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Detection of escherichia coli using the bacteriophage-based fluorescent technique

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Detection of *Escherichia coli* Using the Bacteriophage-Based Fluorescent Technique

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ABSTRACT

The detection of viable *Escherichia coli* (*E. coli)* by the bacteriophage-based fluorescent technique using the enhanced green fluorescent protein (EGFP) was tested in this project. EGFP, a mutant of green fluorescent protein (GFP), was used due to its high fluorescent efficiency compared to wild-type GFP. First, *E. coli* bacteria were cultured to the mid-exponential phase in the 2× YT medium and then they were infected with the M13 bacteriophages containing the EGFP gene. Infected bacteria were incubated for different time intervals, and the expression of EGFP on the *E. coli* membrane was tested by the PerkinElmer LS 55 Luminescence Spectrometer, which measured the relative fluorescence intensity using the emission spectrum technique. The expected emission maximum wavelength for pure EGFP was around 510nm; however, the detected emission maximum wavelength in this project was around 525nm. The emission maximum wavelength of the crude sample in this project was red-shifted about 15nm compared to that of the standard pure EGFP sample. It could be concluded that further mutation of the bacteriophages produced the protein with the longest emission maximum wavelength or yellow fluorescent protein (YFP). Optimization in each intermediate step might help reduce errors or help prevent unwanted mutation to ultimately reduce the red-shift, and hence bring the emission maximum wavelength back to the desired one. It could also be concluded that the cellular autofluorescence from endogenous flavin such as FAD was predominant in the *E. coli* cells. However, cellular autofluorescence problem must be handled, for example, by purifying the sample, using the bandpass fluorescence filter, or by combining with immunofluorescence technique.

Key words: *E. coli* TG1, bacteriophage, fluorescent technique, GFP, EGFP, red-shift, and cellular autofluorescence.

INTRODUCTION

World Health Organization estimated that 76 million people in the United States suffer from foodborne diseases, which results 325,000 hospitalized cases and 5,000 deaths every year (WHO, 2007). Among various causes of foodborne diseases, foodborne pathogenic bacteria are the major contributors accounting for 91% of foodborne illness outbreaks in the United States (Yang, 2006). In the list of about 20 foodborne pathogenic bacteria, *E. coli* O157:H7 is ranked as one of the most common foodborne pathogens since it was found to be a cause of death threatening disease called hemorrhagic colitis (Goodridge et al., 1999). The Centers for Disease Control and Prevention reported that the United States has around 70,000 *E. coli* infections each year (CDC, 2008).

To combat foodborne bacteria, the detection of viable bacteria is critical as only viable bacteria can severely threaten human health and cause a disease outbreak (Unge et al., 1999). Bacteria viability is conventionally tested by plate counting, which is a tedious, inaccurate, and costly method (Lehtinen et al., 2003). Current rapid bacterial detection methods cannot differentiate live bacteria from dead ones. Even modern immunoassay based bacterial detection such as latex agglutination assay, polymerase chain reaction (PCR) based detection, or nucleic acid based detection method can generate false positive results due to dead bacteria (Goodridge et al., 1999). False positive bacterial detection can negatively affect the budget of food industries by causing long delay of product in storage or product recalls (Yao et al., 2008). Therefore, a simple, rapid and low-cost bacteria detection technique with high sensitivity and specificity to detect viable pathogenic bacteria is of prior importance to ensure national food safety and security (Yao et al., 2008).

Since the last decade, GFP cloned from jelly fish *Aequorea victoria* has been used as a reporter in protein localization and monitoring gene expression in many types of cells (Zhang et al., 1996). One advantage of using GFP is that it fluoresces spontaneously without the aid of substrate or specific cofactors (Unge et al., 1999 and Bizzarri et al., 2006). Another advantage is that target cells can be visualized without fixing them with additional solvents or reagents such as paraformaldehyde, and hence can avoid creating artifact in the experiment (Baumann et al., 1998). Chromophore mutations in wild-type (wt) GFP is performed to produce the mutant EGFP, whose excitation maximum is red-shifted, and the fluorescence intensity is increased 35 times as much as the wtGFP (Unge et al., 1999 and Zhang et al., 1996).

In this project, the bacteria were infected with EGFP-displayed recombinant M13 phage, whose plasmid map is shown below in **figure 1,** and then the phagemids, special plasmids that enable phage generation when they are transferred to suitable host cells, produced the EGFP, which would be expressed on the surface of the bacteria and detected by the fluorescence spectrometer. This detection method is based on using specific bacteriophages, viruses that infect bacteria by recognizing membrane receptors of their target bacteria, and hence the method has a high sensitivity and selectivity (Yao et al., 2008 and Goodridge et al., 1999).

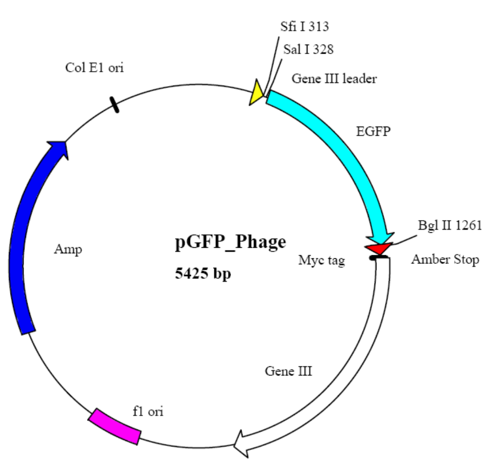


Figure 1. Plasmid of EGFP-displayed M13 phage.

Explanation of important features of the project

E. coli

E. coli bacteria are gram-negative bacilli bacteria, about 2-6μm long, and 1.1-1.5μm wide, occurring in pairs or singly (Krieg, 1984). Unlike gram-positive bacteria, gram-negative ones have cell walls, which contain the pathogenic component called lipopolysaccharide (LPS) or endotoxin layer as in the cell wall of pathogenic *E. coli* O157:H7 (Tortora, 2004). They are facultative anaerobes and non-sporulating bacteria (Tortora, 2004).

***E. coli* TG1**

E. coli TG1 is a typical *E. coli* host strain commonly used in bacteriophage infection. *E. coli* TG1 allows the infection process through its sex pilus. In this process, EGFP produced by the engineered phagemid is fused with pIII phage envelope protein and the expression of EGFP and pIII fusion protein in the bacteriophage infected *E. coli* TG1 renders the expression of EGFP on its surface (Clontech, 1997). The genotype of *E. coli* TG1 bacteria is: *supE thi-1 Δ(lac-proAB) hsdΔ5[F' traD36 proAB*$^{+}$ *lacI*^{q} *LacZΔM15]* (Clontech, 1997).

M13 phage

M13 phage is a filamentous bacteriophage, which has a single-strand (ss) DNA of about 6.4 kilo-bases long (Sambrook, 2001). It can replicate in the host bacteria via lysogenic conversion process (Sambrook, 2001). Unlike lytic activity, lysogenic process allows phages to enter and exit the bacteria without lysing their hosts.

M13 bacteriophages infect bacteria with sex pilli encoded by an F factor, and they do not lyse their hosts (Sambrook, 2001). They can even be released from the infected cells while the cells are growing (Sambrook, 2001). Infection in bacteria without sex pilli can be performed by transfection, but progeny particles produced by transfected cells are not able to infect other cells in the culture, and the yields of particles are very low (Sambrook, 2001).

GFP and EGFP

GFP is cloned from jelly fish *Aequorea victoria* (Tsien, 1998). It is a widely studied protein and used in biosensors, physiological indicators or photochemical memories (Tsien, 1998). Wild type (wt) GFP generates two excitation maxima, 395–397nm (major) and 470–475nm (minor), and the emission maximum at 504nm (Tsien, 1998). A gene mutagenesis of GFP generated an EGFP which is brighter and has a single excitation maximum at 488 nm and emission maximum at 510 nm (Tsien, 1998).

MATERIALS AND METHODS

E. coli TG1 bacteria were purchased from Stratagene Technical Services (La Jolla, CA) and M13 phage were from New England BioLab (Ipswich, MA). Chemicals and reagents necessary for M9 medium, 2× YT medium and 1mM thiamine were ordered from Sigma Aldrich (St. Louis, MO). Phosphate buffered saline (PBS) was prepared in the lab. Agarose (1.5%) was GibCo BRL brand from Life Technologies, Inc. (Gaithersburg, MD). Tryptone and yeast extract for 2× YT medium were from BD Bacto™ (Sparks, MD). Deionized water is produced in the lab by Direct-Q™ 5 Ultrapure Water Systems equipped with Millipore Quantum Filter, which were purchased from Millipore (Billerica, MA). Polystyrene petri dishes (100mmx15mm) and 14ml polystyrene round bottom test tubes were BD Falcon™ brand (Franklin Lakes, NJ). Flexible 1μL disposable inoculating loops, ampicillin stock (100mg/ml) and IPTG stock (1M) were Fisherbrand (Houston, TX). Eppendorf 1.5ml microcentrifuge tubes, 1.5ml disposable cuvettes for spectrophometric measurement, and pipet tips (0.1-10µl, 1-200µl, and 1ml) were purchased from VWR (Batavia, IL). Parafilm M (4in x 250ft) and isotemp incubator for 37^{o}C incubation were purchased from Fisher Sceintific (Pittsburgh, PA). Sterilization of reagents and lab equipments was facilitated by Getinge 522LS gravity steam sterilizer purchased from Getinge USA, Inc. (Rochester, NY). Optical density or OD_{600} were measured by SmartSpecTM Plus spectrophotometer from BIO-RAD (Hercules, CA). Cell culturing was performed in Forma® orbital shaker (model-420) bought from Thermo Electron Corporation (Waltham, MA). Fluorescence detection was performed by LS 55 Luminescence Spectrometer equipped with FL WinLab™ software purchased from PerkinElmer Life and Analytical Sciences, Inc. (Waltham, MA).

Preparation of the M9 Minimal Salts Agar Plates

This media is used to grow recombinant *E. coli* (Clontech, 1997). The M9 minimal salt solution was prepared by dissolving 1.2g of Na_2HPO_4, 0.5g of KH_2PO_4, 0.1g of NaCl, and 0.2g of NH_4Cl in 200ml of deionized water. The prepared M9 salt solution was mixed with 3g of agar (15g/l), and autoclaved at 121^{o}C with liquid cycle and 15 minutes exposure time in gravity steam sterilizer for one hour. After cooling it down at ~50^{o}C, it was mixed with 0.4ml of sterile 1M $MgSO_4$ (final conc. 2mM), 2ml of 200g/l glucose (final conc. 2g/l), 200µl of 0.1mM $CaCl_2$ (final conc. 0.1µM), and 20µl of 10mg/ml thiamine (final conc. 1µg/ml). After mixing it evenly, about 25mL of media was poured into each petri dish and allowed them to solidify at room temperature.

Preparation of 2× YT solution medium

This media contains bacto-tryptone, bacto-yeast extract, NaCl and ddH_2O, and is most suitable for growth and movement of *E. coli* bacteria infected with M13 bacteriophages (Clontech, 1997). The 2× YT solution medium was prepared by dissolving 8g of bacto-tryptone, 5g of bacto-yeast extract, and 2.5g of NaCl in deionized water to make a final volume of 500ml, and was autoclaved at 121^{o}C with liquid cycle and 15 minutes exposure time in gravity steam sterilizer for one hour. It can be stored at 4^{o}C for 3 months.

Culturing *E. coli* TG1 bacteria

Using the aseptic technique, streaked once on the surface of the *E. coli* TG1 stock preserved at -80^{o}C using the inoculating loop and transfer the cells onto the M9+thiamine agar plate by making secondary or tertiary streak. After growing it overnight (approximately 18 hours) in the isotemp incubator at 37^{o}C, the plate was sealed with parafilm and stored in 4^{o}C refrigerator to seize the growth of the cells. A successful culture contained isolable single colonies.

