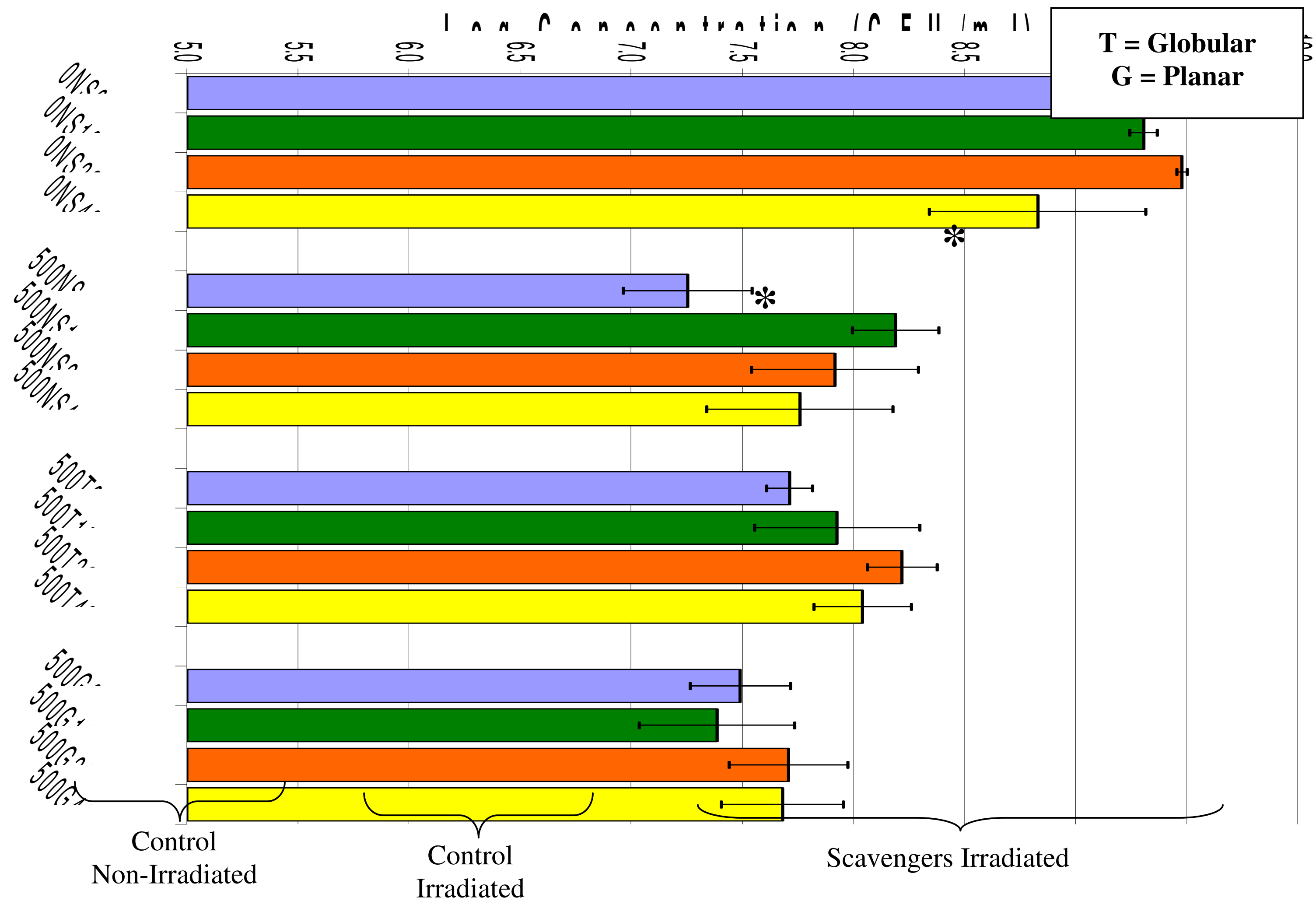


Figure 3.05 Shape analysis; *E. coli* concentration after irradiation to 500Gy. Blue = 0 hours incubation; Green = 10 hours incubation; Orange = 20 hours incubation; yellow = 40 hours incubation. A sample marked with '*' indicates a significant difference from its control group (500NS__).

Tripeptide Analysis

Figure 3.06 shows the effects of a monopeptide versus a tripeptide scavenger, Table 2.02, with *E. coli* irradiated to a dose of 500Gy. Log concentration values are reported with respect to the scavenger and time points 0, 10, 20, and 40 hours incubation. A scavenger treated group that has a higher log concentration value than its corresponding control groups indicates protection of the *E. coli* occurred. Those samples that attained a significant difference, $p<0.05$, than their corresponding control are indicated by a star. Shown in Table 3.04 are the reported p values for the respective parameters. Those p values below 0.05 indicate significant differences among the sample groups. Found in Appendix J is a pair-wise comparison chart between all samples, significance is reported as either significantly different (S) or not significantly different (NS) at a level of $p<0.05$.

Table 3.04 P values for parameters of tripeptide analysis.

	Irradiation	**Scavenger**	**Time**	**Scavenger-Irradiation**	**Scavenger-Time**
Tripeptide	0.000	0.000	0.000	0.000	0.000

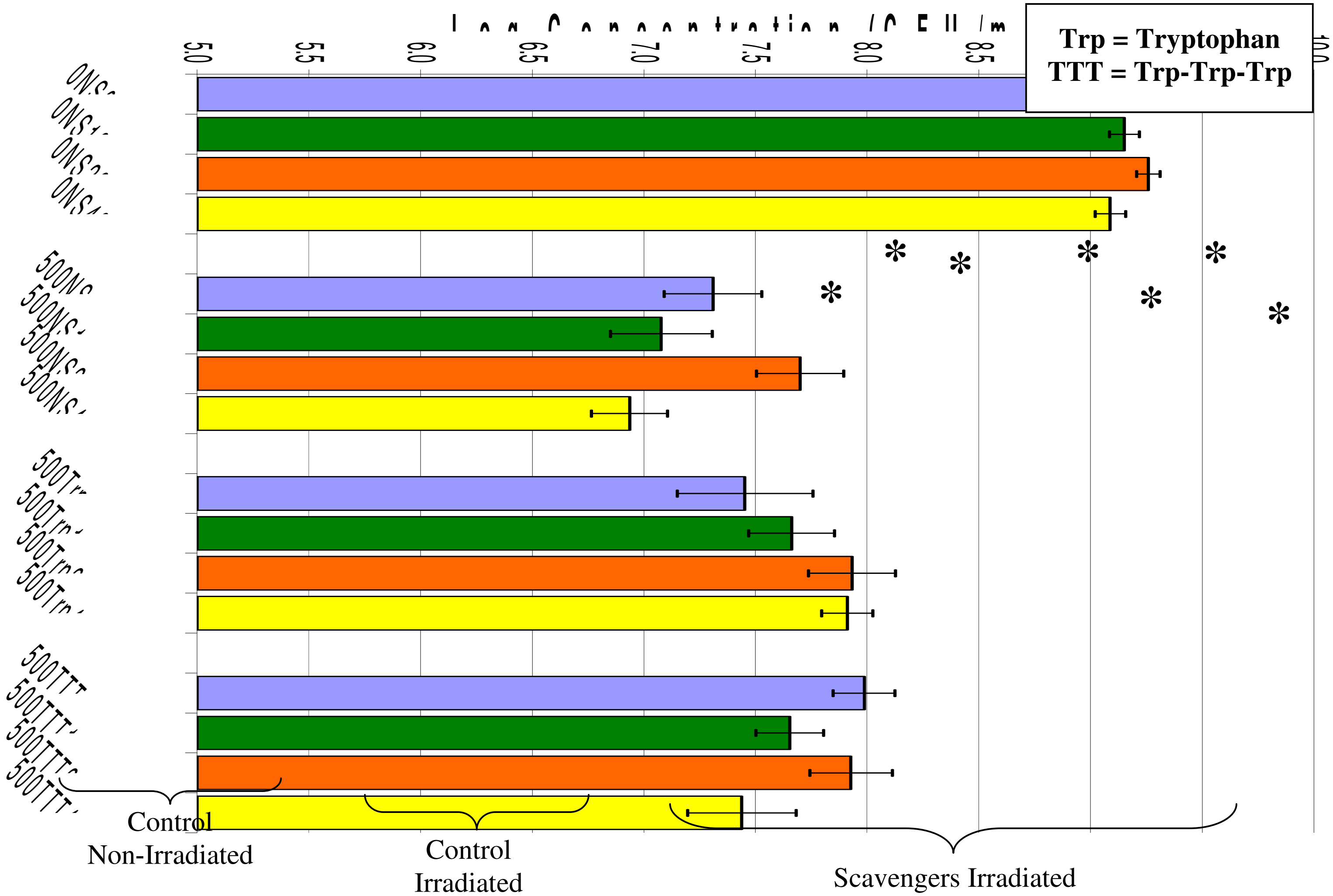


Figure 3.06 Tripeptide analysis; *E. coli* concentration after irradiation to 500Gy. Blue = 0 hours incubation; Green = 10 hours incubation; Orange = 20 hours incubation; yellow = 40 hours incubation. A sample marked with '*' indicates a significant difference from its control group (500NS__).

Chapter Four

Conclusions

After examining the data, one initial conclusion can be drawn on the radiosensitivity of the *E. coli* itself without the presence of a scavenger (control samples). There appears to be a trend of increased resistance to gamma irradiation as time increases. In other words, as the incubation time increases more of the *E. coli* survives the 500Gy dose irradiation. For example in the charge data, at the 0 hour of incubation there is a 2.0-log reduction in the number of *E. coli* whereas at the 40 hours of incubation there is only a 0.8-log reduction in the number of *E. coli*, Figures 3.03 and 3.04.

This increased resistance to the irradiation dose could be the consequence of several factors. Firstly, as time increases, the *E. coli* are running out of nutrition resources, therefore, as a survival technique they are decreasing their metabolic activity. This decrease in metabolic activity results in the cell turning off non-essential cellular processes and implementing ways to conserve resources and enter a state of hibernation. *E. coli* cells become much smaller and almost spherical when they enter stationary phase

[38]. The cytoplasm volume is condensed; which would assume a decrease in intracellular water [38]. Additionally, during this stationary phase there is an increased demand for oxidation management [39]. Several genes are turned on that are devoted to synthesizing proteins with specific roles in the defense against oxidative stress [39]. In other words, as *E. coli* enters a stationary phase, they shrink by removing intracellular water and begin to make proteins with the specific purpose of fighting the oxidative stress of free radicals. Additionally, as incubation time increases and the food supply is depleted, the cells will become less mitotic and are therefore less susceptible to damaging radiation [34]. This would explain the observation of increased viability of *E. coli* in samples with long incubation times as the cells have begun to cope with their compromised environment.

In terms of the charge of a scavenger; the positively charged scavenger, LCEE, provided significant protection to *E. coli*, $p<0.05$. Positively charged LCEE was probably attracted to the overall negatively charged outer capsule of the *E. coli*. Most bacteria, including *E. coli*, contain an outer capsule which is composed mostly of polysaccharides which carries a negative charge [34]. Therefore, LCEE was attracted to *E. coli* and was allowed to enter the intracellular space and thus protected the cellular components from the oxidative stress of the gamma irradiation. The negatively and neutral charged scavengers are repelled from the capsule by the repulsion of charges, and therefore those scavengers do not enter the intracellular space and do not protect the bacteria. Thus, any positively charged scavenger would be a poor choice due to its inherent affinity for the negatively charged capsule of most bacteria. The obvious choice would then be a scavenger with a positive charge or at least a neutral charge.

Maintaining these guidelines will also avoid any interaction that the scavenger may have with the membrane; such that even if the scavenger does not cross into the intracellular space it will not protect the bacteria as a whole from the exterior of the cell. Samples of *E. coli* treated with scavenger alone (no irradiation) showed no decline in cellular viability. This proves that the decline in viability was a consequence of the irradiation and not of the scavenger itself. This data is shown in Appendix C.

In terms of the size of a scavenger; the smallest scavenger tested, U, did not offer any protection to the *E. coli*, $p<0.05$. The medium sized scavenger, AA, significantly protected the *E. coli* at 0 hours incubation time point, but no other time point. Therefore, aside from size, the *E. coli* selectively allowed the scavenger AA to enter the cell based on other parameters besides size. This selectivity was most likely a factor of chemical identity where it recognized AA as a useful molecule and allowed it to pass, where as the smaller U was identified as an unneeded entity and did not allow it to pass into the intracellular space. EGCG was shown to be too large of a molecule to diffuse readily into *E. coli*. This is illustrated as in Figure 3.04 where no significant protection was observed. Interestingly, EGCG has the second highest rate constant of the three scavengers tested for the hydroxyl radical, yet no protection was observed. AA has the highest rate constant for the hydroxyl radical and U the lowest rate constant of the group, AA only protected at the 0 hour incubation time point. Samples of *E. coli* treated with scavenger alone (no irradiation) showed no decline in cellular viability. This proves that the decline in viability was a consequence of the irradiation and not of the scavenger itself. This data is shown in Appendix E.

In terms of the shape of a scavenger; the globular scavenger T significantly protected *E. coli* at the 0 and 40 hours incubation time points, $p<0.05$. The linear scavenger, G, did not offer any significant protection at any of the incubation time points. Therefore, globular scavengers appear to be able to penetrate the bacterial membrane easier than a linearly shaped molecule. This would make sense; it is easier to navigate a tightly packed globular molecule down a channel than it is a linear molecule of the same molecular weight. The globular molecule would simply "tumble" down the channel where as a linear molecule would get stuck like a needle in the side of the channel. Another significant point is that G has a much higher rate constant for the hydroxyl radical than T; about 3-fold higher. Yet G did not protect at any time point, indicating again that regardless of rate constant for the hydroxyl radical physical and chemical features precede reaction rates. Samples of *E. coli* treated with scavenger alone (no irradiation) showed no decline in cellular viability. This proves that the decline in viability was a consequence of the irradiation and not of the scavenger itself. This data is shown in Appendix G.

In terms of a tripeptide molecule versus a singular amino acid; the tripeptide, TTT, significantly protected at 0 hours incubation time whereas the singular amino acid did not, $p<0.05$. Comparing the concentrations tested, the singular amino acid tryptophan was tested at a molarity of 24mM and the tripeptide, tryptohpan-tryptophan-tryptophan, was tested at a molarity of 8.4mM. This means that there were identical amounts of scavenging groups. Interestingly, the tripeptide was preferred over the singular amino acid with no prolonged incubation time. However, at 10, 20, and 40 hours incubation time both the singular amino acid and the tripeptide significantly protected the *E. coli*,

$p<0.05$. As illustrated in Figure 3.06, TTT pretty much protected at the same level for all time points whereas T was able to diffuse through into the bacteria in a time dependent manner; as time increases T protects the bacteria at higher levels. Samples of *E. coli* treated with scavenger alone (no irradiation) showed no decline in cellular viability. This proves that the decline in viability was a consequence of the irradiation and not of the scavenger itself. This data is shown in Appendix I.

To put these results into context of bone allograft sterilization with gamma irradiation; the addition of a scavenger has proven to alleviate biochemical and biomechanical stress to a bone allograft. However, selection of the proper scavenger is essential. From the results of this study, it would be advantageous to avoid positively charged scavengers due to their affinity for negatively charged envelops of bacteria, avoid molecules that cells may view as useful to uptake, avoid very small molecular weight molecules, and avoid globularly shaped molecules as they easily can diffuse into bacteria. Additionally, bacterial cells may show preference over one amino acid to the other, in this case showed to prefer tryptophan over L-cysteine as trpytophan protected and L-cysteine did not at same concentrations (0.1M) and similar rate-constants for the hydroxyl radical (7.9×10^9 $M^{-1}s^{-1}$ and 8.5×10^9 $M^{-1}s^{-1}$ for LC and Trp, respectively), Figures 3.03 and 3.06 . Thus, a well thought smart choice for a scavenger to add to a bone allograft tissue during gamma irradiation would be one that is negatively charged, linear in shape, and somewhat large (~250Da). This would allow for the protection of the collagen structure of the allograft tissue (as molecules below 300Da can penetrate bone fabric) but not allow for protection of the pathogens during irradiation.