Preparation of mid-exponential phase *E. coli* TG1 bacteria

Applying aseptic technique, a single colony of *E. coli* TG1 was taken from plate culture using a sterilized inoculating loop and dipped into 5ml of 2×YT solution medium in the round bottom test tube. After incubating the cell solution in the Forma orbital shaker for 3-5hrs at 37^{o}C with the speed of 250 rpm, transfer it the 0^{o}C ice water bath to seize the growth of the cells. Optical density value at 600nm (OD_{600}) was measured by spectrophotometer to check whether the cells were in the mid-exponential phase. First, 450µl of sterilized water was pipetted into each of two eppendorf tubes: control and sample. Then, 50µl of 2×YT solution medium from 4^{o}C was added into the control tube, and 50µl of *E. coli* TG1 cell from $0^{o}C$ was added into the sample tube. Both eppendorf tubes were vortexed before 0.5ml of both control and sample solutions were transferred to the cuvettes and the OD_{600} value was measured in the spectrophotometer by using the control as a blank. Note that the sample was diluted 10 times before measuring the OD_{600} value to minimize wasting of the sample. The OD_{600} value of 0.3 to 0.5 indicated that the cells were in the mid-exponential phase, and they were ready to be infected with the phage. One cell cycle of *E. coli* TG1 was approximately 20 minutes. The cell solution can be stored at $4^{o}C$ up to one week.

OD_{600} measurement

Optical density at wavelength 600nm (OD_{600}) is a spectrophometric measurement and is used to measure the density of cell in the solution (cell/ml). Wavelength of 600nm is used because the best correlation between the total number of cells and optical density can be obtained at this wavelength. OD_{600} measurement is used to determine the growth state of the cells in the solution whether they are in the desired mid-exponential phase (Clontech, 1997).

Infection of EGFP-displayed M13 phage to *E. coli* TG1

First, 4.5ml of 2× YT, 0.5ml of mid-exponential phase *E. coli* TG1 and 5µl of IPTG were pipetted into control (C1) and two sample tubes (S1 and SA), and then 0.5µl of EGFP-displayed M13 phage was added only into two sample tubes. All test tubes were incubated at room temperature for 30 minutes, and 5µl of ampicillin was added into the sample tube, SA. Then, all three tubes were incubated at $30^{o}C$, an appropriate temperature for EGFP expression, in Forma orbital shaker at 250 rpm for 3 hours, and the first sample was taken to obtain the spectrometric data for 10x 100x and 1000x solution. Each successive sample was taken and performed spectrometric analysis after incubating in the shaker for 5, 7, 9, 11, 13, 15, 18 and 21 hours at 30°C with the speed of 250 rpm. **Figure 2** shown below represents the infection of EGFP-displayed M13 phage to *E. coli* TG1.

Isopropyl-β-D-thiogalactoside (IPTG)

IPTG is used to express high level of pIII fusion protein on the *E. coli* membrane by inducing the *lac* promoter of the fusion gene transcription, the level of which will be low without IPTG induction (Clontech, 1997).

Ampicillin

Normal *E. coli* bacteria are susceptible to the antibiotic ampicillin, and the plasmid of M13 bacteriophage contains the ampicillin resistant (amp^r) gene. After infecting the *E. coli* with the phage, the addition of ampicillin will ensure that only the bacteria which receive ampicillin resistant gene from the phage will survive to the next generation in growth cycle (Clontech, 1997).

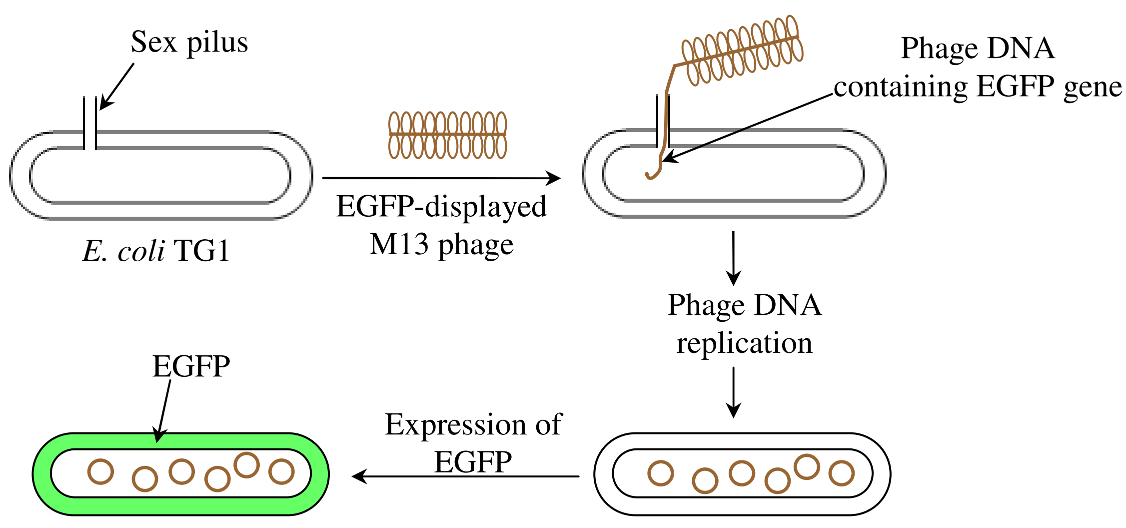


Figure 2. Infection of EGFP-displayed M13 phage to E. coli TG1.

Detection of EGFP expression on *E. coli* surface

Sample of 50µl volume was taken from each of the three test tubes and mixed with 450µl PBS to determine the cellular density (OD_{600}) with the spectrophotometer by blanking with 50µl of 2× YT plus 450µl of PBS. Using the OD_{600} value, calculate the appropriate amount of sample and PBS to be mixed to have the same cellular density in both sample and control of 500µl total volume. Then, after vortexing the sample in an eppendorf tube, the sample was transferred into the appropriate cuvette to measure the fluorescence intensity with PerkinElmer LS 55 Luminiscence Spectrometer. The sample was excited at the wavelength of 480nm and an emission spectrum was recorded from 500nm to 600nm by the FL WinLab™ software using the excitation spectral slit width of 10nm and emission spectral slit width of 5nm. The above procedure was followed for each successive sample taken for different time intervals. The emission maximum for EGFP was expected around 510nm. The samples were scanned with a speed of 500nm/min at 0.5nm intervals. **Figure 3** below shows the schematic of how the fluorescence spectrometer detects fluorescence from the EGFP.

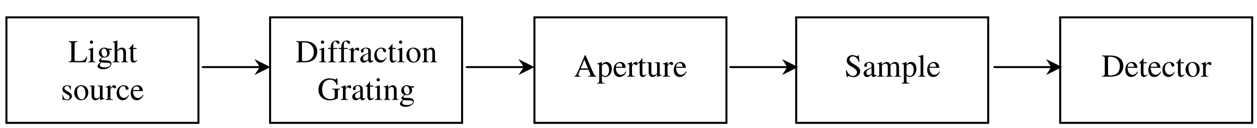
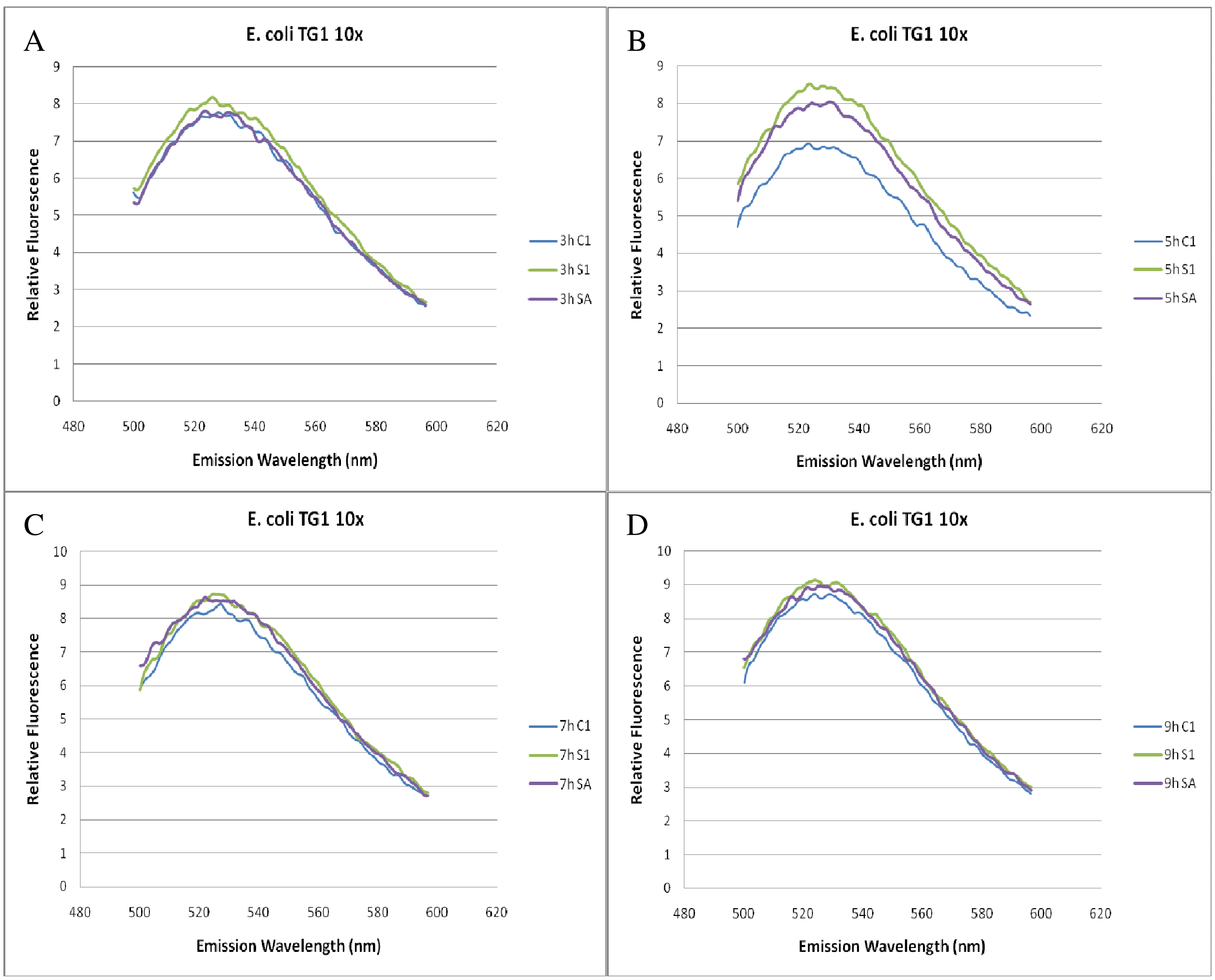
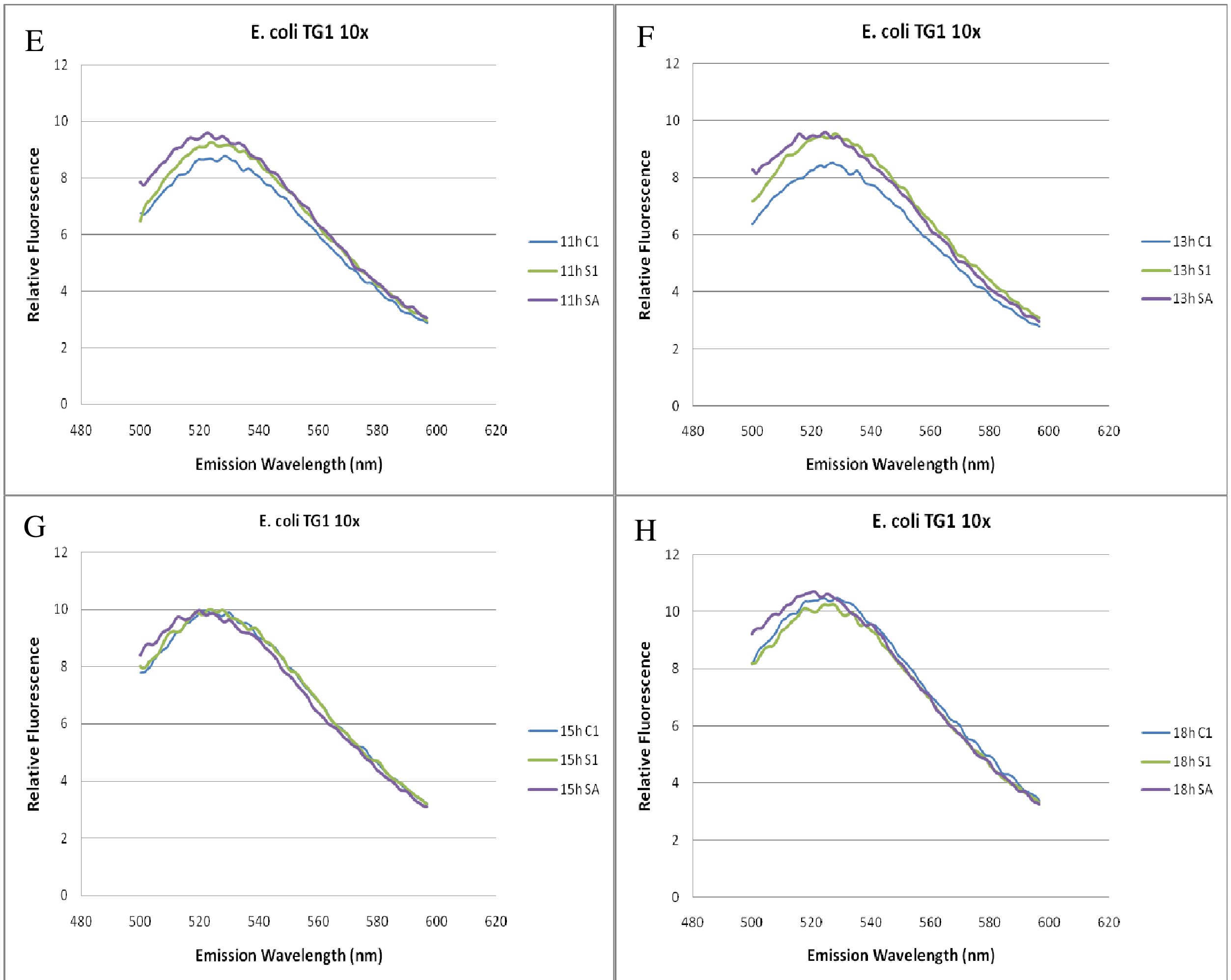