Appendix A

Dose Response *E. coli* Viability

		Control			74Gy			147Gy			294Gy			441Gy			882Gy		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
11	1,024																22	225,280	5.35
	1,024																28	286,720	5.46
12	2,048																21	430,080	5.63
	2,048																20	409,600	5.61
13	4,096																6	245,760	5.39
	4,096																6	245,760	5.39
14	8,192													38	3,112,960	6.49	9	737,280	5.87
	8,192													52	4,259,840	6.63	6	491,520	5.69
15	16,384													33	5,406,720	6.73	3	491,520	5.69
	16,384													30	4,915,200	6.69	1	163,840	5.21
16	32,768										42	13,762,560	7.14	8	2,621,440	6.42	2	655,360	5.82
	32,768										53	17,367,040	7.24	16	5,242,880	6.72			
17	65,536										22	14,417,920	7.16	5	3,276,800	6.52			
	65,536										11	7,208,960	6.86	6	3,932,160	6.59			
18	131,072	130	170,393,600	8.23	67	87,818,240	7.94	24	31,457,280	7.50	14	18,350,080	7.26	5	6,553,600	6.82			
	131,072	124	162,529,280	8.21	97	127,139,840	8.10	26	34,078,720	7.53	15	19,660,800	7.29	5	6,553,600	6.82			
19	262,144	57	149,422,080	8.17	32	83,886,080	7.92	13	34,078,720	7.53									
	262,144	51	133,693,440	8.13	43	112,721,920	8.05	15	39,321,600	7.59									
12	524,288	35	183,500,800	8.26	10	52,428,800	7.72	8	41,943,040	7.62	3	15,728,640	7.20						
	524,288	38	199,229,440	8.30	15	78,643,200	7.90	3	15,728,640	7.20	4	20,971,520	7.32						
		average	166,461,440	8.22	**average**	90,439,680	7.94	**average**	32,768,000	7.50	**average**	15,933,440	7.18	**average**	4,587,520	6.64	**average**	398,429	5.56
		stdev	23,490,794	0.062	**stdev**	26,371,217	0.134	**stdev**	9,193,746	0.154	**stdev**	4,315,071	0.146	**stdev**	1,383,777	0.137	**stdev**	185,310	0.208
		N =	6		**N =**	6		**N =**	6		**N =**	8		**N =**	10		**N =**	11	

Dose Response *E. coli* Viability

N = number of colonies after irradiation
No = number of colonies prior to irradiation

Dose	Conc. (CFU/ml)
0	**170,393,600**
	162,529,280
	149,422,080
	133,693,440
	183,500,800
	199,229,440
74	**87,818,240**
	127,139,840
	83,886,080
	112,721,920
	52,428,800
	78,643,200
147	**31,457,280**
	34,078,720
	34,078,720
	39,321,600
	41,943,040
	15,728,640
294	**13,762,560**
	17,367,040
	14,417,920
	7,208,960
	18,350,080
	19,660,800
441	**3,112,960**
	4,259,840
	5,406,720
	4,915,200
	2,621,440
	5,242,880
882	**225,280**
	286,720
	430,080
	409,600
	245,760
	245,760

	Surviving Fraction	
Dose (Gy)	**N/No**	**log(N/No)**
0	1	0
74	0.5154	-0.288
	0.7823	-0.107
	0.5614	-0.251
	0.8431	-0.074
	0.2857	-0.544
	0.3947	-0.404
	ave	**-0.278**
	stdev	**0.178**
147	0.1846	-0.734
	0.2097	-0.678
	0.2281	-0.642
	0.2941	-0.531
	0.2286	-0.641
	ave	**-0.645**
	stdev	**0.074**
294	0.0808	-1.093
	0.1069	-0.971
	0.0965	-1.016
	0.0539	-1.268
	0.1000	-1.000
	0.0987	-1.006
	ave	**-1.059**
	stdev	**0.110**
441	0.0183	-1.738
	0.0262	-1.582
	0.0362	-1.441
	0.0368	-1.435
	0.0143	-1.845
	0.0263	-1.580
	ave	**-1.603**
	stdev	**0.163**
882	0.0013	-2.879
	0.0018	-2.753
	0.0029	-2.541
	0.0031	-2.514
	0.0013	-2.873
	0.0012	-2.909
	ave	**-2.745**
	stdev	**0.177**

Appendix B

Non-Irradiated Cysteine Preliminary Data

		0ns0			0LC			0DC			0NAC			0LCEE			0LCME		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
9	156,250	210	328,125,000	8.52															
	156,250																		
	156,250																		
	156,250																		
10	312,500	98	306,250,000	8.49															
	312,500	99	309,375,000	8.49															
	312,500	120	375,000,000	8.57															
	312,500	103	321,875,000	8.51															
11	625,000	14	87,500,000	7.94	35	218,750,000	8.34	35	218,750,000	8.34	36	225,000,000	8.35	33	206,250,000	8.31	37	231,250,000	8.36
	625,000	23	143,750,000	8.16	36	225,000,000	8.35	37	231,250,000	8.36	43	268,750,000	8.43	34	212,500,000	8.33	41	256,250,000	8.41
	625,000	49	306,250,000	8.49	37	231,250,000	8.36	34	212,500,000	8.33	34	212,500,000	8.33	33	206,250,000	8.31	36	225,000,000	8.35
	625,000	61	381,250,000	8.58	36	225,000,000	8.35				30	187,500,000	8.27	33	206,250,000	8.31	39	243,750,000	8.39
12	1,250,000	24	300,000,000	8.48	21	262,500,000	8.42	19	237,500,000	8.38	19	237,500,000	8.38	17	212,500,000	8.33	18	225,000,000	8.35
	1,250,000	7	87,500,000	7.94	17	212,500,000	8.33	21	262,500,000	8.42	18	225,000,000	8.35	22	275,000,000	8.44	18	225,000,000	8.35
	1,250,000				21	262,500,000	8.42	21	262,500,000	8.42	17	212,500,000	8.33	18	225,000,000	8.35	16	200,000,000	8.30
	1,250,000	10	125,000,000	8.10	20	250,000,000	8.40	22	275,000,000	8.44	23	287,500,000	8.46	19	237,500,000	8.38	18	225,000,000	8.35
		average	255,989,583	8.34	average	235,937,500	8.37	average	242,857,143	8.38	average	232,031,250	8.36	average	222,656,250	8.35	average	228,906,250	8.36
		stdev	114,058,803	0.252	stdev	19,693,522	0.036	stdev	24,051,965	0.043	stdev	32,292,867	0.059	stdev	23,843,215	0.044	stdev	16,345,018	0.031
		N =	11		N =	8		N =	7		N =	8		N =	8		N =	8	

Irradiated Cysteine Preliminary Data

		500ns0			500LC			500DC			500NAC			500LCEE			500LCME		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125										298	9,312,500	6.97						
	3,125				123	3,843,750	6.58				145	4,531,250	6.66						
	3,125																		
	3,125																		
7	15,625	54	8,437,500	6.93															
	15,625	34	5,312,500	6.73															
	15,625	28	4,375,000	6.64															
	15,625	55	8,593,750	6.93															
8	78,125	9	7,031,250	6.85	9	7,031,250	6.85	17	13,281,250	7.12	19	14,843,750	7.17	55	42,968,750	7.63	44	34,375,000	7.54
	78,125				1			12	9,375,000	6.97	7	5,468,750	6.74				63	49,218,750	7.69
	78,125	5	3,906,250	6.59	8	6,250,000	6.80	10	7,812,500	6.89	18	14,062,500	7.15				65	50,781,250	7.71
	78,125	10	7,812,500	6.89	7	5,468,750	6.74	18	14,062,500	7.15	16	12,500,000	7.10						
9	390,625	1	3,906,250	6.59	2	7,812,500	6.89	3	11,718,750	7.07	3	11,718,750	7.07	10	39,062,500	7.59	12	46,875,000	7.67
	390,625	8	31,250,000	7.49	1	3,906,250	6.59	3	11,718,750	7.07	5	19,531,250	7.29	23	89,843,750	7.95	18	70,312,500	7.85
	390,625	5	19,531,250	7.29				2	7,812,500	6.89				18	70,312,500	7.85	20	78,125,000	7.89
	390,625				1	3,906,250	6.59							12	46,875,000	7.67			
10	781,250	1	7,812,500	6.89				1	7,812,500	6.89	2	15,625,000	7.19				3	23,437,500	7.37
	781,250	2	15,625,000	7.19	2			0						6	46,875,000	7.67			
	781,250							1	7,812,500	6.89	3	23,437,500	7.37	12	93,750,000	7.97	6	46,875,000	7.67
	781,250				1	7,812,500	6.89	2	15,625,000	7.19	2	15,625,000	7.19	8	62,500,000	7.80	3	23,437,500	7.37
		average	10,299,479	6.92	**average**	5,753,906	6.74	**average**	10,703,125	7.01	**average**	13,332,386	7.08	**average**	61,523,438	7.77	**average**	47,048,611	7.64
		stdev	8,110,908	0.283	**stdev**	1,670,383	0.130	**stdev**	2,970,349	0.120	**stdev**	5,009,215	0.177	**stdev**	21,369,926	0.147	**stdev**	18,712,437	0.185
		N =	12		**N =**	8		**N =**	10		**N =**	11		**N =**	8		**N =**	9	

Appendix C

0 hour Charge Data

		0ns0			500ns0			0LC0			500LC0			0NAC0			500NAC0			0LCEE0			500LCEE0		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125										728	22,750,000	7.36				1764.16	55,130,000	7.74						
	3,125																858.4	26,825,000	7.43						
	3,125																								
	3,125																								
7	15,625				320	50,000,000	7.70																		
	15,625				201	31,406,250	7.50																		
	15,625				166	25,937,500	7.41																		
	15,625				326	50,937,500	7.71																		
8	78,125				53	41,406,250	7.62				53	41,406,250	7.62				112	87,500,000	7.94				326	254,687,500	8.41
	78,125				18	14,062,500	7.15				6	4,687,500	6.67				41	32,031,250	7.51						
	78,125				30	23,437,500	7.37				47	36,718,750	7.56				107	83,593,750	7.92						
	78,125				60	46,875,000	7.67				41	32,031,250	7.51				95	74,218,750	7.87						
9	156,250																								
	156,250																								
	156,250																								
	156,250																								
10	312,500	580	1,812,500,000	9.26																					
	312,500	586	1,831,250,000	9.26																					
	312,500	710	2,218,750,000	9.35																					
	312,500	609	1,903,125,000	9.28																					
10b	390,625				6	23,437,500	7.37				11	42,968,750	7.63				18	70,312,500	7.85				59	230,468,750	8.36
	390,625										6	23,437,500	7.37				30	117,187,500	8.07				136	531,250,000	8.73
	390,625																						107	417,968,750	8.62
	390,625										6	23,437,500	7.37										71	277,343,750	8.44
11	625,000	83	518,750,000	8.71				185	1,156,250,000	9.06				196	1,225,000,000	9.09				178	1,112,500,000	9.05			
	625,000	136	850,000,000	8.93				191	1,193,750,000	9.08				185	1,156,250,000	9.06				184	1,150,000,000	9.06			
	625,000	290	1,812,500,000	9.26				199	1,243,750,000	9.09				182	1,137,500,000	9.06				177	1,106,250,000	9.04			
	625,000	361	2,256,250,000	9.35				192	1,200,000,000	9.08				156	975,000,000	8.99				178	1,112,500,000	9.05			
11b	781,250																12	93,750,000	7.97				12	93,750,000	7.97
	781,250				6	46,875,000	7.67				12	93,750,000	7.97										36	281,250,000	8.45
	781,250																18	140,625,000	8.15				71	554,687,500	8.74
	781,250				11	85,937,500	7.93				6	46,875,000	7.67				12	93,750,000	7.97				47	367,187,500	8.56
12	1,250,000	142	1,775,000,000	9.25				113	1,412,500,000	9.15				103	1,287,500,000	9.11				90	1,125,000,000	9.05			
	1,250,000	41	512,500,000	8.71				91	1,137,500,000	9.06				97	1,212,500,000	9.08				119					
	1,250,000	30	375,000,000	8.57				114	1,425,000,000	9.15				103	1,287,500,000	9.11				95	1,187,500,000	9.07			
	1,250,000	59	737,500,000	8.87				109	1,362,500,000	9.13				124	1,550,000,000	9.19				100	1,250,000,000	9.10			
		average	1,383,593,750	9.07	average	40,028,409	7.55	average	1,266,406,250	9.10	average	36,806,250	7.47	average	1,228,906,250	9.09	average	79,538,523	7.86	average	1,149,107,143	9.06	average	334,288,194	8.48
		stdev	718,370,855	0.287	stdev	19,797,414	0.218	stdev	116,301,712	0.039	stdev	23,599,798	0.337	stdev	164,035,925	0.057	stdev	33,730,724	0.221	stdev	52,839,745	0.020	stdev	148,527,648	0.234
		N =	12		N =	11		N =	8		N =	10		N =	8		N =	11		N =	7		N =	9	

10 hour Charge Data

		0ns10			500ns10			0LC10			500LC10		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125												
	3,125												
	3,125												
	3,125												
7	15,625												
	15,625												
	15,625												
	15,625												
8	78,125										40	31,250,000	7.49
	78,125										38	29,687,500	7.47
	78,125										36	28,125,000	7.45
	78,125										44	34,375,000	7.54
9	156,250				56	87,500,000	7.94				20	31,250,000	7.49
	156,250				43	67,187,500	7.83				23	35,937,500	7.56
	156,250				47	73,437,500	7.87				31	48,437,500	7.69
	156,250				58	90,625,000	7.96				14	21,875,000	7.34
10	312,500				18	56,250,000	7.75				6	18,750,000	7.27
	312,500				31	96,875,000	7.99				11	34,375,000	7.54
	312,500				29	90,625,000	7.96				5	15,625,000	7.19
	312,500				37	115,625,000	8.06				9	28,125,000	7.45
11	625,000				9	56,250,000	7.75				8	50,000,000	7.70
	625,000				12	75,000,000	7.88				6	37,500,000	7.57
	625,000				10	62,500,000	7.80				8	50,000,000	7.70
	625,000				20	125,000,000	8.10				4	25,000,000	7.40
12	1,250,000	153	1,912,500,000	9.28	9	112,500,000	8.05	130	1,625,000,000	9.21	1	12,500,000	7.10
	1,250,000	149	1,862,500,000	9.27	8	100,000,000	8.00	145	1,812,500,000	9.26	2	25,000,000	7.40
	1,250,000	145	1,812,500,000	9.26	3	37,500,000	7.57	163	2,037,500,000	9.31	5	62,500,000	7.80
	1,250,000	151	1,887,500,000	9.28	2	25,000,000	7.40	151	1,887,500,000	9.28	5	62,500,000	7.80
		average	1,868,750,000	9.27	**average**	79,492,188	7.87	**average**	1,840,625,000	9.26	**average**	34,140,625	7.50
		stdev	42,695,628	0.010	**stdev**	28,060,545	0.186	**stdev**	171,505,284	0.041	**stdev**	14,151,536	0.186
		N =	4		**N =**	16		**N =**	4		**N =**	20	

		0NAC10			500NAC10			0LCEE10			500LCEE10		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125												
	3,125												
	3,125												
	3,125												
7	15,625												
	15,625												
	15,625												
	15,625												
8	78,125				31	24,218,750	7.38						
	78,125				25	19,531,250	7.29						
	78,125				31	24,218,750	7.38						
	78,125				28	21,875,000	7.34						
9	156,250				18	28,125,000	7.45				79	123,437,500	8.09
	156,250				16	25,000,000	7.40				83	129,687,500	8.11
	156,250				11	17,187,500	7.24				90	140,625,000	8.15
	156,250				12	18,750,000	7.27				67	104,687,500	8.02
10	312,500				10	31,250,000	7.49				58	181,250,000	8.26
	312,500				6	18,750,000	7.27				59	184,375,000	8.27
	312,500				11	34,375,000	7.54				80	250,000,000	8.40
	312,500				7	21,875,000	7.34				77	240,625,000	8.38
11	625,000				4	25,000,000	7.40				29	181,250,000	8.26
	625,000				6	37,500,000	7.57				14	87,500,000	7.94
	625,000				7	43,750,000	7.64				11	68,750,000	7.84
	625,000				8	50,000,000	7.70				34	212,500,000	8.33
12	1,250,000	142	1,775,000,000	9.25	1	12,500,000	7.10	170	2,125,000,000	9.33	6	75,000,000	7.88
	1,250,000	130	1,625,000,000	9.21	4	50,000,000	7.70	160	2,000,000,000	9.30	9	112,500,000	8.05
	1,250,000	135	1,687,500,000	9.23				167	2,087,500,000	9.32	7	87,500,000	7.94
	1,250,000	129	1,612,500,000	9.21	1	12,500,000	7.10	164	2,050,000,000	9.31	16	200,000,000	8.30
		average	1,675,000,000	9.22	**average**	27,179,276	7.40	**average**	2,065,625,000	9.31	**average**	148,730,469	8.14
		stdev	74,302,310	0.019	**stdev**	11,320,127	0.176	**stdev**	53,400,023	0.011	**stdev**	59,122,871	0.182
		N =	4		**N =**	19		**N =**	4		**N =**	16	