Figure 3. Schematic of the detection of fluorescence by spectrometer.

RESULTS AND DISCUSSIONS

Figure 4 (A-I) show the spectrometric data of the control (C1), and the two samples: sample without ampicillin (S1) and sample with ampicillin (SA) for different incubation time intervals. Relative fluorescence values were plotted over the range of emission wavelengths where the emission maximum wavelength of EGFP was expected. The data for *E. coli* TG1 sample with dilution factor 10x are presented here, but not for 100x and 1000x dilutions due to high noise level.





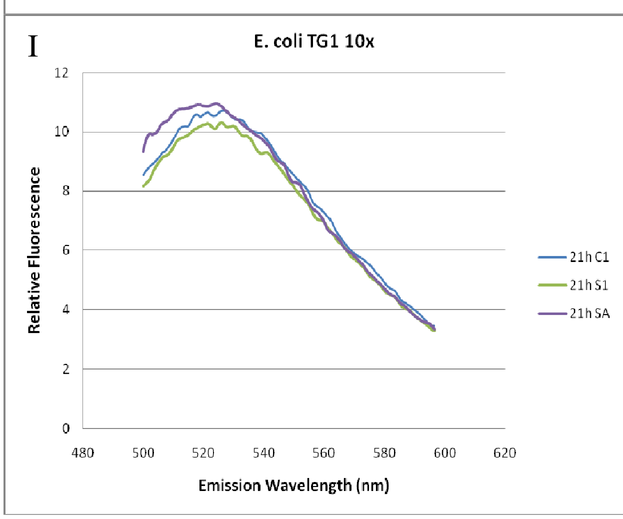


Figure 4 (A-I). Spectrometric data of *E. coli* TG1 for 3h, 5h, 7h, 9h, 11h, 13h, 15h, 18h, and 21h incubation time intervals respectively. C1: Control, S1: Sample without ampicillin, and SA: Sample with ampicillin.

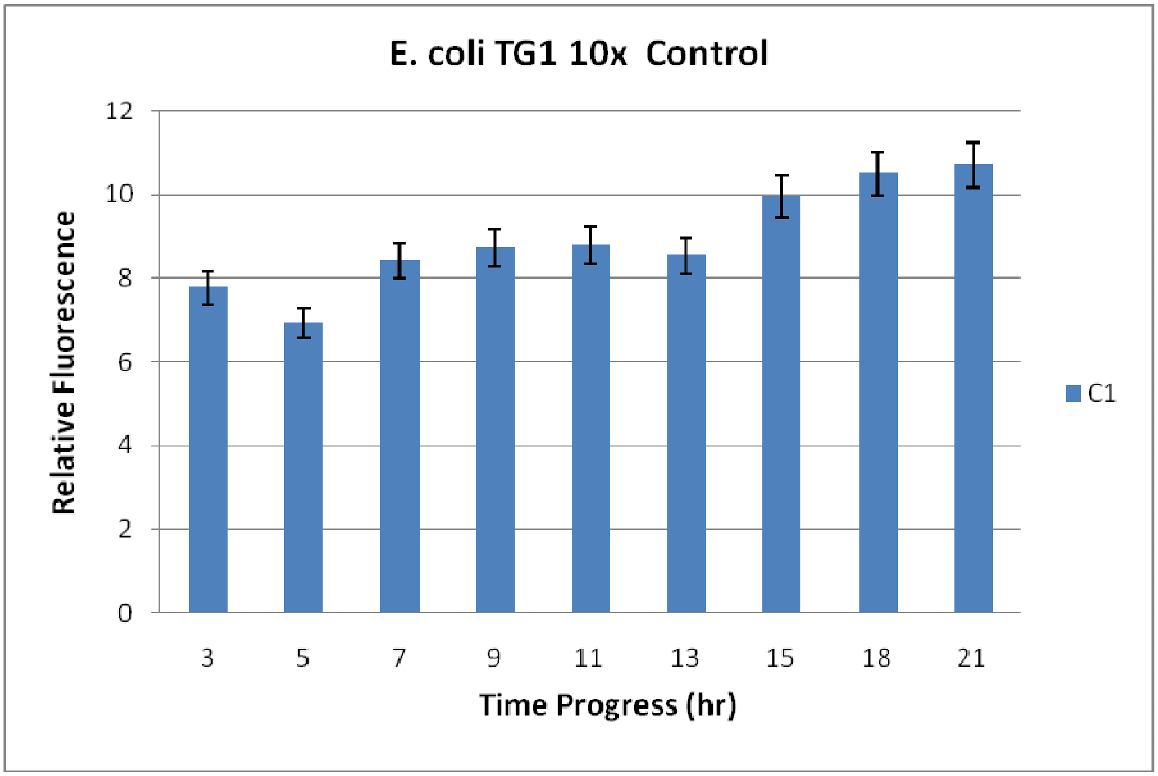


Figure 5. Maximum relative fluorescence at different time intervals for the control.

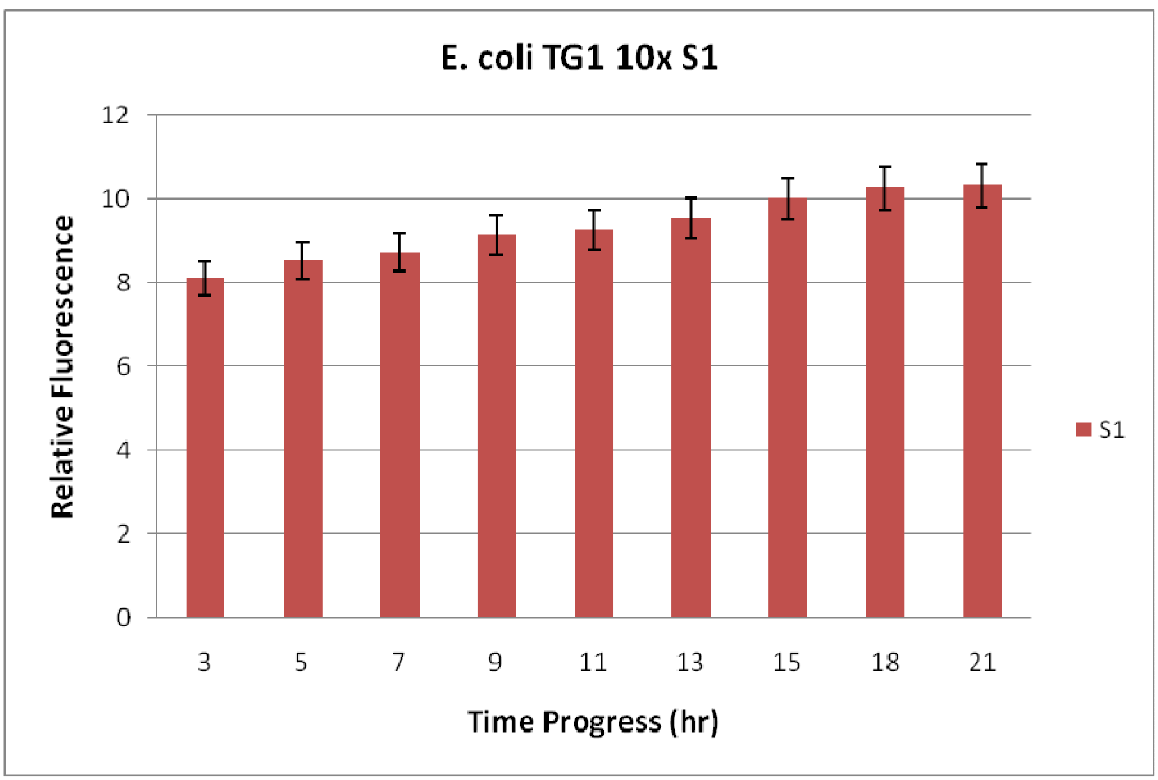


Figure 6. Maximum relative fluorescence at different time intervals for the sample S1.

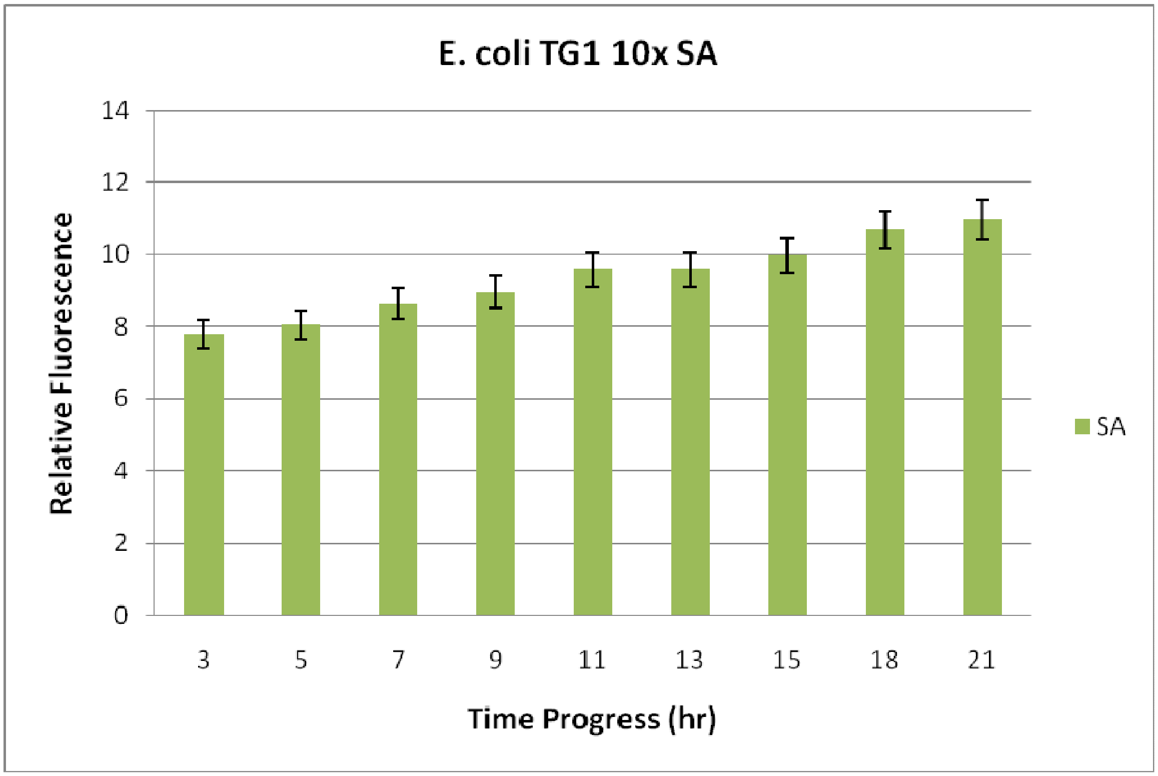


Figure 7. Maximum relative fluorescence at different time intervals for the sample SA.