20 hour Charge Data

		0ns20			500ns20			0LC20			500LC20		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125												
	3,125												
	3,125												
	3,125												
7	15,625												
	15,625												
	15,625												
	15,625												
8	78,125				109	85,156,250	7.93				117	91,406,250	7.96
	78,125				92	71,875,000	7.86				140	109,375,000	8.04
	78,125				100	78,125,000	7.89				104	81,250,000	7.91
	78,125				109	85,156,250	7.93				115	89,843,750	7.95
9	156,250				63	98,437,500	7.99				55	85,937,500	7.93
	156,250				57	89,062,500	7.95				62	96,875,000	7.99
	156,250				53	82,812,500	7.92				49	76,562,500	7.88
	156,250				40	62,500,000	7.80				56	87,500,000	7.94
10	312,500				19	59,375,000	7.77				23	71,875,000	7.86
	312,500				27	84,375,000	7.93				44	137,500,000	8.14
	312,500				37	115,625,000	8.06				26	81,250,000	7.91
	312,500				27	84,375,000	7.93				38	118,750,000	8.07
11	625,000	221	1,381,250,000	9.14	11	68,750,000	7.84	205	1,281,250,000	9.11	4	25,000,000	7.40
	625,000	216	1,350,000,000	9.13	14	87,500,000	7.94	217	1,356,250,000	9.13	8	50,000,000	7.70
	625,000	215	1,343,750,000	9.13	11	68,750,000	7.84	207	1,293,750,000	9.11	10	62,500,000	7.80
	625,000	228	1,425,000,000	9.15	14	87,500,000	7.94	208	1,300,000,000	9.11	21	131,250,000	8.12
12	1,250,000	147	1,837,500,000	9.26	8	100,000,000	8.00	111	1,387,500,000	9.14	2	25,000,000	7.40
	1,250,000	145	1,812,500,000	9.26	10	125,000,000	8.10	114	1,425,000,000	9.15	3	37,500,000	7.57
	1,250,000	119	1,487,500,000	9.17	12	150,000,000	8.18	87			4	50,000,000	7.70
	1,250,000	119	1,487,500,000	9.17	8	100,000,000	8.00	106	1,325,000,000	9.12	5	62,500,000	7.80
		average	1,515,625,000	9.18	average	89,218,750	7.94	average	1,338,392,857	9.13	average	78,593,750	7.85
		stdev	198,768,530	0.054	stdev	21,743,318	0.099	stdev	53,452,248	0.017	stdev	31,797,628	0.211
		N =	8		N =	20		N =	7		N =	20	

		0NAC20			500NAC20			0LCEE20			500LCEE20		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125												
	3,125												
	3,125												
	3,125												
7	15,625				147	22,968,750	7.36						
	15,625				149	23,281,250	7.37						
	15,625				137	21,406,250	7.33						
	15,625				176	27,500,000	7.44						
8	78,125				29	22,656,250	7.36						
	78,125				72	56,250,000	7.75						
	78,125				57	44,531,250	7.65						
	78,125												
9	156,250				8	12,500,000	7.10				66	103,125,000	8.01
	156,250				27	42,187,500	7.63				124	193,750,000	8.29
	156,250				37	57,812,500	7.76				107	167,187,500	8.22
	156,250												
10	312,500				10	31,250,000	7.49				56	175,000,000	8.24
	312,500				10	31,250,000	7.49				39	121,875,000	8.09
	312,500				12	37,500,000	7.57				55	171,875,000	8.24
	312,500				15	46,875,000	7.67				47	146,875,000	8.17
11	625,000	217	1,356,250,000	9.13	2	12,500,000	7.10	149	931,250,000	8.97	37	231,250,000	8.36
	625,000	239	1,493,750,000	9.17	1	6,250,000	6.80	150	937,500,000	8.97	36	225,000,000	8.35
	625,000	205	1,281,250,000	9.11	3	18,750,000	7.27	168	1,050,000,000	9.02	28	175,000,000	8.24
	625,000	183	1,143,750,000	9.06				152	950,000,000	8.98	32	200,000,000	8.30
12	1,250,000	109	1,362,500,000	9.13	2	25,000,000	7.40	72	900,000,000	8.95	11	137,500,000	8.14
	1,250,000	107	1,337,500,000	9.13	5	62,500,000	7.80	71	887,500,000	8.95	17	212,500,000	8.33
	1,250,000	92	1,150,000,000	9.06	3	37,500,000	7.57	67	837,500,000	8.92	18	225,000,000	8.35
	1,250,000	86	1,075,000,000	9.03	3	37,500,000	7.57	80	1,000,000,000	9.00	12	150,000,000	8.18
		average	1,275,000,000	9.10	average	32,284,226	7.45	average	936,718,750	8.97	average	175,729,167	8.23
		stdev	140,867,846	0.048	stdev	15,463,420	0.248	stdev	66,180,683	0.031	stdev	39,115,440	0.103
		N =	8		N =	20		N =	8		N =	15	

40 hour Charge Data

		0ns40			500ns40			0LC40			500LC40			0NAC40			500NAC40			0LCEE40			500LCEE40		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125	[redacted]						[redacted]						[redacted]						[redacted]					
	3,125																								
	3,125																								
	3,125																								
7	15,625																								
	15,625																								
	15,625																								
	15,625																								
8	78,125																								
	78,125																								
	78,125																								
	78,125																								
9	156,250				71	110,937,500	8.05				164	256,250,000	8.41				30	46,875,000	7.67				67	104,687,500	8.02
	156,250				73	114,062,500	8.06				142	221,875,000	8.35				40	62,500,000	7.80				61	95,312,500	7.98
	156,250				70	109,375,000	8.04				165	257,812,500	8.41				32	50,000,000	7.70				71	110,937,500	8.05
	156,250				66	103,125,000	8.01				149	232,812,500	8.37				38	59,375,000	7.77				68	106,250,000	8.03
10	312,500				41	128,125,000	8.11				60	187,500,000	8.27				20	62,500,000	7.80				30	93,750,000	7.97
	312,500				32	100,000,000	8.00				60	187,500,000	8.27				20	62,500,000	7.80				25	78,125,000	7.89
	312,500				36	112,500,000	8.05				68	212,500,000	8.33				15	46,875,000	7.67				41	128,125,000	8.11
	312,500				27	84,375,000	7.93				90	281,250,000	8.45				18	56,250,000	7.75				24	75,000,000	7.88
11	625,000	181	1,131,250,000	9.05	16	100,000,000	8.00	198	1,237,500,000	9.09	32	200,000,000	8.30	160	1,000,000,000	9.00	9	56,250,000	7.75	125	781,250,000	8.89	12	75,000,000	7.88
	625,000	185	1,156,250,000	9.06	17	106,250,000	8.03	194	1,212,500,000	9.08	36	225,000,000	8.35	161	1,006,250,000	9.00	10	62,500,000	7.80	117	731,250,000	8.86	16	100,000,000	8.00
	625,000	152	950,000,000	8.98	12	75,000,000	7.88	208	1,300,000,000	9.11	36	225,000,000	8.35	176	1,100,000,000	9.04	9	56,250,000	7.75	103	643,750,000	8.81	19	118,750,000	8.07
	625,000	138	862,500,000	8.94	21	131,250,000	8.12	203	1,268,750,000	9.10	29	181,250,000	8.26	158	987,500,000	8.99	5	31,250,000	7.49				14	87,500,000	7.94
12	1,250,000	110	1,375,000,000	9.14	8	100,000,000	8.00	92	1,150,000,000	9.06	13	162,500,000	8.21	67	837,500,000	8.92	4	50,000,000	7.70	73	912,500,000	8.96	4	50,000,000	7.70
	1,250,000	100	1,250,000,000	9.10	9	112,500,000	8.05	96	1,200,000,000	9.08	10	125,000,000	8.10	90	1,125,000,000	9.05	4	50,000,000	7.70	67	837,500,000	8.92	5	62,500,000	7.80
	1,250,000	90	1,125,000,000	9.05	8	100,000,000	8.00	138	1,725,000,000	9.24	32	400,000,000	8.60	97	1,212,500,000	9.08	2	25,000,000	7.40	81	1,012,500,000	9.01	6	75,000,000	7.88
	1,250,000	90	1,125,000,000	9.05	8	100,000,000	8.00	114	1,425,000,000	9.15	13	162,500,000	8.21				6	75,000,000	7.88	51	637,500,000	8.80			
		average	1,121,875,000	9.05	**average**	105,468,750	8.02	**average**	1,314,843,750	9.12	**average**	219,921,875	8.33	**average**	1,038,392,857	9.01	**average**	53,320,313	7.71	**average**	793,750,000	8.89	**average**	90,729,167	7.95
		stdev	159,729,123	0.064	**stdev**	13,963,774	0.060	**stdev**	184,981,222	0.056	**stdev**	62,620,977	0.115	**stdev**	120,460,735	0.052	**stdev**	12,314,707	0.119	**stdev**	138,349,648	0.075	**stdev**	21,565,627	0.110
		N =	8		**N =**	16		**N =**	8		**N =**	16		**N =**	7		**N =**	16		**N =**	7		**N =**	15	

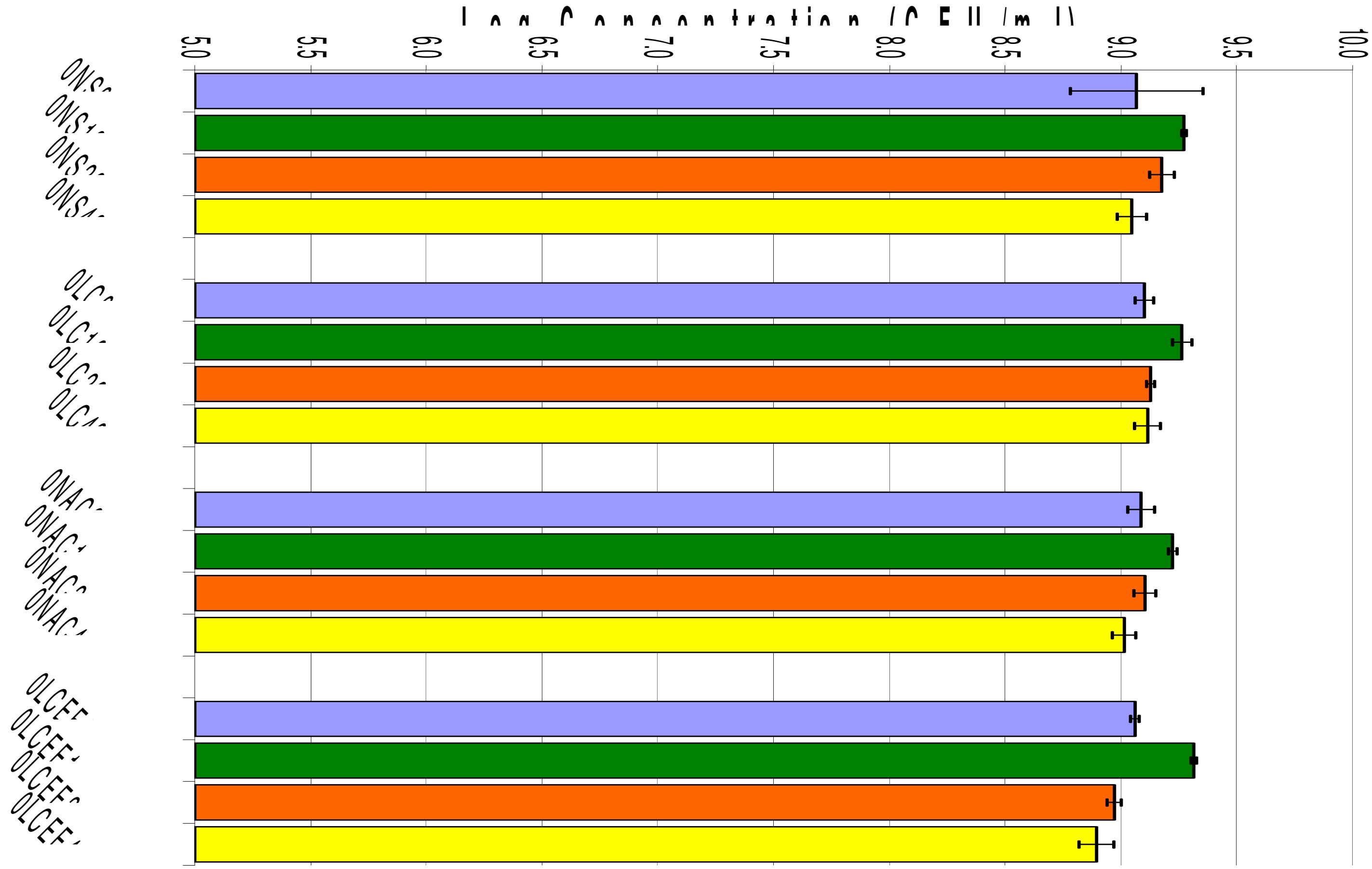


Figure C.01 Charge analysis; *E. coli* concentration control groups non-irradiated Blue = 0 hours incubation; Green = 10 hours incubation; Orange = 20 hours incubation; yellow = 40 hours incubation. A sample marked with ‘*’ indicates a significant difference from its control group (500NS__).

Appendix D

Charge Significance Chart

	0ns0	0ns10	0ns20	0ns40	500ns0	500ns10	500ns20	500ns40	0Lc0	0Lc10	0Lc20	0Lc40	500Lc0	500Lc10	500Lc20	500Lc40
0ns0		NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0ns10	NS		NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0ns20	NS	NS		NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0ns40	NS	NS	NS		S	S	S	S	NS	NS	NS	NS	S	S	S	S
500ns0	S	S	S	S		S	S	S	S	S	S	S	NS	NS	S	S
500ns10	S	S	S	S	S		NS	NS	S	S	S	S	S	S	NS	S
500ns20	S	S	S	S	S	NS		NS	S	S	S	S	S	S	NS	S
500ns40	S	S	S	S	S	NS	NS		S	S	S	S	S	S	NS	S
0Lc0	NS	NS	NS	NS	S	S	S	S		NS	NS	NS	S	S	S	S
0Lc10	NS	NS	NS	NS	S	S	S	S	NS		NS	NS	S	S	S	S
0Lc20	NS	NS	NS	NS	S	S	S	S	NS	NS		NS	S	S	S	S
0Lc40	NS	NS	NS	NS	S	S	S	S	NS	NS	NS		S	S	S	S
500Lc0	S	S	S	S	NS	S	S	S	S	S	S	S		NS	S	S
500Lc10	S	S	S	S	NS	S	S	S	S	S	S	S	NS		S	S
500Lc20	S	S	S	S	S	NS	NS	NS	S	S	S	S	S	S		S
500Lc40	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
0NAC0	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0NAC10	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0NAC20	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0NAC40	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
500NAC0	S	S	S	S	NS	NS	NS	NS	S	S	S	S	S	S	NS	S
500NAC10	S	S	S	S	S	S	S	S	S	S	S	S	NS	NS	S	S
500NAC20	S	S	S	S	NS	S	S	S	S	S	S	S	NS	NS	S	S
500NAC40	S	S	S	S	NS	NS	S	S	S	S	S	S	NS	S	NS	S
0Lcee0	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0Lcee10	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0Lcee20	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0Lcee40	NS	S	NS	NS	S	S	S	S	NS	S	NS	NS	S	S	S	S
500Lcee0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	NS
500Lcee10	S	S	S	S	NS	NS	NS	S	S	S	S	S	S	S	S	NS
500Lcee20	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	NS
500Lcee40	S	S	S	S	S	NS	NS	NS	S	S	S	S	S	S	NS	S

	0NAC0	0NAC10	0NAC20	0NAC40	500NAC0	500NAC10	500NAC20	500NAC40	0Lcee0	0Lcee10	0Lcee20	0Lcee40	500Lceel0	500Lcee10	500Lcee20	500Lcee40
0ns0	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0ns10	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	S	S	S	S	S
0ns20	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0ns40	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
500ns0	S	S	S	S	NS	S	NS	NS	S	S	S	S	S	NS	S	S
500ns10	S	S	S	S	NS	S	S	NS	S	S	S	S	S	NS	S	NS
500ns20	S	S	S	S	NS	S	S	S	S	S	S	S	S	NS	S	NS
500ns40	S	S	S	S	NS	S	S	S	S	S	S	S	S	S	S	NS
0Lc0	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0Lc10	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	S	S	S	S	S
0Lc20	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0Lc40	NS	NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
500Lc0	S	S	S	S	S	NS	NS	NS	S	S	S	S	S	S	S	S
500Lc10	S	S	S	S	S	NS	NS	S	S	S	S	S	S	S	S	S
500Lc20	S	S	S	S	NS	S	S	NS	S	S	S	S	S	S	S	NS
500Lc40	S	S	S	S	S	S	S	S	S	S	S	S	NS	NS	NS	S
0NAC0		NS	NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0NAC10	NS		NS	NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0NAC20	NS	NS		NS	S	S	S	S	NS	NS	NS	NS	S	S	S	S
0NAC40	NS	NS	NS		S	S	S	S	NS	NS	NS	NS	S	S	S	S
500NAC0	S	S	S	S		S	S	NS	S	S	S	S	S	S	S	NS
500NAC10	S	S	S	S	S		NS	S	S	S	S	S	S	NS	S	S
500NAC20	S	S	S	S	S	NS		S	S	S	S	S	S	NS	S	S
500NAC40	S	S	S	S	NS	S	S		S	S	S	S	S	NS	S	S
0Lcee0	NS	NS	NS	NS	S	S	S	S		NS	NS	NS	S	S	S	S
0Lcee10	NS	NS	NS	NS	S	S	S	S	NS		S	S	S	S	S	S
0Lcee20	NS	NS	NS	NS	S	S	S	S	NS	S		NS	S	S	S	S
0Lcee40	NS	NS	NS	NS	S	S	S	S	NS	S	NS		S	S	S	S
500Lcee0	S	S	S	S	S	S	S	S	S	S	S	S		S	S	S
500Lcee10	S	S	S	S	S	NS	NS	NS	S	S	S	S	S		NS	NS
500Lcee20	S	S	S	S	S	S	S	S	S	S	S	S	S	NS		S
500Lcee40	S	S	S	S	NS	S	S	S	S	S	S	S	S	NS	S	

Appendix E

0 hour Size Data

		0ns0			500ns0			0U0			500U0		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125				110	3,437,500	6.54				85	2,656,250	6.42
	3,125				74	2,312,500	6.36				105	3,281,250	6.52
	3,125				115	3,593,750	6.56				116	3,625,000	6.56
	3,125				88	2,750,000	6.44				105	3,281,250	6.52
7	15,625				26	4,062,500	6.61				24	3,750,000	6.57
	15,625				10	1,562,500	6.19				47	7,343,750	6.87
	15,625				22	3,437,500	6.54				38	5,937,500	6.77
	15,625				18	2,812,500	6.45				27	4,218,750	6.63
8	78,125				1	781,250	5.89				5	3,906,250	6.59
	78,125				1	781,250	5.89				4	3,125,000	6.49
	78,125				3	2,343,750	6.37				3	2,343,750	6.37
	78,125				1	781,250	5.89				6	4,687,500	6.67
9	156,250										2	3,125,000	6.49
	156,250				3	4,687,500	6.67				3	4,687,500	6.67
	156,250				1	1,562,500	6.19				2	3,125,000	6.49
	156,250				2	3,125,000	6.49				2	3,125,000	6.49
10	312,500										1	3,125,000	6.49
	312,500				1	3,125,000	6.49				1	3,125,000	6.49
	312,500										1	3,125,000	6.49
	312,500				3								
11	625,000	56	350,000,000	8.54				152	950,000,000	8.98	2		
	625,000	54	337,500,000	8.53				47					
	625,000	155	968,750,000	8.99				126	787,500,000	8.90			
	625,000	160	1,000,000,000	9.00				169	1,056,250,000	9.02	2		
12	1,250,000	71	887,500,000	8.95				73	912,500,000	8.96			
	1,250,000	90	1,125,000,000	9.05				95	1,187,500,000	9.07			
	1,250,000	100	1,250,000,000	9.10				91	1,137,500,000	9.06			
	1,250,000	82	1,025,000,000	9.01				67	837,500,000	8.92	1		
		average	867,968,750	8.90	average	2,572,266	6.35	average	981,250,000	8.99	average	3,768,092	6.56
		stdev	341,076,249	0.226	stdev	1,203,279	0.260	stdev	150,822,398	0.067	stdev	1,203,218	0.120
		N =	8		N =	16		N =	7		N =	19	

		0AA0			500AA0			0EGCG0			500EGCG0		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125										100	3,125,000	6.49
	3,125										96	3,000,000	6.48
	3,125										50	1,562,500	6.19
	3,125										128	4,000,000	6.60
7	15,625				74	11,562,500	7.06				15	2,343,750	6.37
	15,625				64	10,000,000	7.00				14	2,187,500	6.34
	15,625				65	10,156,250	7.01				30	4,687,500	6.67
	15,625				78	12,187,500	7.09				17	2,656,250	6.42
8	78,125				18	14,062,500	7.15				2	1,562,500	6.19
	78,125				22	17,187,500	7.24				5	3,906,250	6.59
	78,125				23	17,968,750	7.25				6	4,687,500	6.67
	78,125				13	10,156,250	7.01				2	1,562,500	6.19
9	156,250				6	9,375,000	6.97				3	4,687,500	6.67
	156,250				12	18,750,000	7.27				1	1,562,500	6.19
	156,250				8	12,500,000	7.10						
	156,250				4	6,250,000	6.80				1	1,562,500	6.19
10	312,500				5	15,625,000	7.19						
	312,500				3	9,375,000	6.97						
	312,500				3	9,375,000	6.97				2	6,250,000	6.80
	312,500				10	31,250,000	7.49				1	3,125,000	6.49
11	625,000	150	937,500,000	8.97				153	956,250,000	8.98			
	625,000	102	637,500,000	8.80							1	6,250,000	6.80
	625,000	145	906,250,000	8.96	1	6,250,000	6.80						
	625,000	150	937,500,000	8.97	1	6,250,000	6.80				1	6,250,000	6.80
12	1,250,000	57	712,500,000	8.85				80	1,000,000,000	9.00	1		
	1,250,000	75	937,500,000	8.97	1	12,500,000	7.10	71	887,500,000	8.95	1		
	1,250,000	70	875,000,000	8.94	2	25,000,000	7.40	70	875,000,000	8.94			
	1,250,000							86	1,075,000,000	9.03			
		average	849,107,143	8.92	average	13,289,063	7.08	average	958,750,000	8.98	average	3,419,408	6.48
		stdev	123,027,593	0.068	stdev	6,337,884	0.189	stdev	82,632,469	0.037	stdev	1,671,358	0.221
		N =	7		N =	20		N =	5		N =	19	

10 hour Size Data

		0ns10			500ns10			0U10			500U10			0AA10			500AA10			0EGCG10			500EGCG10		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125	[redacted]						[redacted]			260	8,125,000	6.91	[redacted]						[redacted]			102	3,187,500	6.50
	3,125										312	9,750,000	6.99										150	4,687,500	6.67
	3,125										328	10,250,000	7.01										150	4,687,500	6.67
	3,125										305	9,531,250	6.98												
7	15,625				29	4,531,250	6.66				20	3,125,000	6.49				57	8,906,250	6.95				14	2,187,500	6.34
	15,625										25	3,906,250	6.59										23	3,593,750	6.56
	15,625										38	5,937,500	6.77										15	2,343,750	6.37
	15,625										59	9,218,750	6.96										40	6,250,000	6.80
8	78,125				7	5,468,750	6.74				3	2,343,750	6.37				14	10,937,500	7.04				3	2,343,750	6.37
	78,125				15	11,718,750	7.07				6	4,687,500	6.67				19	14,843,750	7.17						
	78,125				33	25,781,250	7.41				5	3,906,250	6.59				17	13,281,250	7.12				2	1,562,500	6.19
	78,125										10	7,812,500	6.89				4	3,125,000	6.49				9	7,031,250	6.85
9	156,250	199	310,937,500	8.49				284	443,750,000	8.65	8	12,500,000	7.10	236	368,750,000	8.57	3	4,687,500	6.67	225	351,562,500	8.55	2	3,125,000	6.49
	156,250	180	281,250,000	8.45	11	17,187,500	7.24	254	396,875,000	8.60	2	3,125,000	6.49	213	332,812,500	8.52	13	20,312,500	7.31	166	259,375,000	8.41	1	1,562,500	6.19
	156,250	219	342,187,500	8.53	5	7,812,500	6.89	278	434,375,000	8.64	5	7,812,500	6.89	241	376,562,500	8.58	10	15,625,000	7.19	223	348,437,500	8.54	1	1,562,500	6.19
	156,250	204	318,750,000	8.50	7	10,937,500	7.04	298	465,625,000	8.67	10	15,625,000	7.19	329	514,062,500	8.71	14	21,875,000	7.34	320	500,000,000	8.70	6	9,375,000	6.97
10	312,500	117	365,625,000	8.56	5	15,625,000	7.19	146	456,250,000	8.66	9	28,125,000	7.45	95	296,875,000	8.47									
	312,500				1	3,125,000	6.49	153	478,125,000	8.68	2	6,250,000	6.80	40	125,000,000	8.10	1	3,125,000	6.49						
	312,500				1	3,125,000	6.49				6	18,750,000	7.27	80	250,000,000	8.40	1	3,125,000	6.49						
	312,500				1	3,125,000	6.49				4	12,500,000	7.10	78	243,750,000	8.39	10	31,250,000	7.49				1	3,125,000	6.49
11	625,000	84	525,000,000	8.72				27	168,750,000	8.23				62	387,500,000	8.59	1	6,250,000	6.80	94	587,500,000	8.77	1	6,250,000	6.80
	625,000	70	437,500,000	8.64				29	181,250,000	8.26				51	318,750,000	8.50	15	93,750,000	7.97	80	500,000,000	8.70			
	625,000	65	406,250,000	8.61	1	6,250,000	6.80				4	25,000,000	7.40	75	468,750,000	8.67	2	12,500,000	7.10	101	631,250,000	8.80			
	625,000																1	6,250,000	6.80	60	375,000,000	8.57			
12	1,250,000	51	637,500,000	8.80	4	50,000,000	7.70	49	612,500,000	8.79				29	362,500,000	8.56				49	612,500,000	8.79			
	1,250,000	58	725,000,000	8.86	1	12,500,000	7.10	29	362,500,000	8.56				14	175,000,000	8.24				37	462,500,000	8.67	1	12,500,000	7.10
	1,250,000	24	300,000,000	8.48				47	587,500,000	8.77	1	12,500,000	7.10	21	262,500,000	8.42	4	50,000,000	7.70	23	287,500,000	8.46			
	1,250,000	17	212,500,000	8.33				30	375,000,000	8.57				5	62,500,000	7.80				20	250,000,000	8.40	1	12,500,000	7.10
		average	405,208,333	8.58	average	12,656,250	6.95	average	413,541,667	8.59	average	10,035,511	6.91	average	303,020,833	8.43	average	18,814,338	7.07	average	430,468,750	8.61	average	4,881,944	6.59
		stdev	152,878,880	0.154	stdev	12,572,058	0.366	stdev	134,215,242	0.175	stdev	6,833,698	0.293	stdev	121,736,796	0.237	stdev	22,727,398	0.423	stdev	137,218,041	0.144	stdev	3,502,946	0.295
		N =	12		N =	14		N =	12		N =	22		N =	15		N =	17		N =	12		N =	18	

20 hour Size Data

		0ns20			500ns20			0U20			500U20			0AA20			500AA20			0EGCG20			500EGCG20		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125	[redacted]			260	8,125,000	6.91	[redacted]			481	15,031,250	7.18	[redacted]						[redacted]					
	3,125	[redacted]			235	7,343,750	6.87	[redacted]						[redacted]						[redacted]					
	3,125	[redacted]						[redacted]						[redacted]						[redacted]					
	3,125	[redacted]						[redacted]						[redacted]						[redacted]					
7	15,625	[redacted]			120	18,750,000	7.27	[redacted]						[redacted]			333	52,031,250	7.72	[redacted]			40	6,250,000	6.80
	15,625	[redacted]			93	14,531,250	7.16	[redacted]						[redacted]						[redacted]			98	15,312,500	7.19
	15,625	[redacted]			76	11,875,000	7.07	[redacted]						[redacted]						[redacted]			55	8,593,750	6.93
	15,625	[redacted]						[redacted]						[redacted]						[redacted]			81	12,656,250	7.10
8	78,125	[redacted]			14	10,937,500	7.04	[redacted]			45	35,156,250	7.55	[redacted]			75	58,593,750	7.77	[redacted]			14	10,937,500	7.04
	78,125	[redacted]			17	13,281,250	7.12	[redacted]			36	28,125,000	7.45	[redacted]						[redacted]			8	6,250,000	6.80
	78,125	[redacted]			23	17,968,750	7.25	[redacted]						[redacted]						[redacted]			14	10,937,500	7.04
	78,125	[redacted]			55	42,968,750	7.63	[redacted]						[redacted]						[redacted]					
9	156,250				26	40,625,000	7.61	240	375,000,000	8.57							28	43,750,000	7.64						
	156,250				13	20,312,500	7.31	186	290,625,000	8.46	26	40,625,000	7.61				28	43,750,000	7.64				7	10,937,500	7.04
	156,250				25	39,062,500	7.59	215	335,937,500	8.53	27	42,187,500	7.63				17	26,562,500	7.42				17	26,562,500	7.42
	156,250				22	34,375,000	7.54	204	318,750,000	8.50	14	21,875,000	7.34				19	29,687,500	7.47				13	20,312,500	7.31
10	312,500	337	1,053,125,000	9.02	3	9,375,000	6.97	85	265,625,000	8.42	16	50,000,000	7.70	315	984,375,000	8.99	6	18,750,000	7.27						
	312,500	335	1,046,875,000	9.02	4	12,500,000	7.10	265	828,125,000	8.92	7	21,875,000	7.34	185	578,125,000	8.76	2	6,250,000	6.80						
	312,500	375	1,171,875,000	9.07	9	28,125,000	7.45				1	3,125,000	6.49	220	687,500,000	8.84	10	31,250,000	7.49				1	3,125,000	6.49
	312,500	314	981,250,000	8.99	7	21,875,000	7.34				2	6,250,000	6.80	130	406,250,000	8.61	20	62,500,000	7.80				4	12,500,000	7.10
11	625,000	59	368,750,000	8.57	10	62,500,000	7.80	13	81,250,000	7.91	1	6,250,000	6.80				2	12,500,000	7.10	164	1,025,000,000	9.01	6	37,500,000	7.57
	625,000	29	181,250,000	8.26	19	118,750,000	8.07	24	150,000,000	8.18	18						10	62,500,000	7.80				2	12,500,000	7.10
	625,000	29	181,250,000	8.26	15	93,750,000	7.97	13	81,250,000	7.91							9	56,250,000	7.75				1	6,250,000	6.80
	625,000	61	381,250,000	8.58				67	418,750,000	8.62	8	50,000,000	7.70				2	12,500,000	7.10				6	37,500,000	7.57
12	1,250,000	39	487,500,000	8.69	11	137,500,000	8.14	105	1,312,500,000	9.12				61	762,500,000	8.88	3	37,500,000	7.57	81	1,012,500,000	9.01	3	37,500,000	7.57
	1,250,000	9	112,500,000	8.05	10	125,000,000	8.10	54	675,000,000	8.83	1	12,500,000	7.10	46	575,000,000	8.76	3	37,500,000	7.57	82	1,025,000,000	9.01	4	50,000,000	7.70
	1,250,000	3	37,500,000	7.57	3	37,500,000	7.57	14	175,000,000	8.24	1	12,500,000	7.10	49	612,500,000	8.79				20	250,000,000	8.40	2	25,000,000	7.40
	1,250,000	51	637,500,000	8.80	3	37,500,000	7.57							33	412,500,000	8.62	1	12,500,000	7.10	39	487,500,000	8.69	2	25,000,000	7.40
		average	553,385,417	8.57	**average**	40,188,802	7.44	**average**	408,293,269	8.48	**average**	24,678,571	7.27	**average**	627,343,750	8.78	**average**	35,551,471	7.47	**average**	760,000,000	8.82	**average**	18,781,250	7.17
		stdev	412,475,159	0.463	**stdev**	38,981,791	0.383	**stdev**	347,455,984	0.360	**stdev**	16,441,194	0.377	**stdev**	188,899,169	0.129	**stdev**	18,864,811	0.299	**stdev**	366,934,088	0.275	**stdev**	13,177,039	0.321
		N =	12		**N =**	24		**N =**	13		**N =**	14		**N =**	8		**N =**	17		**N =**	5		**N =**	20	