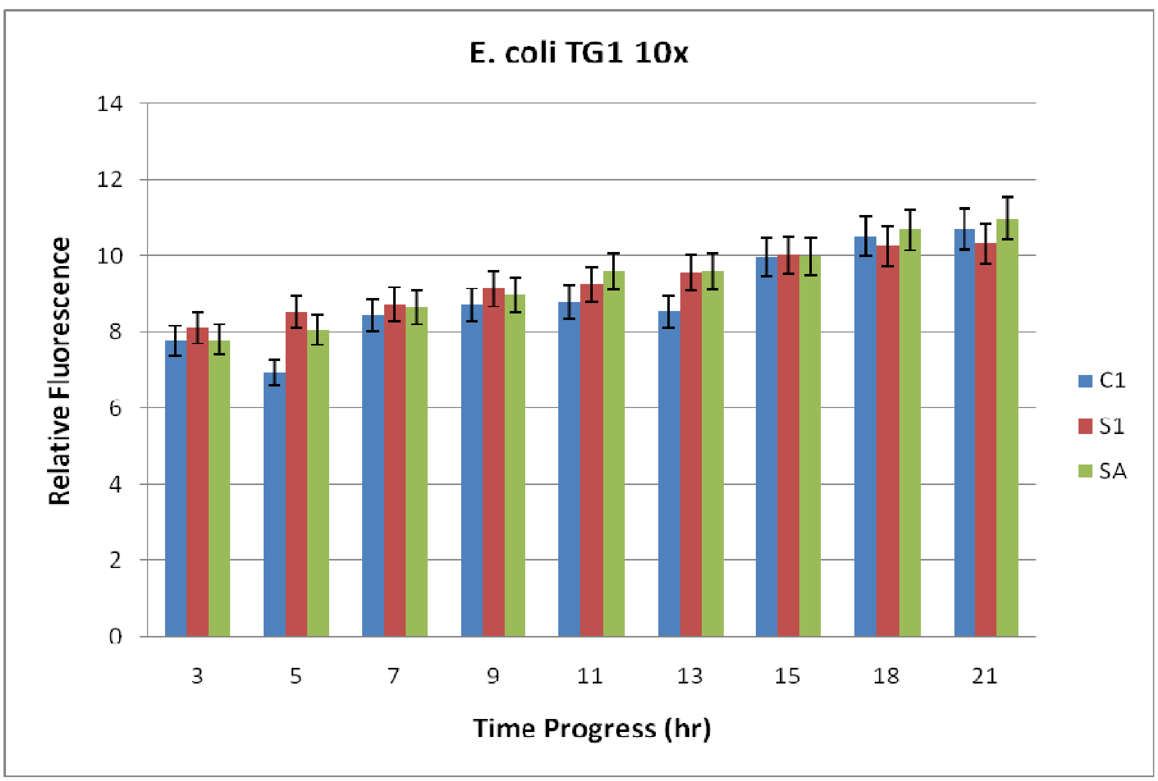


Figure 8. Maximum relative fluorescence at different time intervals for C1, S1, and SA.

The above **figures 5 – 7** show the maximum relative fluorescence at various incubation time for the control (C1) and the two samples (S1 and SA). **Figure 8** shows the comparison of maximum relative fluorescence among the control and the samples. Except for the control, the two samples show the steady increase in the fluorescence level as time progress. The relative fluorescence levels at 3 hours of incubation are 7.78 for the control, 8.10 for the sample S1, and 7.79 for the sample SA. The relative fluorescence levels at 21 hours of incubation are 10.71 for the control, 10.32 for the sample S1 and 10.97 for the sample SA. The low level of signals at 5 hour and 13 hour of the control might be due to error in dilution. The following **figures 9 – 11** show the linear relationship between maximum relative fluorescence levels and incubation time for the control (C1) and the two samples (S1 and SA). The correlation coefficient (R^2) values are greater than 0.95 except for the control. The level of fluorescence increase with a rate of 0.19 for C1, 0.13 for S1, and 0.18 for SA.

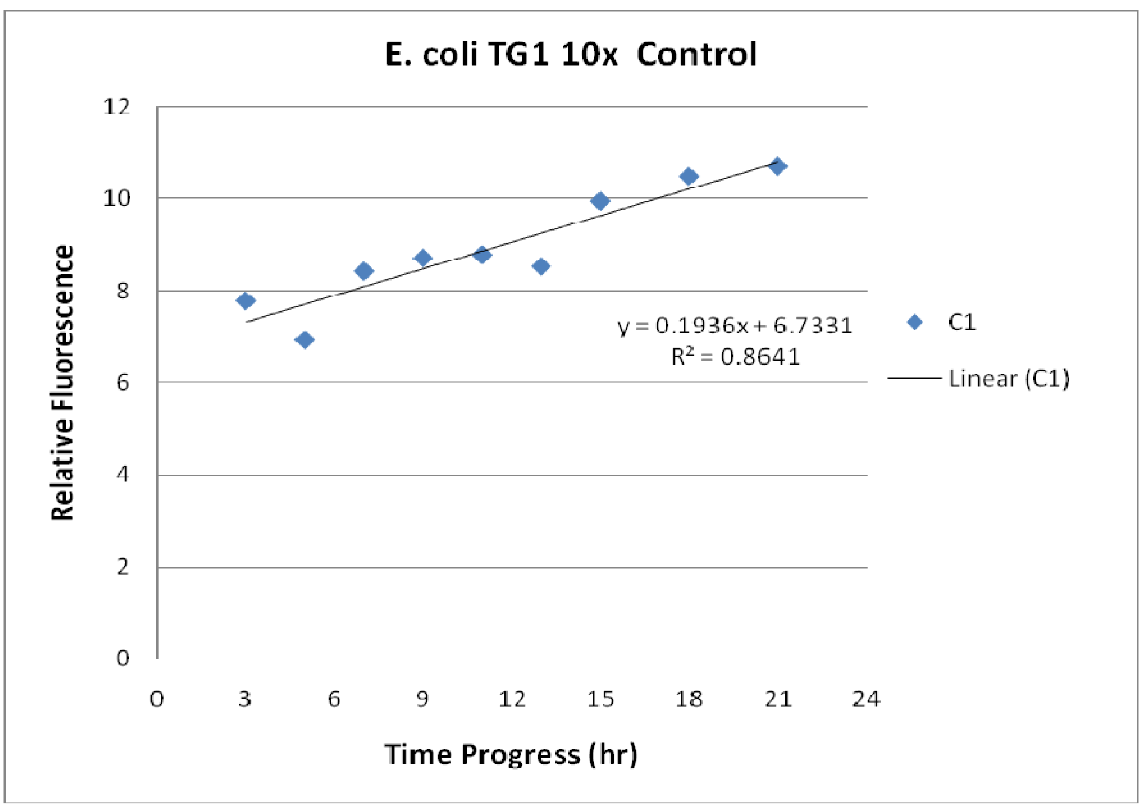


Figure 9. Linear relationship between max. relative fluorescence and incubation time for C1.

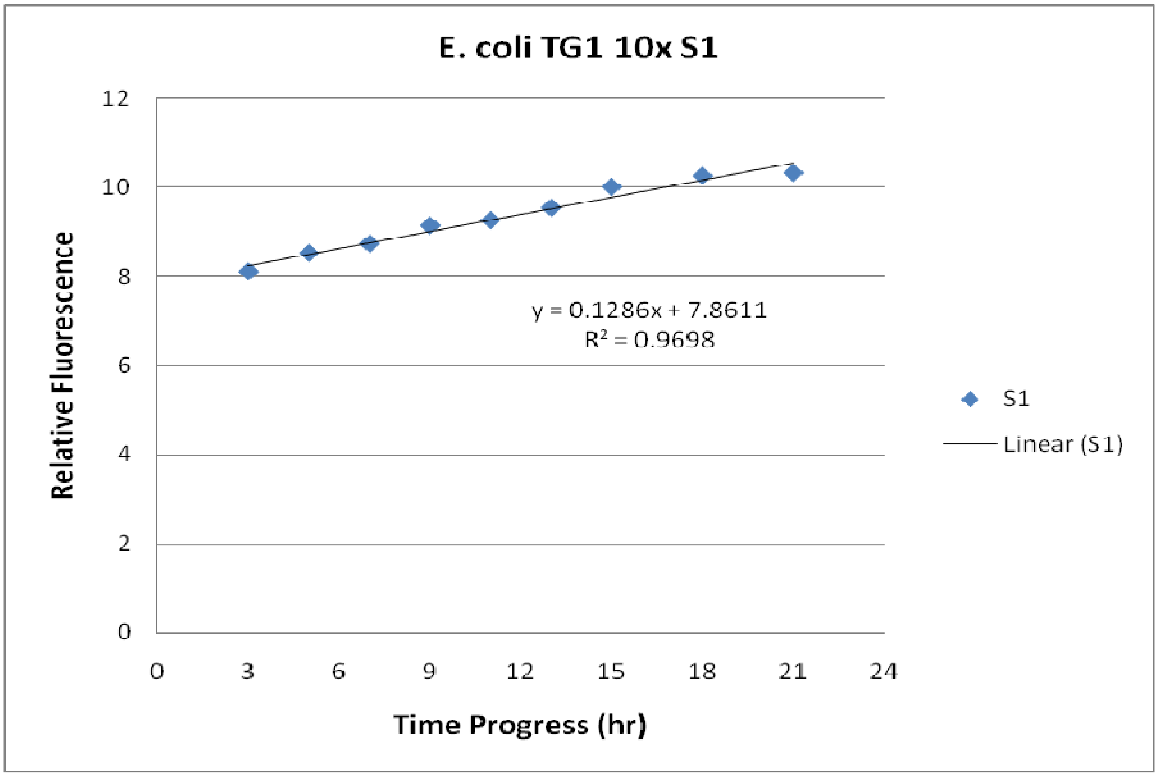


Figure 10. Linear relationship between max. relative fluorescence and incubation time for S1.