40 hour Size Data

		0ns40			500ns40			0U40			500U40		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125	[redacted]						[redacted]					
	3,125												
	3,125												
	3,125												
7	15,625				211	32,968,750	7.52				88	13,750,000	7.14
	15,625				298	46,562,500	7.67				109	17,031,250	7.23
	15,625				352	55,000,000	7.74				86	13,437,500	7.13
	15,625				97	15,156,250	7.18						
8	78,125				23	17,968,750	7.25				51	39,843,750	7.60
	78,125				40	31,250,000	7.49				26	20,312,500	7.31
	78,125				45	35,156,250	7.55						
	78,125				135	105,468,750	8.02						
9	156,250							318	496,875,000	8.70	25	39,062,500	7.59
	156,250							303	473,437,500	8.68	39	60,937,500	7.78
	156,250							320	500,000,000	8.70	36	56,250,000	7.75
	156,250							88	137,500,000	8.14	29	45,312,500	7.66
10	312,500	240	750,000,000	8.88				103	321,875,000	8.51	15	46,875,000	7.67
	312,500	204	637,500,000	8.80				98	306,250,000	8.49	2	6,250,000	6.80
	312,500	216	675,000,000	8.83				128	400,000,000	8.60	6	18,750,000	7.27
	312,500							176	550,000,000	8.74	4	12,500,000	7.10
11	625,000	124	775,000,000	8.89				310	1,937,500,000	9.29	8	50,000,000	7.70
	625,000	140	875,000,000	8.94							1	6,250,000	6.80
	625,000	93	581,250,000	8.76							5	31,250,000	7.49
	625,000										8	50,000,000	7.70
12	1,250,000	148			61	762,500,000	8.88	130	1,625,000,000	9.21	3	37,500,000	7.57
	1,250,000	33	412,500,000	8.62	48	600,000,000	8.78	106	1,325,000,000	9.12	1	12,500,000	7.10
	1,250,000	65	812,500,000	8.91	100	1,250,000,000	9.10				4	50,000,000	7.70
	1,250,000	30	375,000,000	8.57	87	1,087,500,000	9.04						
		average	654,861,111	8.80	**average**	336,627,604	8.02	**average**	733,948,864	8.74	**average**	31,390,625	7.40
		stdev	173,198,816	0.129	**stdev**	461,904,511	0.723	**stdev**	602,037,593	0.343	**stdev**	18,109,602	0.316
		N =	9		**N =**	12		**N =**	11		**N =**	20	

		0AA40			500AA40			0EGCG40			500EGCG40		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125	[redacted]						[redacted]					
	3,125												
	3,125												
	3,125												
7	15,625										74	11,562,500	7.06
	15,625												
	15,625												
	15,625												
8	78,125										6	4,687,500	6.67
	78,125										5	3,906,250	6.59
	78,125										4	3,125,000	6.49
	78,125										1	781,250	5.89
9	156,250				64	100,000,000	8.00				10	15,625,000	7.19
	156,250				48	75,000,000	7.88				11	17,187,500	7.24
	156,250				51	79,687,500	7.90				10	15,625,000	7.19
	156,250				31	48,437,500	7.69						
10	312,500	272	850,000,000	8.93	17	53,125,000	7.73				3	9,375,000	6.97
	312,500				14	43,750,000	7.64				5	15,625,000	7.19
	312,500				11	34,375,000	7.54				2	6,250,000	6.80
	312,500				7	21,875,000	7.34				1	3,125,000	6.49
11	625,000	166	1,037,500,000	9.02	22	137,500,000	8.14	36	225,000,000	8.35			
	625,000	190	1,187,500,000	9.07	40	250,000,000	8.40	43	268,750,000	8.43			
	625,000	152	950,000,000	8.98	30	187,500,000	8.27						
	625,000	212	1,325,000,000	9.12									
12	1,250,000	172			2	25,000,000	7.40	153	1,912,500,000	9.28	1	12,500,000	7.10
	1,250,000				5	62,500,000	7.80	140	1,750,000,000	9.24			
	1,250,000				8	100,000,000	8.00	21	262,500,000	8.42	1	12,500,000	7.10
	1,250,000				13	162,500,000	8.21	23	287,500,000	8.46	1	12,500,000	7.10
		average	1,070,000,000	9.02	**average**	92,083,333	7.86	**average**	784,375,000	8.70	**average**	9,625,000	6.87
		stdev	188,869,995	0.076	**stdev**	66,023,626	0.315	**stdev**	812,786,007	0.439	**stdev**	5,509,668	0.379
		N =	5		**N =**	15		**N =**	6		**N =**	15	

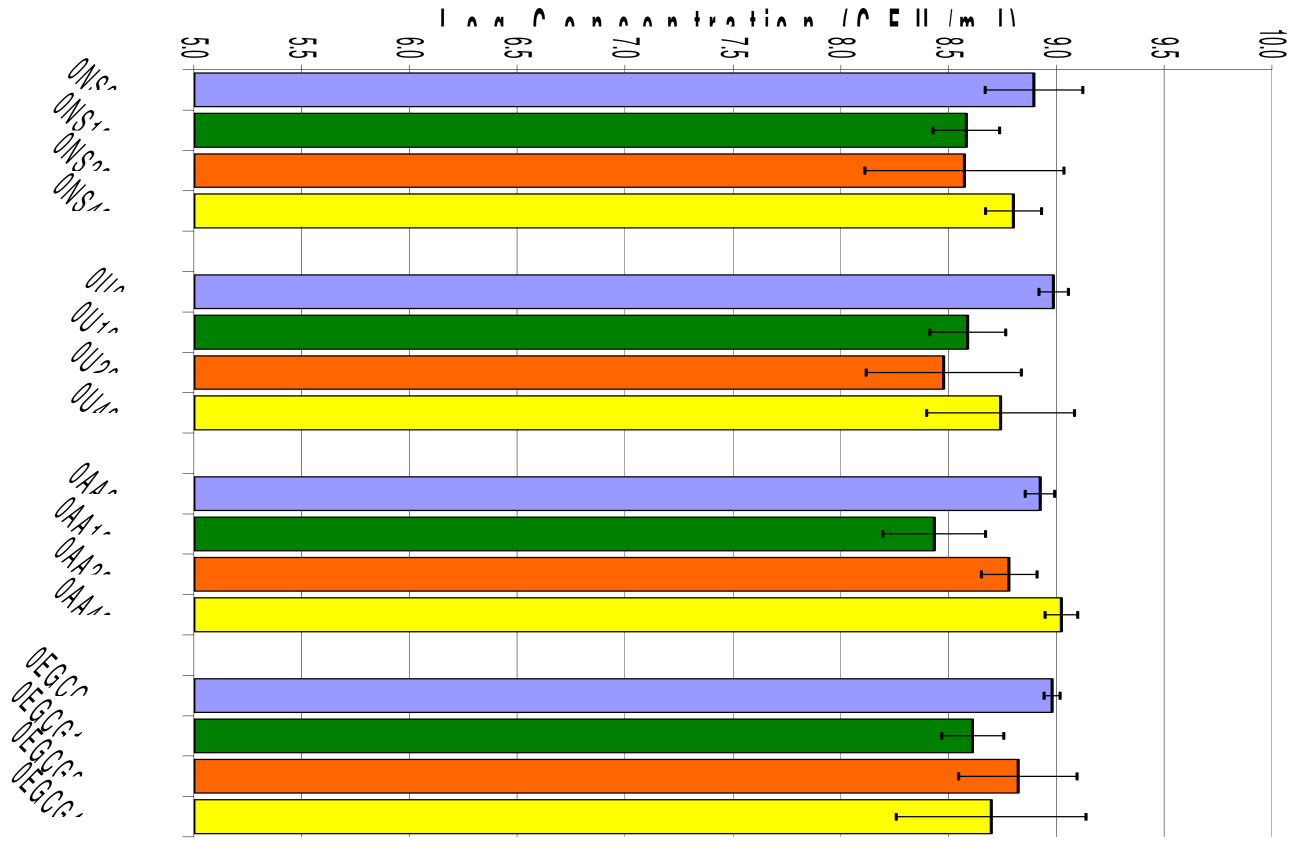


Figure E.01 Size analysis; *E. coli* concentration control groups non-irradiated Blue = 0 hours incubation; Green = 10 hours incubation; Orange = 20 hours incubation; yellow = 40 hours incubation. A sample marked with ‘*’ indicates a significant difference from its control group (500NS__).

Appendix F

Size Significance Chart

	0ns0	500ns0	0U0	500U0	0AA0	500AA0	0EGCG0	500EGCG0	0ns10	500ns10	0U10	500U10	0AA10	500AA10	0EGCG10	500EGCG10
0ns0		S	NS	S	NS	S	NS	S	NS	S	NS	S	S	S	NS	S
500ns0	S		S	NS	S	S	S	NS	S	S	S	S	S	S	S	NS
0U0	NS	S		S	S	S	NS	S	NS	S	NS	S	S	S	NS	S
500U0	S	NS	S		S	S	S	NS	S	NS	S	NS	S	S	S	NS
0AA0	NS	S	S	S		S	NS	S	NS	S	NS	S	NS	S	NS	S
500AA0	S	S	S	S	S		S	S	S	NS	S	NS	S	NS	S	S
0EGCG0	NS	S	NS	S	NS	S		S	NS	S	NS	S	NS	S	NS	S
500EGCG0	S	NS	S	NS	S	S	S		S	S	S	S	S	S	S	NS
0ns10	NS	S	NS	S	NS	S	NS	S		S	NS	S	NS	S	NS	S
500ns10	S	S	S	NS	S	NS	S	S	S		S	NS	S	NS	S	NS
0U10	NS	S	NS	S	NS	S	NS	S	NS	S		S	NS	S	NS	S
500U10	S	S	S	NS	S	NS	S	S	S	NS	S		S	NS	S	NS
0AA10	S	S	S	S	NS	S	NS	S	NS	S	NS	S		S	NS	S
500AA10	S	S	S	S	S	NS	S	S	S	NS	S	NS	S		S	S
0EGCG10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S		S
500EGCG10	S	NS	S	NS	S	S	S	NS	S	NS	S	NS	S	S	S	
0ns20	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns20	S	S	S	S	S	NS	S	S	S	S	S	S	S	NS	S	NS
0U20	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500U20	S	S	S	S	S	NS	S	S	S	NS	S	NS	S	NS	S	S
0AA20	NS	S	NS	S	NS	S	NS	S	S	S	NS	S	NS	S	NS	S
500AA20	S	NS	S	S	S	NS	S	S	S	NS	S	S	S	NS	S	S
0EGCG20	NS	S	NS	S	NS	NS	NS	S	NS	S	NS	S	NS	S	NS	S
500EGCG20	S	S	S	NS	S	NS	S	S	S	NS	S	NS	S	NS	S	S
0ns40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns40	S	S	S	S	S	S	S	S	S	S	S	S	NS	S	S	NS
0U40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500U40	S	S	S	S	S	NS	S	S	S	S	S	S	S	NS	S	S
0AA40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	S	S
500AA40	S	S	S	S	S	NS	S	S	S	S	S	S	S	S	S	S
0EGCG40	NS	S	NS	S	NS	S	NS	S	S	S	NS	S	NS	NS	NS	S
500EGCG40	S	S	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS

	0ns20	500ns20	0U20	500U20	0AA20	500AA20	0EGCG20	500EGCG20	0ns40	500ns40	0U40	500U40	0AA40	500AA40	0EGCG40	500EGCG40
0ns0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns0	S	S	S	S	S	NS	S	S	S	S	S	S	S	S	S	S
0U0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500U0	S	S	S	S	S	S	S	NS	S	S	S	S	S	S	S	NS
0AA0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500AA0	S	NS	S	NS	S	NS	NS	NS	S	S	S	NS	S	NS	S	NS
0EGCG0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500EGCG0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	NS
0ns10	NS	S	NS	S	S	S	NS	S	NS	S	NS	S	NS	S	S	S
500ns10	S	S	S	NS	S	NS	S	NS	S	S	S	S	S	S	S	NS
0U10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500U10	S	S	S	NS	S	S	S	NS	S	S	S	S	S	S	S	NS
0AA10	NS	S	NS	S	NS	S	NS	S	NS	NS	NS	S	NS	S	NS	S
500AA10	S	NS	S	NS	S	NS	S	NS	S	S	S	NS	S	S	NS	NS
0EGCG10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	S	S	NS	S
500EGCG10	S	NS	S	S	S	S	S	S	S	NS	S	S	S	S	S	NS
0ns20		S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns20	S		S	NS	S	NS	S	NS	S	S	S	NS	S	S	S	S
0U20	NS	S		S	NS	S	NS	S	NS	NS	NS	S	NS	S	NS	S
500U20	S	NS	S		S	NS	S	NS	S	S	S	NS	S	S	S	NS
0AA20	NS	S	NS	S		S	NS	S	NS	S	NS	S	NS	S	S	NS
500AA20	S	NS	S	NS	S		S	NS	S	NS	S	NS	S	NS	S	NS
0EGCG20	NS	S	NS	S	NS	S		S	NS	S	NS	S	NS	S	NS	S
500EGCG20	S	NS	S	NS	S	NS	S		S	S	S	NS	S	S	S	NS
0ns40	NS	S	NS	S	NS	S	NS	S		S	NS	S	S	S	S	S
500ns40	S	S	NS	S	S	NS	S	S	S		S	S	S	NS	S	S
0U40	NS	S	NS	S	NS	S	NS	S	NS	S		S	NS	S	NS	S
500U40	S	NS	S	NS	S	NS	S	NS	S	S	S		S	S	S	S
0AA40	NS	S	NS	S	NS	S	NS	S	S	S	NS	S		S	NS	S
500AA40	S	S	S	S	S	NS	S	S	S	NS	S	S	S		S	S
0EGCG40	NS	S	NS	S	S	S	NS	S	S	S	NS	S	NS	S		S
500EGCG40	S	S	S	NS	NS	NS	S	NS	S	S	S	S	S	S	S	

Appendix G

0 hour Shape Data

		0ns0			500ns0			0T0			500T0			0G0			500G0		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125																		
	3,125																		
	3,125																		
	3,125																		
7	15,625				70	10,937,500	7.04												
	15,625				100	15,625,000	7.19												
	15,625				65	10,156,250	7.01												
	15,625				70	10,937,500	7.04												
8	78,125				20	15,625,000	7.19				67	52,343,750	7.72				54	42,187,500	7.63
	78,125				13	10,156,250	7.01				64	50,000,000	7.70				52	40,625,000	7.61
	78,125				16	12,500,000	7.10				79	61,718,750	7.79				60	46,875,000	7.67
	78,125				24	18,750,000	7.27				73	57,031,250	7.76				65	50,781,250	7.71
9	156,250				8	12,500,000	7.10				35	54,687,500	7.74				19	29,687,500	7.47
	156,250				6	9,375,000	6.97				35	54,687,500	7.74				13	20,312,500	7.31
	156,250				5	7,812,500	6.89				31	48,437,500	7.69				20	31,250,000	7.49
	156,250				6	9,375,000	6.97				38	59,375,000	7.77				28	43,750,000	7.64
10	312,500				4	12,500,000	7.10				14	43,750,000	7.64				14	43,750,000	7.64
	312,500				8	25,000,000	7.40				18	56,250,000	7.75				12	37,500,000	7.57
	312,500				4	12,500,000	7.10				15	46,875,000	7.67				2	6,250,000	6.80
	312,500				8	25,000,000	7.40				17	53,125,000	7.73				8	25,000,000	7.40
11	625,000	367	2,293,750,000	9.36	12	75,000,000	7.88	152	950,000,000	8.98	7	43,750,000	7.64	214	1,337,500,000	9.13	5	31,250,000	7.49
	625,000	381	2,381,250,000	9.38	6	37,500,000	7.57	222	1,387,500,000	9.14	13	81,250,000	7.91	209	1,306,250,000	9.12	7	43,750,000	7.64
	625,000	421	2,631,250,000	9.42	9	56,250,000	7.75	179	1,118,750,000	9.05	9	56,250,000	7.75	228	1,425,000,000	9.15	5	31,250,000	7.49
	625,000	434	2,712,500,000	9.43	8	50,000,000	7.70	204	1,275,000,000	9.11	5	31,250,000	7.49	212	1,325,000,000	9.12	8	50,000,000	7.70
12	1,250,000				3	37,500,000	7.57	91	1,137,500,000	9.06	3	37,500,000	7.57	108	1,350,000,000	9.13	2	25,000,000	7.40
	1,250,000				4	50,000,000	7.70	99	1,237,500,000	9.09	7	87,500,000	7.94	82	1,025,000,000	9.01	7		
	1,250,000				1	12,500,000	7.10	115	1,437,500,000	9.16	4	50,000,000	7.70	23	287,500,000	8.46	1	12,500,000	7.10
	1,250,000				1	12,500,000	7.10	94	1,175,000,000	9.07	3	37,500,000	7.57	54	675,000,000	8.83	3	37,500,000	7.57
		average	2,504,687,500	9.40	average	22,916,667	7.26	average	1,214,843,750	9.08	average	53,164,063	7.71	average	1,091,406,250	8.99	average	34,169,408	7.49
		stdev	199,110,980	0.035	stdev	18,305,514	0.290	stdev	156,051,213	0.057	stdev	13,276,351	0.104	stdev	407,695,745	0.241	stdev	12,352,214	0.227
		N =	4		N =	24		N =	8		N =	20		N =	8		N =	19	