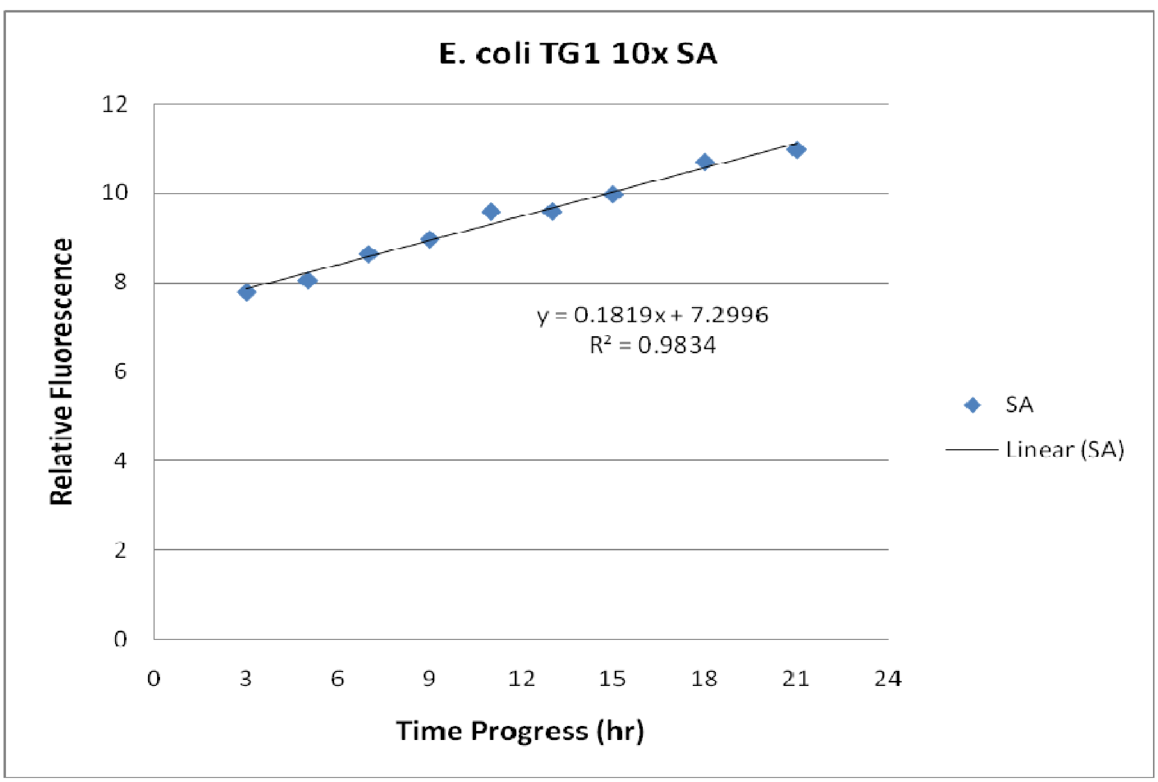


Figure 11. Linear relationship between max. relative fluorescence and incubation time for SA.

Table 1. Maximum emission wavelengths of *E. coli* TG1 for control.

Control - C1		
Time (hr)	Relative Fluorescence	Maximum wavelength (nm)
3	7.78	528
5	6.93	524
7	8.43	527
9	8.72	524
11	8.79	529
13	8.53	527
15	9.95	522
18	10.50	529
21	10.71	527
Mean maximum wavelength (nm)		526

Table 2. Maximum emission wavelengths of *E. coli* TG1 for sample without ampicillin.

Sample without ampicillin - S1		
Time (hr)	Relative Fluorescence	Maximum wavelength (nm)
3	8.10	525
5	8.52	524
7	8.73	525
9	9.14	524
11	9.26	524
13	9.54	528
15	10.01	524
18	10.26	527
21	10.32	526
Mean maximum wavelength (nm)		525

Table 3. Maximum emission wavelengths of *E. coli* TG1 for sample with ampicillin.

Sample with ampicillin - SA		
Time (hr)	Relative Fluorescence	Maximum wavelength (nm)
3	7.79	524
5	8.05	530
7	8.64	522
9	8.97	526
11	9.58	523
13	9.59	525
15	9.97	520
18	10.69	520
21	10.97	524
Mean maximum wavelength (nm)		524

The above **table 1, 2 and 3** show the maximum emission wavelengths with corresponding relative fluorescence at different incubation time intervals of the control (C1), and the two samples (S1 and SA). Although maximum fluorescence levels were detected at different maximum wavelengths over the range of 520nm to 530nm, the mean maximum emission wavelengths for three sets of data were only 1nm apart: 526nm for C1, 525nm for S1, and 524nm for SA. Considering that the error was existed only in the control, but not in the samples, the data for the samples suggested that the maximum emission wavelength detected in this experiment was approximately 15nm higher than that of pure EGFP (510nm). In other words, the maximum wavelengths in this experiment were red-shifted, in which the emission maximum wavelength was increased compared to the standard spectrum. Red-shift can occur due to the unpurified nature of the sample (Spiess, 2005). In standard experiment as in Bizzarri's 2006, fluorescent proteins are first purified before the detection.

The shift in maximum wavelength also depends on the pH of the environment where the cells are grown (Shu, 2007). The sensitivity to pH is even increased in the mutated GFP or EGFP, and the red-shifting of maximum wavelength occurs at high or basic pH (Shu, 2007). An increase in pH can cause deprotonation in the chromophores of the EGFP. Deprotonation in chromophore of fluorescent protein can further create the electron delocalization in the protein, and cause the red-shift (Bizzarri et al., 2006). Bizzarri et al. mentioned that deprotonated protein will create a red-shifted spectrum with approximately 15nm shift, which matched with the emission maximum wavelength of 525nm detected in this experiment because standard purified EGFP generates an emission maximum at 510nm.

EGFP required in this experiment is a mutant of wtGFP, and the emission maximum wavelength of EGFP (510 nm) is normally red-shifted from that of wtGFP (504 nm) (Tsien, 1998). Further increase in emission maximum wavelength might be due to undesired mutation of the phage during the storage time, because the range of emission maximum wavelengths detected here suggested the expression of yellow fluorescent protein (YFP), which is a mutant of wtGFP with the longest emission maximum wavelength (Moerner, 2002).

However, considering the fact that the control and the samples generated similar maximum wavelengths, the detected signals could be due to intracellular autofluorescence. In this case, the error might exist in the process of generating the EGFP-displayed phage. Intracellular autofluorescence is the emission of fluorescence from the endogenous fluorophores instead of from the EGFP. Autofluorescent signals in cells arise mainly from reduced nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FAD), the latter of which is the better candidate for the autofluorescence of this experiment because FAD is an important coenzyme in the cells involving in various redox processes of metabolic reactions, and also because the emission maximum wavelength of FAD is around 525nm (Rhee, 2007).

CONCLUSION

This experiment found out that detection of EGFP on *E. coli* bacteria from crude sample can be challenging due to interference of cellular autofluorescence in addition to the errors that could arise from various sources such as in generating the bacteriophage with EGFP gene, during the storage of the stock phage and bacteria, during the transfection of *E. coli* with phage, and/or from the detection device. In other words, the autofluorescence of endogenous flavins such as FAD was so predominant in the cells that it interfered with fluorescence of the EGFP. One possible solution suggested by Benson et al. was using the bandpass fluorescence filter centered at the expected emission maximum wavelength (Benson, 1979). Cellular autofluorescence can be reduced, but difficult to avoid, and optimization in each of the above steps is crucial to reduce the errors. Purity of the fluorescent species is also important especially when detecting the fluorescent species that has the emission maximum wavelength close to that of the autofluorescent species in the cells. Another possible solution is the detection of EGFP by the immunofluorescent technique, in which antibodies are coupled to other fluorescent dyes with the emission maximum wavelength far from that of the autofluorescent species.

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Appendix – Spectrometric data for control (C1)

Em. WL	C1 - Control								
(nm)	3h	5h	7h	9h	11h	13h	15h	18h	21h
500	5.62	4.709	5.896	6.088	6.761	6.382	7.786	8.17	8.541
500.5	5.5465	4.8826	5.9817	6.3253	6.7257	6.4492	7.7909	8.2934	8.6272
501	5.4932	5.0238	6.049	6.4863	6.7187	6.516	7.7938	8.4175	8.6878
501.5	5.4647	5.1276	6.1227	6.5951	6.7389	6.5856	7.8126	8.5345	8.7554
502	5.4763	5.2073	6.1808	6.6803	6.7818	6.6748	7.8665	8.6299	8.816
502.5	5.5369	5.2409	6.231	6.7163	6.844	6.7414	7.9235	8.7015	8.8731
503	5.6283	5.2481	6.2592	6.7421	6.9123	6.7962	7.975	8.7604	8.9002
503.5	5.7277	5.2689	6.2942	6.8123	6.9806	6.8553	8.0444	8.8028	8.9358
504	5.8301	5.3055	6.3439	6.9292	7.0514	6.9099	8.1383	8.8394	8.9993
504.5	5.9187	5.3598	6.3962	7.0256	7.1341	6.9625	8.2277	8.8851	9.0599
505	5.9772	5.4286	6.4719	7.1062	7.2076	7.0239	8.3058	8.9353	9.1148
505.5	6.0202	5.5242	6.5675	7.1946	7.2749	7.1055	8.3918	8.9864	9.1761
506	6.0681	5.6218	6.6781	7.2604	7.3284	7.1863	8.466	9.0351	9.2482
506.5	6.1213	5.7053	6.7757	7.3175	7.3805	7.2434	8.4953	9.0954	9.3029
507	6.1898	5.7732	6.8732	7.3859	7.4377	7.3122	8.5202	9.1495	9.3321
507.5	6.2657	5.8137	6.9765	7.4756	7.5045	7.3605	8.5655	9.2108	9.3848
508	6.3451	5.8404	7.062	7.5491	7.5724	7.3882	8.6122	9.3004	9.4474
508.5	6.4141	5.8487	7.1341	7.6138	7.6318	7.416	8.6453	9.4073	9.5079
509	6.4788	5.858	7.1851	7.6779	7.6855	7.462	8.6857	9.505	9.5707
509.5	6.5614	5.8786	7.2386	7.7458	7.7106	7.5127	8.7789	9.6	9.6615
510	6.6342	5.9171	7.282	7.8141	7.7336	7.5418	8.8666	9.6851	9.767
510.5	6.7226	5.9723	7.3304	7.8878	7.7777	7.6049	8.9431	9.7303	9.8491
511	6.8088	6.0229	7.3911	7.9654	7.8533	7.6598	9.0369	9.7621	9.928
511.5	6.8713	6.0797	7.4672	8.0171	7.9293	7.716	9.1166	9.8002	10.024
512	6.9188	6.1362	7.5487	8.0577	8.0076	7.7612	9.1707	9.8645	10.108
512.5	6.9426	6.1916	7.6091	8.0704	8.078	7.8092	9.1814	9.8965	10.145
513	6.9751	6.2512	7.6718	8.0907	8.11	7.8459	9.2157	9.9273	10.164
513.5	6.9809	6.3204	7.7152	8.1152	8.1264	7.8587	9.2659	9.9464	10.173
514	7.0095	6.4157	7.755	8.1545	8.1262	7.8948	9.3063	9.9481	10.181
514.5	7.0658	6.4954	7.7954	8.1998	8.1371	7.9181	9.3761	9.9437	10.172
515	7.1182	6.568	7.8464	8.2333	8.1459	7.9527	9.4489	9.9571	10.205
515.5	7.1737	6.6244	7.9127	8.2768	8.172	7.9713	9.5135	10.031	10.289
516	7.2222	6.6564	7.9572	8.3031	8.2185	7.9874	9.5303	10.117	10.387
516.5	7.2872	6.665	8.0025	8.3422	8.2749	7.9919	9.5483	10.215	10.488
517	7.3248	6.663	8.0355	8.3814	8.3529	7.9997	9.5984	10.3	10.539
517.5	7.3507	6.6889	8.0672	8.4383	8.4393	8.0485	9.6339	10.357	10.59
518	7.3827	6.7207	8.1023	8.503	8.5228	8.0923	9.6771	10.374	10.573
518.5	7.4127	6.7547	8.1368	8.5428	8.5886	8.1509	9.719	10.362	10.524
519	7.4175	6.7731	8.1601	8.5608	8.6326	8.2087	9.7766	10.37	10.509
519.5	7.4319	6.7868	8.1688	8.5661	8.6566	8.2529	9.8171	10.373	10.533

Appendix – Spectrometric data for control (C1) (Continued)

Em. WL	C1 - Control								
(nm)	3h	5h	7h	9h	11h	13h	15h	18h	21h
520	7.4658	6.7893	8.1652	8.5683	8.6555	8.2682	9.8487	10.383	10.591
520.5	7.5052	6.7852	8.145	8.5495	8.6446	8.2724	9.9011	10.395	10.613
521	7.5506	6.7962	8.1201	8.5458	8.6471	8.3133	9.9395	10.408	10.644
521.5	7.5846	6.8162	8.1182	8.5679	8.6557	8.3703	9.9537	10.407	10.656
522	7.6482	6.8584	8.1313	8.6143	8.6662	8.405	9.9432	10.407	10.614
522.5	7.6642	6.8959	8.1446	8.6537	8.6711	8.4319	9.9308	10.441	10.565
523	7.6677	6.9245	8.1744	8.6883	8.6818	8.4432	9.8999	10.465	10.528
523.5	7.657	6.9289	8.207	8.7182	8.693	8.4281	9.8552	10.481	10.534
524	7.6504	6.9021	8.2305	8.7055	8.6709	8.3906	9.8586	10.489	10.542
524.5	7.6571	6.8595	8.2354	8.6701	8.6423	8.3916	9.8589	10.475	10.581
525	7.6356	6.8144	8.2661	8.6191	8.6142	8.4427	9.8514	10.437	10.636
525.5	7.6593	6.7887	8.3161	8.592	8.6035	8.4748	9.8366	10.391	10.678
526	7.6763	6.7767	8.3637	8.5824	8.6035	8.5017	9.8142	10.377	10.705
526.5	7.716	6.7775	8.4042	8.5922	8.6346	8.5218	9.7842	10.386	10.714
527	7.7403	6.7993	8.4288	8.6231	8.6922	8.5322	9.7474	10.413	10.707
527.5	7.7588	6.8218	8.4246	8.6569	8.7378	8.5075	9.7641	10.451	10.675
528	7.7821	6.8398	8.3575	8.6959	8.7792	8.4741	9.7903	10.489	10.642
528.5	7.7603	6.8404	8.2766	8.7112	8.7857	8.4592	9.818	10.499	10.583
529	7.7327	6.8272	8.2101	8.706	8.7657	8.449	9.8498	10.479	10.534
529.5	7.6967	6.8157	8.1684	8.6911	8.7239	8.437	9.8777	10.451	10.488
530	7.687	6.8081	8.1384	8.669	8.6815	8.4096	9.8722	10.418	10.45
530.5	7.6773	6.8156	8.1216	8.6502	8.6438	8.3771	9.8216	10.38	10.424
531	7.6768	6.8258	8.1165	8.6118	8.617	8.3265	9.7649	10.355	10.405
531.5	7.6924	6.8323	8.0799	8.5734	8.6205	8.2545	9.7015	10.347	10.422
532	7.6862	6.8266	8.0209	8.556	8.585	8.1739	9.6253	10.339	10.417
532.5	7.6486	6.8105	7.9637	8.527	8.5201	8.1264	9.5611	10.322	10.404
533	7.5904	6.7903	7.9298	8.4929	8.442	8.1207	9.5261	10.305	10.371
533.5	7.5311	6.7569	7.9083	8.465	8.3695	8.1304	9.5075	10.273	10.314
534	7.469	6.7258	7.9082	8.4517	8.3012	8.1617	9.4982	10.232	10.242
534.5	7.408	6.6977	7.9293	8.4137	8.2548	8.2208	9.5057	10.183	10.164
535	7.3817	6.6826	7.9519	8.3473	8.2771	8.258	9.521	10.13	10.111
535.5	7.3729	6.664	7.9591	8.3	8.3124	8.2391	9.5189	10.074	10.055
536	7.3767	6.639	7.9647	8.2495	8.3383	8.1667	9.4944	10.001	10.019
536.5	7.393	6.6183	7.944	8.1894	8.3389	8.0769	9.4679	9.945	9.9995
537	7.4058	6.6004	7.8955	8.1495	8.3085	7.9794	9.4191	9.8659	9.9862
537.5	7.4112	6.5826	7.8284	8.1564	8.2536	7.8863	9.3378	9.7852	9.9805
538	7.3818	6.5671	7.7449	8.1644	8.1858	7.824	9.2488	9.7149	9.9685
538.5	7.355	6.5593	7.659	8.1661	8.1453	7.7887	9.1596	9.6595	9.9684
539	7.3136	6.5539	7.5756	8.1568	8.1186	7.7813	9.0826	9.6209	9.9502
539.5	7.2774	6.5284	7.511	8.1354	8.0855	7.7752	9.0193	9.5931	9.905

Appendix – Spectrometric data for control (C1) (Continued)

Em. WL	C1 - Control								
(nm)	3h	5h	7h	9h	11h	13h	15h	18h	21h
540	7.2513	6.4785	7.4633	8.079	8.0423	7.7593	8.9901	9.5869	9.8442
540.5	7.2395	6.404	7.4279	8.0227	7.9809	7.7476	8.9801	9.5724	9.7793
541	7.2523	6.3223	7.424	7.984	7.9161	7.725	8.9556	9.5434	9.7207
541.5	7.2392	6.2458	7.4141	7.9381	7.8489	7.69	8.9254	9.4996	9.6461
542	7.2257	6.1743	7.4068	7.9111	7.8007	7.6498	8.8774	9.4522	9.5811
542.5	7.186	6.1378	7.373	7.8662	7.7749	7.6031	8.8318	9.4059	9.5152
543	7.1352	6.122	7.3105	7.8125	7.753	7.5527	8.7826	9.3512	9.4346
543.5	7.0628	6.1063	7.2307	7.7383	7.7415	7.4747	8.7541	9.2885	9.3373
544	6.9884	6.0924	7.1415	7.6665	7.7156	7.4125	8.7337	9.2257	9.2448
544.5	6.9339	6.0806	7.0781	7.6136	7.6724	7.3608	8.7197	9.1554	9.171
545	6.8505	6.0751	7.0207	7.5555	7.5983	7.3172	8.688	9.0908	9.0946
545.5	6.7662	6.0464	6.9996	7.527	7.5195	7.2938	8.627	9.0321	9.0286
546	6.6876	6.0112	6.9928	7.506	7.4475	7.2561	8.5552	8.97	8.9834
546.5	6.6147	5.9755	6.9912	7.4843	7.3996	7.2156	8.4834	8.9233	8.9466
547	6.5462	5.9198	6.9747	7.4484	7.3631	7.1511	8.3991	8.8506	8.8896
547.5	6.4934	5.8426	6.939	7.3956	7.3438	7.1023	8.3105	8.764	8.8242
548	6.4812	5.7646	6.8884	7.3266	7.3331	7.0496	8.236	8.6611	8.7636
548.5	6.4784	5.7	6.8237	7.2378	7.3036	7.0047	8.16	8.5719	8.7
549	6.4673	5.6483	6.7656	7.155	7.2793	6.989	8.0803	8.485	8.633
549.5	6.4691	5.5973	6.6971	7.0847	7.2103	6.9654	8.011	8.4166	8.5744
550	6.4753	5.5718	6.6446	7.0283	7.1486	6.9314	7.9704	8.3799	8.5271
550.5	6.4686	5.5457	6.5927	6.9925	7.0738	6.8668	7.9353	8.3338	8.4686
551	6.4265	5.5213	6.5428	6.959	6.995	6.8038	7.8901	8.2849	8.4133
551.5	6.3742	5.5038	6.4817	6.9253	6.9288	6.7145	7.8507	8.2199	8.3634
552	6.3046	5.4917	6.4224	6.89	6.851	6.6191	7.8019	8.1625	8.3078
552.5	6.2088	5.4881	6.3796	6.8512	6.7965	6.5513	7.7323	8.0972	8.2528
553	6.1163	5.4584	6.3473	6.8079	6.7166	6.4945	7.6491	8.0394	8.2087
553.5	6.045	5.4273	6.3323	6.7629	6.6677	6.4339	7.5613	7.9906	8.1654
554	6.0023	5.3952	6.3223	6.7296	6.6206	6.3689	7.4838	7.9269	8.1023
554.5	5.9605	5.3446	6.3024	6.6982	6.5617	6.3208	7.4031	7.846	8.0192
555	5.9311	5.2768	6.2594	6.6562	6.5252	6.2604	7.3371	7.758	7.9197
555.5	5.9006	5.2085	6.1819	6.6026	6.4699	6.1955	7.2812	7.6807	7.7958
556	5.8615	5.1514	6.0813	6.5477	6.422	6.1374	7.2249	7.5974	7.6734
556.5	5.8172	5.0719	6.0003	6.4829	6.3566	6.0904	7.1753	7.5284	7.592
557	5.7793	4.9817	5.9261	6.4028	6.3246	6.0232	7.1043	7.4619	7.5303
557.5	5.7489	4.9076	5.8679	6.3137	6.3016	5.9681	7.0527	7.3961	7.4862
558	5.6997	4.8437	5.8114	6.225	6.2437	5.9375	6.995	7.3157	7.4566
558.5	5.6502	4.7844	5.7646	6.1396	6.1893	5.9044	6.9386	7.2334	7.4279
559	5.5834	4.7476	5.7181	6.0645	6.1282	5.8599	6.8716	7.1613	7.387
559.5	5.497	4.7519	5.6452	6.0156	6.0644	5.8112	6.8106	7.1007	7.3202

Appendix – Spectrometric data for control (C1) (Continued)

Em. WL (nm)	C1 - Control								
	3h	5h	7h	9h	11h	13h	15h	18h	21h
560	5.4161	4.7691	5.5822	5.9785	5.966	5.7659	6.7594	7.0518	7.263
560.5	5.3444	4.7721	5.5155	5.9518	5.893	5.7037	6.6942	6.9996	7.2071
561	5.2762	4.775	5.4611	5.906	5.847	5.6508	6.6411	6.952	7.1468
561.5	5.2163	4.7654	5.4139	5.8553	5.7935	5.6182	6.5921	6.8919	7.0961
562	5.1741	4.7224	5.3756	5.7889	5.7401	5.5828	6.5453	6.8191	7.0327
562.5	5.1431	4.6526	5.3592	5.7079	5.6921	5.5273	6.4645	6.7641	6.9565
563	5.0947	4.5746	5.3344	5.6311	5.6596	5.4711	6.364	6.7158	6.8547
563.5	5.041	4.4987	5.3156	5.5623	5.6034	5.4174	6.2663	6.6708	6.7521
564	4.9955	4.4195	5.2746	5.5185	5.5435	5.354	6.177	6.6121	6.6602
564.5	4.9273	4.351	5.2438	5.4775	5.4903	5.3051	6.0887	6.5469	6.57
565	4.834	4.3003	5.2115	5.4501	5.4351	5.2795	6.0282	6.4732	6.4932
565.5	4.7375	4.2571	5.1611	5.4197	5.3769	5.2658	5.9969	6.3797	6.4239
566	4.6612	4.2173	5.115	5.3749	5.3298	5.231	5.9621	6.3073	6.354
566.5	4.5847	4.164	5.0723	5.3258	5.2841	5.1878	5.9193	6.248	6.2888
567	4.5352	4.0988	5.0423	5.2624	5.238	5.1317	5.8848	6.2025	6.2131
567.5	4.5307	4.0317	4.9864	5.1927	5.1877	5.0547	5.8545	6.1734	6.1417
568	4.5337	3.9712	4.9262	5.1254	5.1298	4.9829	5.8069	6.1587	6.0824
568.5	4.5282	3.9224	4.8608	5.0764	5.0577	4.9234	5.7607	6.1482	6.0328
569	4.5001	3.8871	4.7849	5.0397	4.9729	4.8696	5.7012	6.1047	5.9881
569.5	4.4655	3.8763	4.7009	5.0045	4.915	4.8098	5.6306	6.0521	5.9407
570	4.4123	3.8631	4.6131	4.9662	4.8592	4.7582	5.5516	5.9724	5.9014
570.5	4.3539	3.8284	4.5527	4.9085	4.8188	4.7271	5.4873	5.8635	5.8617
571	4.3028	3.7807	4.5006	4.8368	4.8013	4.6919	5.425	5.7557	5.817
571.5	4.2545	3.7363	4.4497	4.7718	4.7845	4.6508	5.3686	5.6624	5.7885
572	4.2152	3.6901	4.4167	4.7184	4.7566	4.6119	5.3237	5.6	5.7652
572.5	4.1652	3.657	4.395	4.678	4.7076	4.5602	5.287	5.5502	5.731
573	4.124	3.6458	4.3791	4.6403	4.6608	4.4874	5.2432	5.5296	5.6883
573.5	4.0879	3.6445	4.3474	4.6085	4.5943	4.4011	5.2095	5.5225	5.6472
574	4.0664	3.6256	4.3121	4.5471	4.516	4.327	5.1975	5.5041	5.6067
574.5	4.0463	3.5861	4.2774	4.459	4.4432	4.2719	5.1813	5.4762	5.5641
575	4.0147	3.5361	4.2178	4.3761	4.3785	4.2209	5.166	5.4333	5.5196
575.5	3.9888	3.4675	4.1493	4.306	4.3309	4.1936	5.1248	5.3835	5.475
576	3.9412	3.3977	4.0839	4.2626	4.3086	4.1845	5.0784	5.3126	5.4101
576.5	3.8828	3.347	4.0338	4.2552	4.305	4.1815	4.9877	5.2233	5.3481
577	3.8255	3.3252	3.986	4.2666	4.31	4.1649	4.8937	5.1367	5.2838
577.5	3.7798	3.3231	3.9511	4.2558	4.3037	4.1444	4.8246	5.0634	5.215
578	3.7405	3.326	3.9288	4.2195	4.2828	4.1182	4.7637	5.005	5.1639
578.5	3.7049	3.3277	3.8919	4.1744	4.2371	4.0762	4.7339	4.9691	5.1144
579	3.6781	3.3139	3.8464	4.1098	4.1713	4.0204	4.6973	4.9572	5.0609
579.5	3.6538	3.2818	3.7912	4.0357	4.1032	3.9468	4.6798	4.9538	4.9905