10 hour Shape Data

		0ns10			500ns10			0T10			500T10			0G10			500G10		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125	[redacted]						[redacted]						[redacted]					
	3,125	[redacted]						[redacted]						[redacted]					
	3,125	[redacted]						[redacted]						[redacted]					
	3,125	[redacted]						[redacted]						[redacted]					
7	15,625	[redacted]						[redacted]						[redacted]					
	15,625	[redacted]						[redacted]						[redacted]					
	15,625	[redacted]						[redacted]						[redacted]					
	15,625	[redacted]						[redacted]						[redacted]					
8	78,125	[redacted]						[redacted]			226	176,562,500	8.25	[redacted]			21	16,406,250	7.22
	78,125	[redacted]						[redacted]			238	185,937,500	8.27	[redacted]			17	13,281,250	7.12
	78,125	[redacted]						[redacted]			260	203,125,000	8.31	[redacted]			50	39,062,500	7.59
	78,125	[redacted]						[redacted]			228	178,125,000	8.25	[redacted]					
9	156,250				139	217,187,500	8.34	857	1,339,062,500	9.13	60	93,750,000	7.97	736	1,150,000,000	9.06	51	79,687,500	7.90
	156,250				120	187,500,000	8.27	432	675,000,000	8.83	109	170,312,500	8.23	797	1,245,312,500	9.10	54	84,375,000	7.93
	156,250				75	117,187,500	8.07	281	439,062,500	8.64	29	45,312,500	7.66	796	1,243,750,000	9.09	24	37,500,000	7.57
	156,250				73	114,062,500	8.06	295	460,937,500	8.66	117	182,812,500	8.26	616	962,500,000	8.98	19	29,687,500	7.47
10	312,500				95	296,875,000	8.47	622	1,943,750,000	9.29	12	37,500,000	7.57	435	1,359,375,000	9.13	9	28,125,000	7.45
	312,500				99	309,375,000	8.49	98	306,250,000	8.49	34	106,250,000	8.03	424	1,325,000,000	9.12	9	28,125,000	7.45
	312,500				95	296,875,000	8.47	526	1,643,750,000	9.22	37	115,625,000	8.06	392	1,225,000,000	9.09	4	12,500,000	7.10
	312,500				70	218,750,000	8.34	311	971,875,000	8.99				496	1,550,000,000	9.19	31	96,875,000	7.99
11	625,000	305	1,906,250,000	9.28	16	100,000,000	8.00	301	1,881,250,000	9.27	2	12,500,000	7.10	265	1,656,250,000	9.22	6	37,500,000	7.57
	625,000	291	1,818,750,000	9.26	19	118,750,000	8.07	320	2,000,000,000	9.30	10	62,500,000	7.80	245	1,531,250,000	9.19	1	6,250,000	6.80
	625,000	287	1,793,750,000	9.25	28	175,000,000	8.24				16	100,000,000	8.00	252	1,575,000,000	9.20	1	6,250,000	6.80
	625,000				25	156,250,000	8.19				6	37,500,000	7.57	242	1,512,500,000	9.18			
12	1,250,000	163	2,037,500,000	9.31	7	87,500,000	7.94	194	2,425,000,000	9.38	1	12,500,000	7.10	132	1,650,000,000	9.22	1	12,500,000	7.10
	1,250,000	150	1,875,000,000	9.27	7	87,500,000	7.94	175	2,187,500,000	9.34	9	112,500,000	8.05	133	1,662,500,000	9.22	1	12,500,000	7.10
	1,250,000	180	2,250,000,000	9.35	7	87,500,000	7.94	185	2,312,500,000	9.36	11	137,500,000	8.14	147	1,837,500,000	9.26	2	25,000,000	7.40
	1,250,000	212	2,650,000,000	9.42	13	162,500,000	8.21	177	2,212,500,000	9.34	8	100,000,000	8.00	137	1,712,500,000	9.23	2	25,000,000	7.40
		average	2,047,321,429	9.31	**average**	170,800,781	8.19	**average**	1,485,602,679	9.09	**average**	108,963,816	7.93	**average**	1,449,902,344	9.16	**average**	32,812,500	7.39
		stdev	308,365,507	0.061	**stdev**	77,740,133	0.195	**stdev**	770,706,055	0.310	**stdev**	62,212,538	0.372	**stdev**	240,755,152	0.076	**stdev**	27,136,161	0.351
		N =	7		**N =**	16		**N =**	14		**N =**	19		**N =**	16		**N =**	18	

20 hour Shape Data

		0ns20			500ns20			0T20			500T20			0G20			500G20		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125																		
	3,125																		
	3,125																		
	3,125																		
7	15,625				221	34,531,250	7.54										237	37,031,250	7.57
	15,625				241	37,656,250	7.58										181	28,281,250	7.45
	15,625				200	31,250,000	7.49										289	45,156,250	7.65
	15,625				245	38,281,250	7.58										202	31,562,500	7.50
8	78,125				37	28,906,250	7.46				173	135,156,250	8.13				133	103,906,250	8.02
	78,125				56	43,750,000	7.64				171	133,593,750	8.13						
	78,125				122	95,312,500	7.98				102	79,687,500	7.90						
	78,125				42	32,812,500	7.52				196	153,125,000	8.19						
9	156,250										141	220,312,500	8.34				32	50,000,000	7.70
	156,250										151	235,937,500	8.37				19	29,687,500	7.47
	156,250										129	201,562,500	8.30				36	56,250,000	7.75
	156,250										73	114,062,500	8.06				71	110,937,500	8.05
10	312,500				73	228,125,000	8.36				63	196,875,000	8.29				31	96,875,000	7.99
	312,500				91	284,375,000	8.45				34	106,250,000	8.03				20	62,500,000	7.80
	312,500				47	146,875,000	8.17				73	228,125,000	8.36				8	25,000,000	7.40
	312,500				66	206,250,000	8.31				68	212,500,000	8.33				23	71,875,000	7.86
11	625,000				37	231,250,000	8.36	373	2,331,250,000	9.37	39	243,750,000	8.39	321	2,006,250,000	9.30	16	100,000,000	8.00
	625,000				38	237,500,000	8.38	367	2,293,750,000	9.36	40	250,000,000	8.40	315	1,968,750,000	9.29	9	56,250,000	7.75
	625,000							280	1,750,000,000	9.24				324	2,025,000,000	9.31			
	625,000							317	1,981,250,000	9.30				180	1,125,000,000	9.05			
12	1,250,000	251	3,137,500,000	9.50	6	75,000,000	7.88	263	3,287,500,000	9.52	10	125,000,000	8.10	200	2,500,000,000	9.40	8	100,000,000	8.00
	1,250,000	255	3,187,500,000	9.50	8	100,000,000	8.00	213	2,662,500,000	9.43	49						1	12,500,000	7.10
	1,250,000	236	2,950,000,000	9.47				219	2,737,500,000	9.44									
	1,250,000	228	2,850,000,000	9.45				179	2,237,500,000	9.35									
		average	3,031,250,000	9.48	**average**	115,742,188	7.92	**average**	2,410,156,250	9.37	**average**	175,729,167	8.22	**average**	1,925,000,000	9.27	**average**	59,871,324	7.71
		stdev	158,278,499	0.023	**stdev**	91,706,931	0.376	**stdev**	479,570,789	0.085	**stdev**	56,900,739	0.156	**stdev**	497,277,746	0.130	**stdev**	31,937,469	0.268
		N =	4		**N =**	16		**N =**	8		**N =**	15		**N =**	5		**N =**	17	

40 hour Shape Data

		0ns40			500ns40			0T40			500T40			0G40			500G40		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
6	3,125	[redacted]						[redacted]						[redacted]					
	3,125																		
	3,125																		
	3,125																		
7	15,625										525	82,031,250	7.91				292	45,625,000	7.66
	15,625										473	73,906,250	7.87				113	17,656,250	7.25
	15,625										460	71,875,000	7.86				370	57,812,500	7.76
	15,625										464	72,500,000	7.86				437	68,281,250	7.83
8	78,125				29	22,656,250	7.36				159	124,218,750	8.09				44	34,375,000	7.54
	78,125				1						93	72,656,250	7.86				99	77,343,750	7.89
	78,125				34	26,562,500	7.42				101	78,906,250	7.90				80	62,500,000	7.80
	78,125				54	42,187,500	7.63				247	192,968,750	8.29						
9	156,250				48	75,000,000	7.88				176	275,000,000	8.44				32	50,000,000	7.70
	156,250				12	18,750,000	7.27				127	198,437,500	8.30				20	31,250,000	7.49
	156,250				9	14,062,500	7.15				53	82,812,500	7.92				12	18,750,000	7.27
	156,250				35	54,687,500	7.74				80	125,000,000	8.10				51	79,687,500	7.90
10	312,500	529	1,653,125,000	9.22				489	1,528,125,000	9.18	27	84,375,000	7.93	689	2,153,125,000	9.33	14	43,750,000	7.64
	312,500	427	1,334,375,000	9.13				480	1,500,000,000	9.18	21	65,625,000	7.82	620	1,937,500,000	9.29	6	18,750,000	7.27
	312,500	129	403,125,000	8.61				426	1,331,250,000	9.12	19	59,375,000	7.77	680	2,125,000,000	9.33	7	21,875,000	7.34
	312,500	623	1,946,875,000	9.29				525	1,640,625,000	9.22	35	109,375,000	8.04	480	1,500,000,000	9.18	9	28,125,000	7.45
11	625,000	83	518,750,000	8.71	1			110	687,500,000	8.84	7	43,750,000	7.64	373	2,331,250,000	9.37	8	50,000,000	7.70
	625,000	123	768,750,000	8.89	8	50,000,000	7.70	260	1,625,000,000	9.21	24	150,000,000	8.18	305	1,906,250,000	9.28	15	93,750,000	7.97
	625,000	15	93,750,000	7.97	1			244	1,525,000,000	9.18	15	93,750,000	7.97	213	1,331,250,000	9.12	6	37,500,000	7.57
	625,000	20	125,000,000	8.10	17	106,250,000	8.03	296	1,850,000,000	9.27	19	118,750,000	8.07	298	1,862,500,000	9.27			
12	1,250,000	142	1,775,000,000	9.25	14	175,000,000	8.24	144	1,800,000,000	9.26	16	200,000,000	8.30	163	2,037,500,000	9.31	12	150,000,000	8.18
	1,250,000	29	362,500,000	8.56	19	237,500,000	8.38	185	2,312,500,000	9.36	20	250,000,000	8.40	150	1,875,000,000	9.27	10	125,000,000	8.10
	1,250,000	206	2,575,000,000	9.41	17	212,500,000	8.33	154	1,925,000,000	9.28	11	137,500,000	8.14	55	687,500,000	8.84	22		
	1,250,000							162	2,025,000,000	9.31	19	237,500,000	8.38	175	2,187,500,000	9.34	8	100,000,000	8.00
		average	1,050,568,182	8.83	average	86,263,021	7.76	average	1,645,833,333	9.20	average	125,013,021	8.04	average	1,827,864,583	9.24	average	57,715,774	7.68
		stdev	843,962,726	0.487	stdev	79,111,287	0.419	stdev	404,424,073	0.132	stdev	66,340,479	0.219	stdev	456,956,680	0.145	stdev	36,088,997	0.274
		N =	11		N =	12		N =	12		N =	24		N =	12		N =	21	

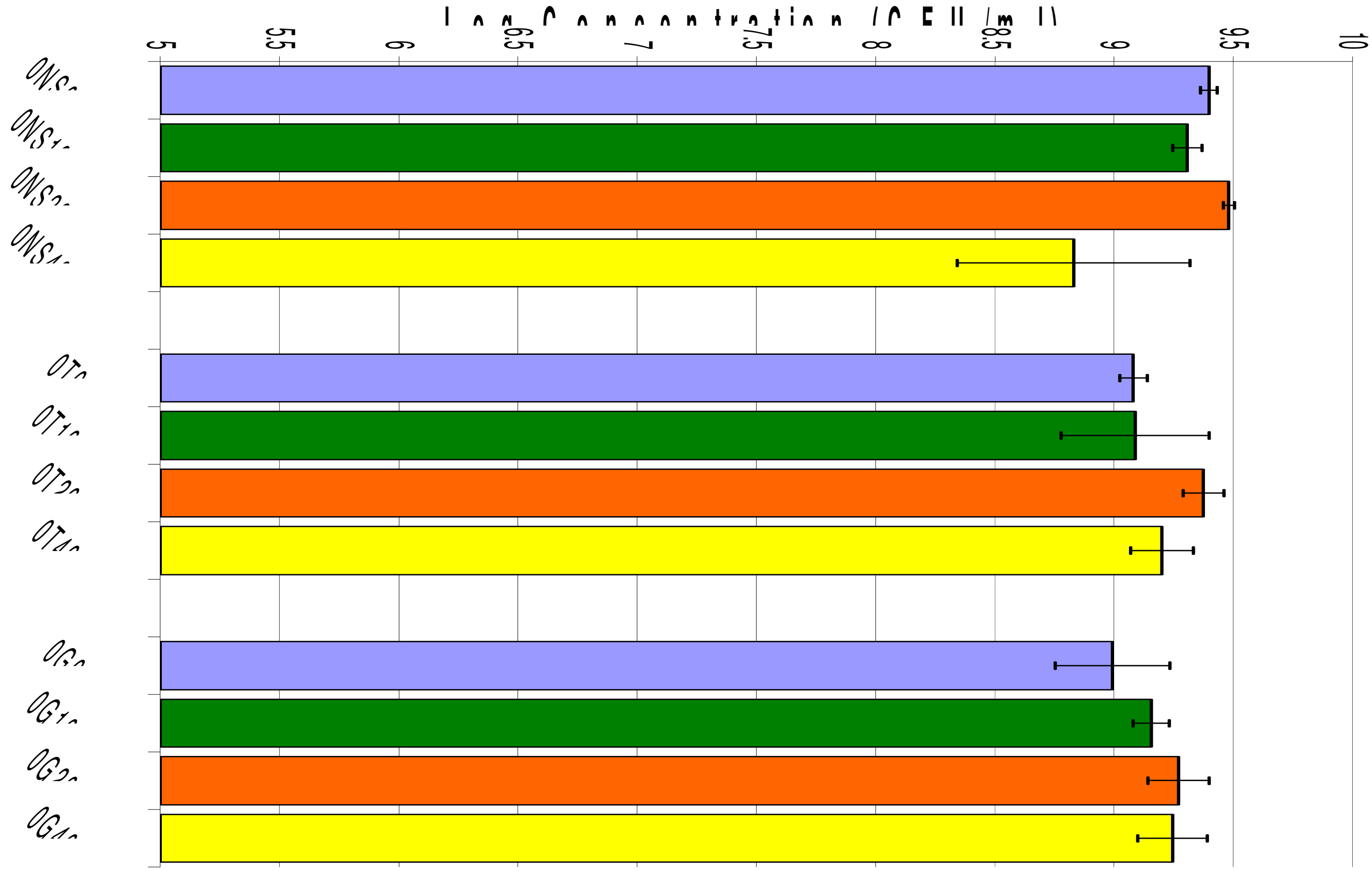


Figure G.01 Size analysis; *E. coli* concentration control groups non-irradiated Blue = 0 hours incubation; Green = 10 hours incubation; Orange = 20 hours incubation; yellow = 40 hours incubation. A sample marked with ‘*’ indicates a significant difference from its control group (500NS__).

Appendix H

Shape Significance Chart

	0ns0	500ns0	0T0	500T0	0G0	500G0	0ns10	500ns10	0T10	500T10	0G10	500G10
0ns0		S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns0	S		S	S	S	NS	S	S	S	S	S	NS
0T0	NS	S		S	NS	S	NS	S	NS	S	NS	S
500T0	S	S	S		S	NS	S	S	S	NS	S	NS
0G0	NS	S	NS	S		S	NS	S	NS	S	NS	S
500G0	S	NS	S	NS	S		S	S	S	S	S	NS
0ns10	NS	S	NS	S	NS	S		S	NS	S	NS	S
500ns10	S	S	S	S	S	S	S		S	NS	S	S
0T10	NS	S	NS	S	NS	S	NS	S		S	NS	S
500T10	S	S	S	NS	S	S	S	NS	S		S	S
0G10	NS	S	NS	S	NS	S	NS	S	NS	S		S
500G10	S	NS	S	NS	S	NS	S	S	S	S	S	
0ns20	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns20	S	S	S	NS	S	S	S	NS	S	NS	S	S
0T20	NS	S	NS	S	NS	S	NS	S	NS	S	NS	
500T20	S	S	S	S	S	S	S	NS	S	NS	S	S
0G20	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500G20	S	S	S	NS	S	NS	S	S	S	NS	S	NS
0ns40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns40	S	NS	S	S	S	NS	S	S	S	S	S	NS
0T40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500T40	S	S	S	S	S	S	S	NS	S	NS	S	S
0G40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500G40	S	S	S	NS	S	NS	S	S	S	NS	S	NS

	0ns20	500ns20	0T20	500T20	0G20	500G20	0ns40	500ns40	0T40	500T40	0G40	500G40
0ns0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns0	S	S	S	S	S	S	S	NS	S	S	S	S
0T0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500T0	S	NS	S	S	S	NS	S	S	S	S	S	NS
0G0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500G0	S	S	S	S	S	NS	S	NS	S	S	S	NS
0ns10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns10	S	NS	S	NS	S	S	S	S	S	NS	S	S
0T10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500T10	S	NS	S	NS	S	NS	S	S	S	NS	S	NS
0G10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500G10	S	S		S	S	NS	S	NS	S	S	S	NS
0ns20		S	NS	S	NS	S	S	S	NS	S	NS	S
500ns20	S		S	NS	S	NS	S	S	S	NS	S	NS
0T20	NS	S		S	NS	S	S	S	NS	S	NS	S
500T20	S	NS	S		S	S	S	S	S	NS	S	S
0G20	NS	S	NS	S		S	NS	S	NS	S	NS	S
500G20	S	NS	S	S	S		S	S	S	NS	S	NS
0ns40	S	S	S	S	NS	S		S	NS	S	NS	S
500ns40	S	S	S	S	S	S	S		S	S	S	NS
0T40	NS	S	NS	S	NS	S	NS	S		S	NS	S
500T40	S	NS	S	NS	S	NS	S	S	S		S	S
0G40	NS	S	NS	S	NS	S	NS	S	NS	S		S
500G40	S	NS	S	S	S	NS	S	S	S	S	S	

Appendix I

0 hour Tripeptide Data

	DF	0ns0 Count	0ns0 Conc.	0ns0 log(Conc.)	500ns0 Count	500ns0 Conc.	500ns0 log(Conc.)	0Trp0 Count	0Trp0 Conc.	0Trp0 log(Conc.)	500Trp0 Count	500Trp0 Conc.	500Trp0 log(Conc.)	0TTT0 Count	0TTT0 Conc.	0TTT0 log(Conc.)	500TTT0 Count	500TTT0 Conc.	500TTT0 log(Conc.)
7	15,625																		
	15,625																		
	15,625																		
	15,625																		
8	78,125				21	16,406,250	7.22				42	32,812,500	7.52				127	99,218,750	8.00
	78,125				23	17,968,750	7.25				45	35,156,250	7.55				128	100,000,000	8.00
	78,125				36	28,125,000	7.45				47	36,718,750	7.56				115	89,843,750	7.95
	78,125				24	18,750,000	7.27				25	19,531,250	7.29				117	91,406,250	7.96
9	390,625				4	15,625,000	7.19				2	7,812,500	6.89				26	101,562,500	8.01
	390,625				8	31,250,000	7.49				11	42,968,750	7.63				23	89,843,750	7.95
	390,625				6	23,437,500	7.37				5	19,531,250	7.29				24	93,750,000	7.97
	390,625				8	31,250,000	7.49				12	46,875,000	7.67				25	97,656,250	7.99
10	781,250				1	7,812,500	6.89				1	7,812,500	6.89				13	101,562,500	8.01
	781,250				4	31,250,000	7.49				3	23,437,500	7.37				14	109,375,000	8.04
	781,250										6	46,875,000	7.67				8	62,500,000	7.80
	781,250				1	7,812,500	6.89				5	39,062,500	7.59				16	125,000,000	8.10
11	1,562,500	93	1,453,125,000	9.16	2	31,250,000	7.49	90	1,406,250,000	9.15	1	15,625,000	7.19	74	1,156,250,000	9.06	10	156,250,000	8.19
	1,562,500	84	1,312,500,000	9.12				83	1,296,875,000	9.11	1	15,625,000	7.19	81	1,265,625,000	9.10	4	62,500,000	7.80
	1,562,500	125	1,953,125,000	9.29				86	1,343,750,000	9.13	1	15,625,000	7.19	88	1,375,000,000	9.14	5	78,125,000	7.89
	1,562,500	76	1,187,500,000	9.07	4			93	1,453,125,000	9.16	1	15,625,000	7.19	86	1,343,750,000	9.13	11	171,875,000	8.24
12	3,125,000	42	1,312,500,000	9.12				43	1,343,750,000	9.13	2	62,500,000	7.80	70	2,187,500,000	9.34	2	62,500,000	7.80
	3,125,000	63	1,968,750,000	9.29				45	1,406,250,000	9.15	2	62,500,000	7.80	42	1,312,500,000	9.12	2	62,500,000	7.80
	3,125,000	48	1,500,000,000	9.18	1	31,250,000	7.49	47	1,468,750,000	9.17	3	93,750,000	7.97	59	1,843,750,000	9.27	6	187,500,000	8.27
	3,125,000	50	1,562,500,000	9.19				39	1,218,750,000	9.09	2	62,500,000	7.80	42	1,312,500,000	9.12	3	93,750,000	7.97
		average	1,531,250,000	9.18	**average**	22,475,962	7.31	**average**	1,367,187,500	9.14	**average**	35,117,188	7.45	**average**	1,474,609,375	9.16	**average**	101,835,938	7.99
		stdev	290,641,801	0.080	**stdev**	8,971,083	0.218	**stdev**	83,519,138	0.027	**stdev**	22,573,828	0.303	**stdev**	352,361,443	0.094	**stdev**	34,871,632	0.138
		N =	8		**N =**	13		**N =**	8		**N =**	20		**N =**	8		**N =**	20	

10 hour Tripeptide Data

		0ns10			500ns10			0Trp10			500Trp10			0TTT10			500TTT10		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
7	15,625	[redacted]			69	10,781,250	7.03	[redacted]						[redacted]					
	15,625	[redacted]			66	10,312,500	7.01	[redacted]						[redacted]					
	15,625	[redacted]			70	10,937,500	7.04	[redacted]						[redacted]					
	15,625	[redacted]			93	14,531,250	7.16	[redacted]						[redacted]					
8	78,125	[redacted]			16	12,500,000	7.10	[redacted]			58	45,312,500	7.66	[redacted]			75	58,593,750	7.77
	78,125	[redacted]			18	14,062,500	7.15	[redacted]			46	35,937,500	7.56	[redacted]			58	45,312,500	7.66
	78,125	[redacted]			14	10,937,500	7.04	[redacted]			65	50,781,250	7.71	[redacted]			56	43,750,000	7.64
	78,125	[redacted]			12	9,375,000	6.97	[redacted]			62	48,437,500	7.69	[redacted]			49	38,281,250	7.58
9	390,625				3	11,718,750	7.07				15	58,593,750	7.77				11	42,968,750	7.63
	390,625				1	3,906,250	6.59				8	31,250,000	7.49				9	35,156,250	7.55
	390,625				2	7,812,500	6.89				8	31,250,000	7.49				13	50,781,250	7.71
	390,625				4	15,625,000	7.19				8	31,250,000	7.49				15	58,593,750	7.77
10	781,250				5	39,062,500	7.59				8	62,500,000	7.80				9	70,312,500	7.85
	781,250				1	7,812,500	6.89				8	62,500,000	7.80				4	31,250,000	7.49
	781,250				2	15,625,000	7.19				6	46,875,000	7.67				6	46,875,000	7.67
	781,250				1	7,812,500	6.89				6	46,875,000	7.67				3	23,437,500	7.37
11	1,562,500	102	1,593,750,000	9.20				105	1,640,625,000	9.22	3	46,875,000	7.67	100	1,562,500,000	9.19	4	62,500,000	7.80
	1,562,500	99	1,546,875,000	9.19	2	31,250,000	7.49	90	1,406,250,000	9.15	8	125,000,000	8.10	93	1,453,125,000	9.16	2	31,250,000	7.49
	1,562,500	80	1,250,000,000	9.10				87	1,359,375,000	9.13	1	15,625,000	7.19	89	1,390,625,000	9.14	3	46,875,000	7.67
	1,562,500	89	1,390,625,000	9.14				75	1,171,875,000	9.07	5	78,125,000	7.89	79	1,234,375,000	9.09	4	62,500,000	7.80
12	3,125,000	38	1,187,500,000	9.07				42	1,312,500,000	9.12	1	31,250,000	7.49	33	1,031,250,000	9.01	1	31,250,000	7.49
	3,125,000	39	1,218,750,000	9.09				39	1,218,750,000	9.09	1	31,250,000	7.49	35	1,093,750,000	9.04	1	31,250,000	7.49
	3,125,000	44	1,375,000,000	9.14				42	1,312,500,000	9.12	2	62,500,000	7.80	39	1,218,750,000	9.09	3	93,750,000	7.97
	3,125,000	60	1,875,000,000	9.27				46	1,437,500,000	9.16	2	62,500,000	7.80	32	1,000,000,000	9.00			
		average	1,429,687,500	9.15	**average**	13,768,382	7.08	**average**	1,357,421,875	9.13	**average**	50,234,375	7.66	**average**	1,248,046,875	9.09	**average**	47,615,132	7.65
		stdev	232,657,397	0.068	**stdev**	8,727,280	0.229	**stdev**	144,764,833	0.045	**stdev**	23,289,141	0.192	**stdev**	205,164,469	0.071	**stdev**	17,125,190	0.150
		N =	8		**N =**	17		**N =**	8		**N =**	20		**N =**	8		**N =**	19	

20 hour Tripeptide Data

		0ns20			500ns20			0Trp20			500Trp20			0TTT20			500TTT20		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
7	15,625																		
	15,625																		
	15,625																		
	15,625																		
8	78,125				72	56,250,000	7.75				42	32,812,500	7.52				111	86,718,750	7.94
	78,125				54	42,187,500	7.63				103	80,468,750	7.91				129	100,781,250	8.00
	78,125				64	50,000,000	7.70				136	106,250,000	8.03				111	86,718,750	7.94
	78,125				63	49,218,750	7.69				111	86,718,750	7.94				101	78,906,250	7.90
9	390,625				15	58,593,750	7.77				24	93,750,000	7.97				20	78,125,000	7.89
	390,625				19	74,218,750	7.87				19	74,218,750	7.87				21	82,031,250	7.91
	390,625				11	42,968,750	7.63				18	70,312,500	7.85				13	50,781,250	7.71
	390,625				7	27,343,750	7.44				24	93,750,000	7.97				25	97,656,250	7.99
10	781,250				10	78,125,000	7.89				11	85,937,500	7.93				11	85,937,500	7.93
	781,250				2	15,625,000	7.19				4	31,250,000	7.49				12	93,750,000	7.97
	781,250				5	39,062,500	7.59				16	125,000,000	8.10				13	101,562,500	8.01
	781,250				9	70,312,500	7.85				10	78,125,000	7.89				9	70,312,500	7.85
11	1,562,500	111	1,734,375,000	9.24	2	31,250,000	7.49	103	1,609,375,000	9.21	5	78,125,000	7.89	104	1,625,000,000	9.21	4	62,500,000	7.80
	1,562,500	109	1,703,125,000	9.23	5	78,125,000	7.89	120	1,875,000,000	9.27	11	171,875,000	8.24	95	1,484,375,000	9.17	5	78,125,000	7.89
	1,562,500	134	2,093,750,000	9.32	3	46,875,000	7.67	113	1,765,625,000	9.25	11	171,875,000	8.24	100	1,562,500,000	9.19	11	171,875,000	8.24
	1,562,500	93	1,453,125,000	9.16	3	46,875,000	7.67	100	1,562,500,000	9.19	7	109,375,000	8.04	92	1,437,500,000	9.16	16	250,000,000	8.40
12	3,125,000	56	1,750,000,000	9.24	3	93,750,000	7.97	54	1,687,500,000	9.23	2	62,500,000	7.80	59	1,843,750,000	9.27	2	62,500,000	7.80
	3,125,000	68	2,125,000,000	9.33	1	31,250,000	7.49	61	1,906,250,000	9.28	2	62,500,000	7.80	48	1,500,000,000	9.18	3	93,750,000	7.97
	3,125,000	59	1,843,750,000	9.27	2	62,500,000	7.80	64	2,000,000,000	9.30	3	93,750,000	7.97	54	1,687,500,000	9.23	1	31,250,000	7.49
	3,125,000	60	1,875,000,000	9.27	3	93,750,000	7.97	41	1,281,250,000	9.11	5	156,250,000	8.19	38	1,187,500,000	9.07			
		average	1,822,265,625	9.26	**average**	54,414,063	7.70	**average**	1,710,937,500	9.23	**average**	93,242,188	7.93	**average**	1,541,015,625	9.18	**average**	92,804,276	7.93
		stdev	217,781,257	0.053	**stdev**	21,688,104	0.195	**stdev**	229,639,663	0.062	**stdev**	38,908,640	0.195	**stdev**	193,169,042	0.056	**stdev**	47,073,926	0.185
		N =	8		**N =**	20		**N =**	8		**N =**	20		**N =**	8		**N =**	19	

40 hour Tripeptide Data

		0ns40			500ns40			0Trp40			500Trp40			0TTT40			500TTT40		
	DF	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)	Count	Conc.	log(Conc.)
7	15,625	[redacted]			46	7,187,500	6.86	[redacted]						[redacted]					
	15,625				59	9,218,750	6.96												
	15,625				53	8,281,250	6.92												
	15,625				50	7,812,500	6.89												
8	78,125				12	9,375,000	6.97				91	71,093,750	7.85				40	31,250,000	7.49
	78,125				8	6,250,000	6.80				92	71,875,000	7.86				41	32,031,250	7.51
	78,125				6	4,687,500	6.67				98	76,562,500	7.88				44	34,375,000	7.54
	78,125				9	7,031,250	6.85				80	62,500,000	7.80				37	28,906,250	7.46
9	390,625				2	7,812,500	6.89				17	66,406,250	7.82				9	35,156,250	7.55
	390,625				3	11,718,750	7.07				20	78,125,000	7.89				10	39,062,500	7.59
	390,625				1	3,906,250	6.59				25	97,656,250	7.99				6	23,437,500	7.37
	390,625				3	11,718,750	7.07				18	70,312,500	7.85				10	39,062,500	7.59
10	781,250	169	1,320,312,500	9.12	1	7,812,500	6.89	131	1,023,437,500	9.01	9	70,312,500	7.85	119	929,687,500	8.97	1	7,812,500	6.89
	781,250	158	1,234,375,000	9.09	2	15,625,000	7.19	157	1,226,562,500	9.09	9	70,312,500	7.85	137	1,070,312,500	9.03	1	7,812,500	6.89
	781,250	138	1,078,125,000	9.03	1	7,812,500	6.89	127	992,187,500	9.00	11	85,937,500	7.93	157	1,226,562,500	9.09	3	23,437,500	7.37
	781,250	170	1,328,125,000	9.12				81	632,812,500	8.80	17	132,812,500	8.12	126	984,375,000	8.99	3	23,437,500	7.37
11	1,562,500	77	1,203,125,000	9.08	1	15,625,000	7.19	78	1,218,750,000	9.09	7	109,375,000	8.04	85	1,328,125,000	9.12	2	31,250,000	7.49
	1,562,500	79	1,234,375,000	9.09	1	15,625,000	7.19	96	1,500,000,000	9.18	7	109,375,000	8.04	71	1,109,375,000	9.05	4	62,500,000	7.80
	1,562,500	72	1,125,000,000	9.05				78	1,218,750,000	9.09	5	78,125,000	7.89	73	1,140,625,000	9.06			
	1,562,500	85	1,328,125,000	9.12				82	1,281,250,000	9.11	4	62,500,000	7.80	85	1,328,125,000	9.12	1	15,625,000	7.19
12	3,125,000	43	1,343,750,000	9.13				50	1,562,500,000	9.19	2	62,500,000	7.80	28	875,000,000	8.94			
	3,125,000	55	1,718,750,000	9.24				36	1,125,000,000	9.05	5	156,250,000	8.19	33	1,031,250,000	9.01	2	62,500,000	7.80
	3,125,000	32	1,000,000,000	9.00				31	968,750,000	8.99	3	93,750,000	7.97	28	875,000,000	8.94	1	31,250,000	7.49
	3,125,000	30	937,500,000	8.97				28	875,000,000	8.94	2	62,500,000	7.80	29	906,250,000	8.96	1	31,250,000	7.49
		average	1,237,630,208	9.09	average	9,264,706	6.94	average	1,135,416,667	9.04	average	84,414,063	7.91	average	1,067,057,292	9.02	average	31,119,792	7.44
		stdev	202,672,637	0.069	stdev	3,630,403	0.171	stdev	259,396,677	0.107	stdev	25,475,377	0.115	stdev	163,925,752	0.066	stdev	14,616,463	0.244
		N =	12		N =	17		N =	12		N =	20		N =	12		N =	18	