Appendix – Spectrometric data for control (C1) (Continued)

Em. WL (nm)	C1 - Control								
	3h	5h	7h	9h	11h	13h	15h	18h	21h
580	3.6353	3.2356	3.7411	3.9719	4.0333	3.8798	4.6263	4.9353	4.9219
580.5	3.6159	3.1835	3.6979	3.9227	3.9752	3.8183	4.5514	4.9004	4.8537
581	3.594	3.1292	3.6695	3.8766	3.9252	3.7742	4.4897	4.853	4.7847
581.5	3.5566	3.0913	3.6535	3.8394	3.8751	3.7385	4.4312	4.7822	4.7477
582	3.5213	3.0677	3.631	3.8174	3.8194	3.7087	4.3779	4.6863	4.7116
582.5	3.4763	3.0463	3.6003	3.7935	3.7689	3.687	4.3175	4.585	4.6908
583	3.4284	3.0244	3.5514	3.754	3.7311	3.6495	4.2824	4.4899	4.6629
583.5	3.39	2.9966	3.4925	3.7159	3.6997	3.6023	4.2391	4.4063	4.6277
584	3.3589	2.961	3.4255	3.6784	3.6819	3.554	4.1798	4.3452	4.5567
584.5	3.3389	2.9141	3.372	3.6378	3.6779	3.5163	4.1391	4.3109	4.4729
585	3.2978	2.8713	3.3506	3.6011	3.6653	3.4899	4.1117	4.3039	4.4074
585.5	3.2622	2.839	3.3455	3.5692	3.6244	3.4673	4.0889	4.3047	4.3338
586	3.2137	2.8059	3.3512	3.5406	3.569	3.4549	4.0572	4.3064	4.2761
586.5	3.1575	2.7733	3.35	3.5067	3.5019	3.4384	4.0202	4.2904	4.2415
587	3.1021	2.7382	3.327	3.4535	3.4301	3.4126	3.9797	4.2573	4.2257
587.5	3.0554	2.7001	3.2725	3.3959	3.3542	3.3727	3.9225	4.217	4.1897
588	3.0345	2.655	3.2056	3.3383	3.3042	3.3208	3.8595	4.1628	4.1475
588.5	3.0088	2.6032	3.1475	3.2876	3.2696	3.2743	3.8013	4.095	4.1165
589	2.9934	2.5769	3.0929	3.2474	3.2536	3.2291	3.7506	4.0226	4.0773
589.5	2.9768	2.5676	3.0522	3.2181	3.247	3.1821	3.7072	3.9537	4.0315
590	2.9553	2.57	3.0329	3.2052	3.2386	3.1479	3.6724	3.886	3.9891
590.5	2.9281	2.5697	3.0245	3.1889	3.2242	3.124	3.6497	3.8186	3.9614
591	2.8983	2.5604	3.004	3.1742	3.1996	3.1003	3.6254	3.7737	3.9162
591.5	2.8659	2.5482	2.9731	3.1571	3.171	3.0694	3.5872	3.748	3.8627
592	2.8331	2.5066	2.9426	3.1344	3.1248	3.0277	3.5405	3.7139	3.8121
592.5	2.7946	2.4652	2.9074	3.1011	3.0885	2.9852	3.4951	3.6824	3.7666
593	2.7444	2.4339	2.8759	3.0578	3.0639	2.9412	3.4482	3.6578	3.7113
593.5	2.6972	2.42	2.8575	3.0197	3.0344	2.913	3.4029	3.6358	3.6509
594	2.6548	2.417	2.8458	2.9748	3.0098	2.9004	3.3789	3.6112	3.5927
594.5	2.6381	2.418	2.8249	2.9305	2.9985	2.8918	3.3678	3.5805	3.5329
595	2.6229	2.4348	2.7991	2.8943	2.9893	2.8857	3.3427	3.5522	3.4853
595.5	2.614	2.4248	2.7726	2.8636	2.9533	2.8685	3.3005	3.5062	3.462
596	2.5957	2.3887	2.737	2.8339	2.9182	2.8411	3.2537	3.4406	3.4561
596.5	2.5583	2.3354	2.7037	2.8015	2.886	2.8012	3.2064	3.3769	3.4497

Appendix – Spectrometric data for sample (S1)

Em. WL	S1 - Sample without ampicillin								
(nm)	3h	5h	7h	9h	11h	13h	15h	18h	21h
500	5.741	5.855	5.861	6.538	6.476	7.176	8.015	8.191	8.18
500.5	5.705	5.9289	6.075	6.6341	6.6782	7.2181	7.9484	8.1902	8.2072
501	5.6987	6.0137	6.2456	6.7391	6.8434	7.2649	7.9436	8.2002	8.2636
501.5	5.7268	6.1077	6.3854	6.8511	6.98	7.3086	7.9616	8.2378	8.3186
502	5.7689	6.225	6.4823	6.9553	7.0778	7.3554	8.01	8.3164	8.377
502.5	5.8372	6.3478	6.5672	7.066	7.1451	7.4199	8.0853	8.4105	8.4656
503	5.9351	6.451	6.6295	7.1718	7.1911	7.4971	8.1731	8.4979	8.5841
503.5	6.0268	6.5331	6.6911	7.248	7.2499	7.5745	8.2227	8.5899	8.6922
504	6.1112	6.5909	6.7575	7.2855	7.3137	7.6603	8.2564	8.6699	8.7859
504.5	6.1896	6.6329	6.7848	7.3156	7.3685	7.7429	8.3016	8.7252	8.88
505	6.28	6.6588	6.7928	7.3308	7.4267	7.8159	8.3383	8.752	8.9586
505.5	6.352	6.7017	6.8108	7.351	7.4919	7.8741	8.3863	8.7715	9.0251
506	6.4174	6.7637	6.8563	7.4189	7.5684	7.9347	8.4457	8.7845	9.0827
506.5	6.493	6.8444	6.9297	7.5297	7.6523	8.005	8.5364	8.7762	9.1451
507	6.5674	6.9344	7.0432	7.6664	7.7549	8.0699	8.6435	8.795	9.1891
507.5	6.6457	7.0276	7.1951	7.7832	7.8501	8.1393	8.767	8.8493	9.2067
508	6.708	7.1223	7.3262	7.8928	7.9333	8.2167	8.8873	8.9281	9.2225
508.5	6.7734	7.1892	7.4173	7.9617	8.0033	8.2946	8.9907	9.0245	9.2592
509	6.8277	7.2495	7.4909	7.9914	8.0682	8.3737	9.0842	9.1337	9.3294
509.5	6.8907	7.2775	7.5335	8.021	8.1307	8.4637	9.1493	9.2458	9.3989
510	6.9537	7.2986	7.5516	8.0536	8.1833	8.5514	9.1895	9.3164	9.4898
510.5	7.0064	7.3025	7.5771	8.1155	8.2441	8.6406	9.219	9.3616	9.5837
511	7.0615	7.3041	7.6385	8.183	8.2846	8.7215	9.2439	9.3961	9.6699
511.5	7.115	7.3507	7.7216	8.2685	8.3245	8.7708	9.238	9.4373	9.7312
512	7.1488	7.4201	7.8012	8.352	8.3676	8.7846	9.2256	9.4858	9.7625
512.5	7.1804	7.5306	7.8874	8.4309	8.4229	8.792	9.2172	9.5393	9.8023
513	7.2335	7.6359	7.9539	8.5122	8.4919	8.7954	9.2276	9.6061	9.8184
513.5	7.2924	7.7467	7.9915	8.5561	8.5614	8.7963	9.2499	9.6664	9.8275
514	7.3526	7.8306	8.0132	8.5909	8.644	8.8238	9.3044	9.7073	9.8504
514.5	7.4182	7.8788	8.0354	8.6075	8.7029	8.8597	9.3935	9.7373	9.8753
515	7.4937	7.9335	8.0686	8.6131	8.7395	8.9048	9.4761	9.7879	9.9217
515.5	7.542	7.9749	8.1087	8.6261	8.7722	8.9465	9.552	9.8513	9.9571
516	7.5993	8.0102	8.1727	8.6645	8.808	9.0032	9.6075	9.9218	10.008
516.5	7.6644	8.0394	8.2424	8.7225	8.8411	9.0638	9.6742	10.001	10.054
517	7.7333	8.0818	8.3156	8.7814	8.8851	9.121	9.7313	10.072	10.086
517.5	7.7935	8.1215	8.3775	8.8388	8.9442	9.1869	9.7886	10.105	10.125
518	7.8311	8.1521	8.439	8.8821	8.9913	9.239	9.8533	10.109	10.147
518.5	7.8647	8.1967	8.4919	8.904	9.0246	9.2767	9.8979	10.093	10.183
519	7.8604	8.2523	8.5175	8.9061	9.056	9.2972	9.9016	10.075	10.201
519.5	7.8465	8.2923	8.5338	8.9283	9.0868	9.3265	9.8657	10.047	10.225

Appendix – Spectrometric data for sample (S1) (Continued)