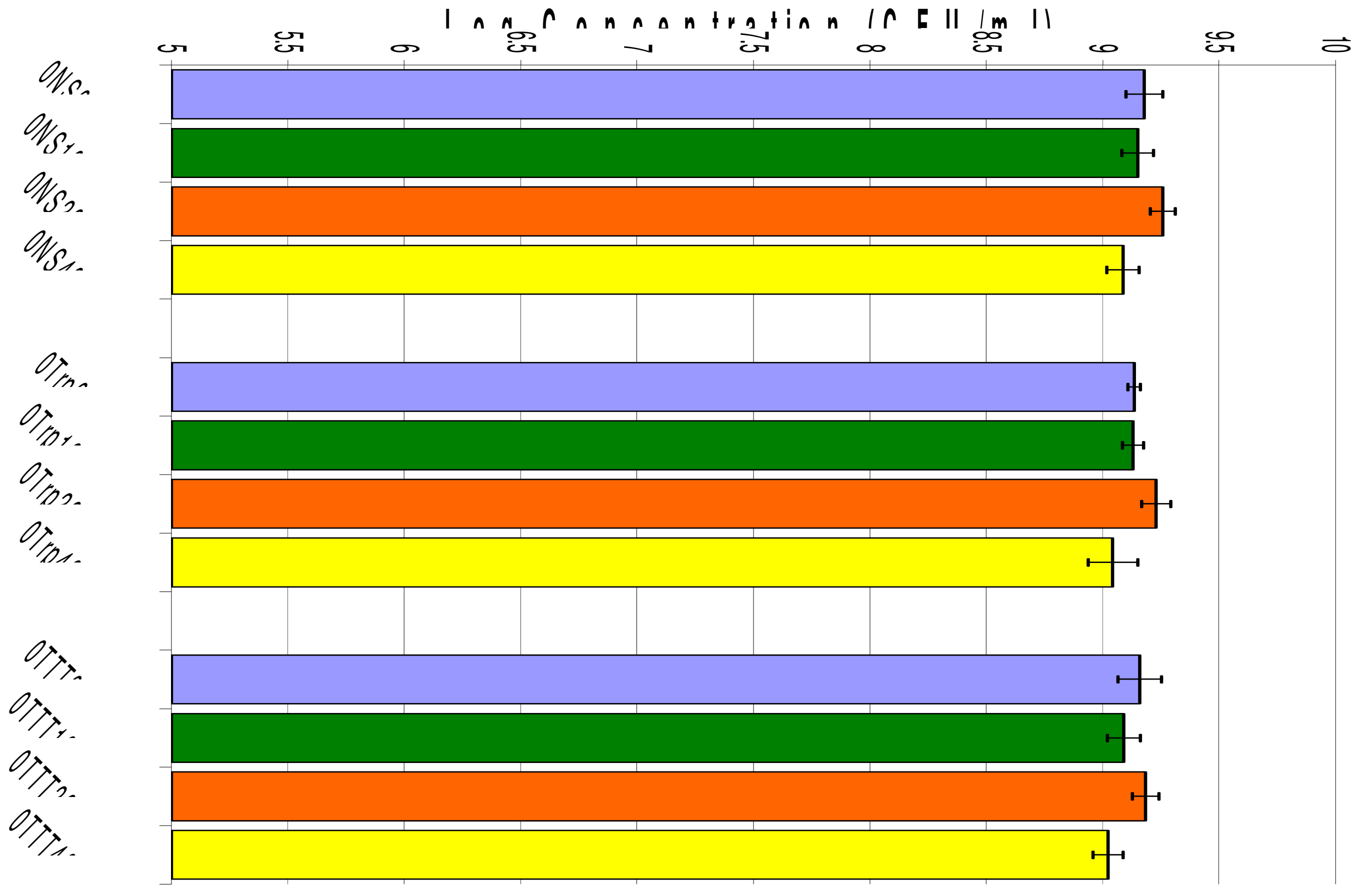


Figure I.01 Size analysis; *E. coli* concentration control groups non-irradiated Blue = 0 hours incubation; Green = 10 hours incubation; Orange = 20 hours incubation; yellow = 40 hours incubation. A sample marked with ‘*’ indicates a significant difference from its control group (500NS__).

Appendix J

Tripeptide Significance Chart

	0ns0	500ns0	0Trp0	500Trp0	0TTT0	500TTT0	0ns10	500ns10	0Trp10	500Trp10	0TTT10	500TTT10
0ns0		S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns0	S		S	NS	S	S	S	S	S	S	S	S
0Trp0	NS	S		S	NS	S	NS	S	NS	S	NS	S
500Trp0	S	NS	S		S	S	S	S	S	S	S	S
0TTT0	NS	S	NS	S		S	NS	S	NS	S	NS	S
500TTT0	S	S	S	S	S		S	S	S	S	S	S
0ns10	NS	S	NS	S	NS	S		S	NS	S	NS	S
500ns10	S	S	S	S	S	S	S		S	S	S	S
0Trp10	NS	S	NS	S	NS	S	NS	S		S	NS	S
500Trp10	S	S	S	S	S	S	S	S	S		S	NS
0TTT10	NS	S	NS	S	NS	S	NS	S	NS	S		S
500TTT10	S	S	S	S	S	S	S	S	S	NS	S	
0ns20	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns20	S	S	S	S	S	S	S	S	S	NS	S	NS
0Trp20	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500Trp20	S	S	S	S	S	NS	S	S	S	S	S	S
0TTT20	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500TTT20	S	S	S	S	S	NS	S	S	S	S	S	S
0ns40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns40	S	S	S	S	S	S	S	NS	S	S	S	S
0Trp40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500Trp40	S	S	S	S	S	NS	S	S	S	S	S	S
0TTT40	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500TTT40	S	NS	S	NS	S	S	S	S	S	S	S	S

	0ns20	500ns20	0Trp20	500Trp20	0TTT20	500TTT20	0ns40	500ns40	0Trp40	500Trp40	0TTT40	500TTT40
0ns0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns0	S	S	S	S	S	S	S	S	S	S	S	NS
0Trp0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500Trp0	S	S	S	S	S	S	S	S	S	S	S	NS
0TTT0	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500TTT0	S	S	S	NS	S	NS	S	S	S	NS	S	S
0ns10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns10	S	S	S	S	S	S	S	NS	S	S	S	S
0Trp10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500Trp10	S	NS	S	S	S	S	S	S	S	S	S	S
0TTT10	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
500TTT10	S	NS	S	S	S	S	S	S	S	S	S	S
0ns20		S	NS	S	NS	S	NS	S	NS	S	NS	S
500ns20	S		S	S	S	S	S	S	S	S	S	S
0Trp20	NS	S		S	NS	S	NS	S	NS	S	NS	S
500Trp20	S	S	S		S	NS	S	S	S	NS	S	S
0TTT20	NS	S	NS	S		S	NS	S	NS	S	NS	S
500TTT20	S	S	S	NS	S		S	S	S	NS	S	S
0ns40	NS	S	NS	S	NS	S		S	NS	S	NS	S
500ns40	S	S	S	S	S	S	S		S	S	S	S
0Trp40	NS	S	NS	S	NS	S	NS	S		S	NS	S
500Trp40	S	S	S	NS	S	NS	S	S	S		S	S
0TTT40	NS	S	NS	S	NS	S	NS	S	NS	S		S
500TTT40	S	S	S	S	S	S	S	S	S	S	S	

Appendix K

General Supplies

Table K.01 List of general supplies.

Item	Vendor	Catalog Number	Price
Petri Dishes 100mm x 15mm	Fisher Scientific	08-757-12	$163.42
20ml Syringe	Becton Dickson	301625	$31.18
0.2µm Filter	Whatman	91816A	$50.54
Sterile Loop	Fisher Scientific	13-075-3	$60.25
Vial with Cap	Fisher Scientific	03-339-21H	$80.05
250ml Flask	Fisher Scientific	n/a	n/a
1L Bottle	Fisher Scientific	n/a	n/a
Incubator	Fisher Scientific	n/a	n/a
Shaker Incubator	New Brunswick	n/a	n/a
pH Meter	Denver Instrument	n/a	n/a
Irradiation Machine	Isotopes	n/a	n/a
200µl - 1ml Pipette	Biomar	n/a	n/a
50µl - 200µl Pipette	Biomar	n/a	n/a
1ml Pipette Tips	Fisher Scientific	21-197-8F	$56.10
200µl Pipette Tips	Fisher Scientific	21-197-8G	$48.45
Bunsen Burner	Fisher Scientific	n/a	n/a

Appendix L

List of Chemicals

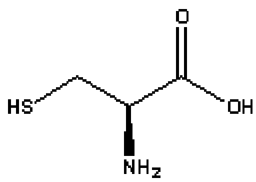
Table L.01 List of chemicals.

Item	Vendor	Catalog Number	Price
L-Cysteine	Alfa Aesar	52-90-4	$20.40
D-Cysteine	MPBio	101438	$125.40
N-Acetyl-L-Cysteine	Acros	616-91-1	$37.90
L-Cysteine-Methyl-Ester	Acros	18598-63-5	$49.00
L-Cysteine-Ethyl-Ester	Acros	868-59-7	$24.10
Tryptophan	Alfa Aesar	153-94-6	$22.90
Trp-Trp-Trp	BAChem	H-6970	$310.00
Uracil	Alfa Aesar	66-22-8	$10.95
Glutathione	Acros	70-18-8	$15.40
Epigallocatechin Gallate	Axxora	989-51-5	$72.00
Ascorbic Acid	Fisher Scientific	50-81-7	$22.99
Trehalose	Sigma-Aldrich	T5251	$27.78
Beef Extract	Sigma-Aldrich	B4888-100	$39.90
Peptone	Fisher Scientific	BP1420-500	$76.95
Tryptone	Fluka	95039	$42.30
Yeast Extract	Fluka	70161	$55.70
Agar	Acros	9002-18-0	$63.85
Sodium Chloride	Sigma-Aldrich	7647-14-5	$7.65
Ethanol	Sigma-Aldrich	E7148-1GA	$33/66

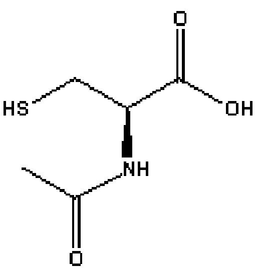
Appendix M

Chemical Structures

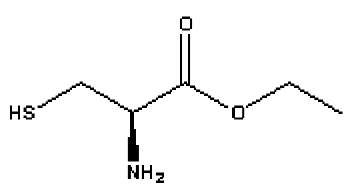
L-Cysteine [40]
mw: 121 Da



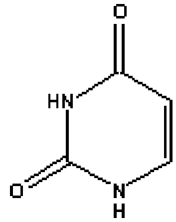
N-Acetyl-L-Cysteine [40]
mw: 163 Da



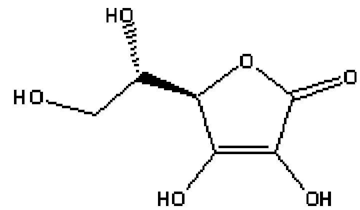
L-Cysteine-Ethyl-Ester [40]
mw: 185 Da



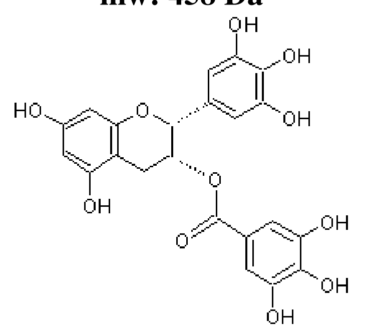
Uracil [40]
mw: 112 Da



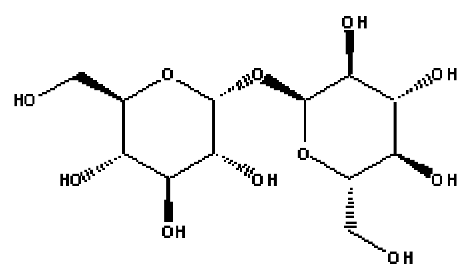
Ascorbic Acid [40]
mw: 176 Da



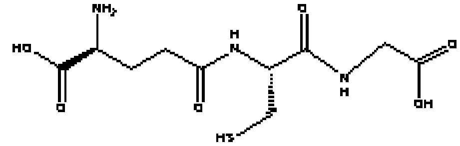
Epigallocatechin Gallate [31]
mw: 458 Da



Trehalose [40]
mw: 342 Da



Glutathione [40]
mw: 307 Da



Appendix N

Colony Count Protocol

Original sample concentration (colony forming units per milliliter, CFU/ml) was determined by counting the number of colonies and dividing by the volume of sample originally plated and accounting for the dilution factor. In all cases 100µl was plated and spread with a glass rod. The concentration formula is shown as Equation 1.

$$concentration(CFU/ml) = \frac{plate\_count}{100\mu l} \times \frac{1000\mu l}{1ml} \times dilution\_factor \qquad \textit{Equation 1.}$$

As an example, the plate in Picture XX contains 38 colonies and was plated at a dilution factor of 625,000. The concentration is calculated as:

$$237{,}500{,}000(CFU/ml) = \frac{38Colonies}{100\mu l} \times \frac{1000\mu l}{1ml} \times 625{,}000$$

Picture N.01 Sample plate containing 38 *E. coli* colonies.

Appendix O

Agar Plate Protocol

Agar plates were prepared using the following recipe:

15.0 g/L Agar
10.0 g/L Tryptone
5.0 g/L Yeast Extract
10 g/L Sodium Chloride

All contents were combined in a one liter autoclavable bottle (Fisher Scientific, St. Louis, MO, USA). The bottle was then filled to volume of one liter with distilled deionized. The pH was adjusted (UB-10, Denver Instrument, Arvada, CO, USA) to 7.0, and the cap was replaced and the contents were gently mixed by bottle inversion. The contents were vented and the cap was left loose for autoclaving. The bottle was autoclaved in a table top pressure vessel (Electric Steroclave, Wisconsin Aluminum Foundry Co., Manitowoc, WI) and was held at 15psi for a minimum of 15 minutes. After the 15 minutes, the vessel was allowed to cool to safely remove the lid. The agar bottles were allowed to cool to a manageable temperature. Once the bottles were cool enough to touch, the agar was poured (using aseptic conditions) into Petri dishes (Fisher Scientific, St. Louis, MO, USA), just enough to cover the bottom of the dish. The dishes were allowed to cool for approximately one hour to allow the agar to completely solidify. At this point the dishes were ready to accept bacterial culture.

Appendix P

Nutrient Broth Protocol

Nutrient Broth was prepared using the following recipe:

3.0 g/L Beef Extract
5 g/L Peptone

All contents were combined in a one liter autoclavable bottle (Fisher Scientific, St. Louis, MO, USA). The bottle was then filled to volume of one liter with distilled deionized. The pH was adjusted (UB-10, Denver Instrument, Arvada, CO, USA) to 7.0, and the cap was replaced and the contents were gently mixed by bottle inversion. The contents were vented and the cap was left loose for autoclaving. The bottle was autoclaved in a table top pressure vessel (Electric Steroclave, Wisconsin Aluminum Foundry Co., Manitowoc, WI) and was held at 15psi for a minimum of 15 minutes. After the 15 minutes, the vessel was allowed to cool to safely remove the lid. The bottles were allowed to continue to room temperature on the bench. Nutrient broth was stored at 4℃ while not in use.

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