Em. WL	S1 - Sample without ampicillin								
(nm)	3h	5h	7h	9h	11h	13h	15h	18h	21h
520	7.8436	8.3112	8.5429	8.9629	9.0897	9.3464	9.8435	10.019	10.247
520.5	7.859	8.3159	8.5442	8.999	9.0858	9.3582	9.8311	9.9957	10.253
521	7.8961	8.3173	8.5265	9.0329	9.0835	9.3834	9.8244	9.9763	10.286
521.5	7.9316	8.3371	8.5257	9.0637	9.084	9.4144	9.8555	9.984	10.293
522	7.9716	8.373	8.5454	9.0888	9.1028	9.4369	9.9116	10.012	10.266
522.5	8.0028	8.4298	8.5717	9.0916	9.1459	9.4368	9.9675	10.071	10.209
523	8.011	8.4889	8.6133	9.1027	9.2109	9.4455	9.9946	10.135	10.156
523.5	8.0261	8.5176	8.6666	9.1288	9.2475	9.4475	10.007	10.2	10.123
524	8.0545	8.5056	8.7076	9.1361	9.2564	9.4261	9.9975	10.241	10.117
524.5	8.102	8.4614	8.7297	9.1182	9.2418	9.3922	9.965	10.249	10.171
525	8.1431	8.4242	8.7263	9.0986	9.2056	9.3789	9.9319	10.24	10.254
525.5	8.1746	8.396	8.7207	9.0778	9.1568	9.3912	9.91	10.222	10.304
526	8.1899	8.377	8.7197	9.0293	9.1261	9.403	9.9108	10.229	10.32
526.5	8.1599	8.392	8.7195	8.9666	9.1104	9.4482	9.9289	10.249	10.299
527	8.1241	8.4293	8.7123	8.9452	9.1157	9.5017	9.9649	10.255	10.249
527.5	8.0652	8.4504	8.7018	8.9367	9.1312	9.5335	9.9859	10.252	10.191
528	8.0075	8.4455	8.7025	8.9364	9.1444	9.5388	9.9737	10.221	10.168
528.5	7.97	8.4457	8.6783	8.9565	9.1565	9.5085	9.9319	10.164	10.184
529	7.961	8.427	8.6307	8.9927	9.1592	9.4586	9.8598	10.066	10.188
529.5	7.958	8.4089	8.5828	9.0353	9.1686	9.3976	9.7902	9.9915	10.207
530	7.9586	8.4099	8.5313	9.0472	9.1571	9.3579	9.7361	9.9464	10.211
530.5	7.9859	8.4195	8.4694	9.0621	9.1443	9.3388	9.7069	9.8919	10.18
531	7.9916	8.4151	8.4103	9.069	9.125	9.3273	9.6931	9.8662	10.114
531.5	7.9691	8.4038	8.3683	9.0559	9.0838	9.3436	9.6864	9.8598	10.037
532	7.9265	8.4013	8.3521	9.0336	9.0271	9.3304	9.6718	9.896	9.9871
532.5	7.8866	8.3668	8.3552	8.9973	8.9614	9.2886	9.6445	9.9112	9.9206
533	7.8262	8.3195	8.3826	8.966	8.9322	9.2379	9.6249	9.9457	9.8853
533.5	7.7666	8.2739	8.4023	8.9255	8.9207	9.183	9.5975	9.9745	9.8725
534	7.7549	8.2223	8.3903	8.8718	8.9333	9.1599	9.5618	9.9444	9.8854
534.5	7.7616	8.1629	8.3642	8.8287	8.9454	9.1335	9.5005	9.858	9.8696
535	7.7711	8.1172	8.2979	8.7864	8.9473	9.1453	9.4341	9.7342	9.8259
535.5	7.7676	8.1017	8.2351	8.7498	8.9108	9.1334	9.3736	9.633	9.7854
536	7.7649	8.0871	8.1902	8.7072	8.8426	9.0852	9.3174	9.5451	9.7061
536.5	7.7393	8.0881	8.1658	8.6556	8.7646	9.0243	9.2974	9.5096	9.6102
537	7.6884	8.0978	8.1575	8.6131	8.7055	8.9213	9.304	9.5119	9.5095
537.5	7.6466	8.0904	8.1594	8.5484	8.6857	8.8515	9.3396	9.5354	9.4362
538	7.6149	8.062	8.1684	8.4901	8.6835	8.7878	9.3606	9.5341	9.3647
538.5	7.6037	8.0283	8.1316	8.4361	8.6931	8.7758	9.3598	9.5	9.3084
539	7.6056	7.9916	8.0748	8.3755	8.6708	8.7795	9.342	9.4438	9.2794
539.5	7.6182	7.9496	7.9999	8.3339	8.6269	8.7828	9.2886	9.387	9.2831

Appendix – Spectrometric data for sample (S1) (Continued)

Em. WL (nm)	S1 - Sample without ampicillin								
	3h	5h	7h	9h	11h	13h	15h	18h	21h
540	7.6274	7.9415	7.906	8.2773	8.5415	8.7983	9.2202	9.3384	9.2955
540.5	7.6152	7.9447	7.8239	8.2461	8.4479	8.7652	9.1308	9.3016	9.3106
541	7.5732	7.9291	7.7853	8.1983	8.3695	8.7123	9.0454	9.2742	9.3151
541.5	7.5171	7.876	7.772	8.1476	8.3152	8.6244	8.9679	9.2396	9.2758
542	7.4678	7.8059	7.7552	8.1337	8.2878	8.5408	8.8973	9.1738	9.2317
542.5	7.4174	7.723	7.7574	8.1186	8.2469	8.4652	8.8569	9.0796	9.1552
543	7.3709	7.6063	7.7671	8.1194	8.2161	8.3998	8.8342	8.9905	9.081
543.5	7.3434	7.5153	7.7632	8.1137	8.1765	8.3713	8.8214	8.8928	9.001
544	7.3203	7.4521	7.7172	8.1252	8.1289	8.3433	8.7924	8.8244	8.9412
544.5	7.2739	7.4096	7.6928	8.1066	8.0721	8.3113	8.7361	8.7628	8.8883
545	7.2097	7.3559	7.676	8.0437	8.0163	8.261	8.664	8.7253	8.8172
545.5	7.1455	7.3023	7.6318	7.9893	7.9641	8.2021	8.5745	8.6888	8.7617
546	7.0729	7.2632	7.577	7.9289	7.89	8.1342	8.502	8.6312	8.6933
546.5	6.994	7.1786	7.5251	7.8618	7.8296	8.0491	8.4482	8.5682	8.619
547	6.9433	7.1093	7.495	7.7914	7.7784	7.9679	8.4074	8.4842	8.5406
547.5	6.9135	7.0675	7.4334	7.7479	7.7308	7.8899	8.3628	8.4167	8.471
548	6.8832	7.0521	7.3767	7.7099	7.6874	7.8232	8.3062	8.3454	8.4077
548.5	6.8474	7.0316	7.3371	7.6567	7.651	7.7627	8.2217	8.2828	8.3329
549	6.8352	7.0232	7.2826	7.6123	7.6122	7.7072	8.1068	8.2243	8.2705
549.5	6.8191	7.0171	7.2193	7.5729	7.556	7.6805	7.9989	8.1642	8.209
550	6.7677	6.9582	7.146	7.5289	7.5088	7.6693	7.9141	8.1034	8.1399
550.5	6.712	6.8822	7.0896	7.4775	7.4736	7.6608	7.8612	8.0294	8.0648
551	6.6442	6.8009	7.0289	7.4108	7.4456	7.6285	7.8281	7.9723	8.0004
551.5	6.5635	6.7257	6.969	7.3494	7.4046	7.5897	7.8287	7.9132	7.93
552	6.4812	6.6384	6.9076	7.2849	7.3609	7.5344	7.8109	7.8628	7.8611
552.5	6.4238	6.573	6.8428	7.2363	7.2978	7.4341	7.7735	7.821	7.8128
553	6.3839	6.5341	6.7883	7.1904	7.2051	7.3278	7.7205	7.7788	7.77
553.5	6.3394	6.4901	6.7429	7.1449	7.1097	7.2316	7.65	7.7305	7.7184
554	6.3223	6.4408	6.6927	7.0895	7.0096	7.1444	7.5695	7.6801	7.6585
554.5	6.296	6.3899	6.6484	6.9985	6.9468	7.0557	7.48	7.6219	7.6008
555	6.2421	6.3563	6.6118	6.9105	6.8802	6.9941	7.417	7.5459	7.5191
555.5	6.1655	6.3132	6.5631	6.8215	6.8239	6.9659	7.3503	7.476	7.4163
556	6.0969	6.2815	6.4956	6.7503	6.7788	6.9159	7.2909	7.4137	7.3118
556.5	6.0326	6.2413	6.409	6.6958	6.7262	6.8517	7.2349	7.3452	7.2108
557	5.9532	6.2125	6.3408	6.6686	6.6846	6.7872	7.1681	7.2834	7.1305
557.5	5.9065	6.1726	6.2769	6.6508	6.6217	6.7314	7.1153	7.2292	7.0668
558	5.8763	6.1168	6.223	6.6019	6.579	6.6688	7.0548	7.1647	7.0415
558.5	5.8283	6.0497	6.1895	6.5399	6.5139	6.6088	6.9983	7.0879	7.026
559	5.7598	5.9772	6.1559	6.4639	6.4371	6.5786	6.9198	7.028	7.0195
559.5	5.6957	5.9103	6.1185	6.3688	6.3787	6.5388	6.8515	6.9757	6.9995

Appendix – Spectrometric data for sample (S1) (Continued)

Em. WL (nm)	S1 - Sample without ampicillin								
	3h	5h	7h	9h	11h	13h	15h	18h	21h
560	5.644	5.832	6.0466	6.2747	6.3082	6.4881	6.7924	6.9101	6.9468
560.5	5.58	5.7609	5.9715	6.1974	6.2437	6.426	6.727	6.8424	6.8796
561	5.5268	5.6892	5.8955	6.1253	6.1658	6.3531	6.6616	6.7821	6.7953
561.5	5.495	5.6289	5.822	6.0595	6.0973	6.2694	6.5998	6.6902	6.7232
562	5.4639	5.5681	5.7563	5.9934	6.0273	6.1861	6.5255	6.5968	6.6522
562.5	5.4056	5.5122	5.7039	5.9355	5.9449	6.1315	6.429	6.5279	6.585
563	5.3282	5.4766	5.6568	5.8748	5.8906	6.084	6.3313	6.4675	6.5321
563.5	5.253	5.445	5.597	5.8102	5.8474	6.045	6.2451	6.4072	6.4577
564	5.1808	5.4141	5.542	5.7576	5.8092	6.0152	6.1729	6.3534	6.3881
564.5	5.1126	5.3666	5.4859	5.7156	5.7792	5.9757	6.1087	6.3174	6.3169
565	5.0738	5.3234	5.4177	5.6924	5.7524	5.9315	6.057	6.2513	6.2654
565.5	5.0525	5.2751	5.3579	5.6661	5.7216	5.8852	6.0124	6.1772	6.2261
566	5.0285	5.2211	5.3103	5.6414	5.6696	5.8312	5.9572	6.1064	6.177
566.5	4.9948	5.1811	5.2568	5.6049	5.6195	5.7539	5.8971	6.0329	6.1354
567	4.9597	5.1483	5.198	5.5574	5.5713	5.6669	5.8385	5.9609	6.0655
567.5	4.9201	5.1083	5.1494	5.4983	5.5089	5.5846	5.8	5.8877	5.9875
568	4.8663	5.0457	5.1097	5.4278	5.4459	5.4818	5.7698	5.8384	5.9063
568.5	4.8241	4.9725	5.0597	5.3605	5.3792	5.3779	5.74	5.7828	5.8311
569	4.7861	4.8977	5.0124	5.295	5.3136	5.3118	5.7049	5.7517	5.78
569.5	4.7446	4.8222	4.9727	5.2254	5.2493	5.2806	5.6581	5.7239	5.7391
570	4.6996	4.7685	4.924	5.1649	5.2047	5.2584	5.5992	5.6936	5.7102
570.5	4.6623	4.7385	4.861	5.1172	5.1747	5.2451	5.5282	5.6507	5.665
571	4.6221	4.7117	4.7906	5.0705	5.137	5.229	5.4547	5.5954	5.6278
571.5	4.5654	4.6908	4.7052	5.0224	5.0952	5.19	5.3997	5.5365	5.5861
572	4.5205	4.6578	4.6068	4.9838	5.0494	5.1178	5.3488	5.4551	5.5368
572.5	4.4886	4.6144	4.5174	4.963	4.983	5.0451	5.2993	5.379	5.4864
573	4.4458	4.5464	4.4501	4.9272	4.8969	4.9965	5.2516	5.3109	5.4262
573.5	4.3876	4.4652	4.405	4.8751	4.8227	4.9545	5.1961	5.2602	5.3604
574	4.3292	4.393	4.3798	4.834	4.7615	4.938	5.1305	5.2016	5.2785
574.5	4.2681	4.3235	4.3705	4.7858	4.7066	4.9249	5.0541	5.1666	5.2064
575	4.1921	4.281	4.3523	4.7251	4.6619	4.9219	4.9905	5.1446	5.1407
575.5	4.1153	4.2524	4.3222	4.6536	4.6378	4.8925	4.9361	5.1108	5.0872
576	4.0591	4.2344	4.2954	4.5889	4.6116	4.8386	4.8788	5.0704	5.0426
576.5	4.0067	4.2144	4.2614	4.5256	4.5741	4.7854	4.8278	5.0293	5.0103
577	3.9594	4.1758	4.2287	4.4615	4.5274	4.7204	4.7855	5.0042	4.9773
577.5	3.9232	4.1275	4.1939	4.4182	4.4763	4.6587	4.7529	4.9566	4.9346
578	3.8872	4.0655	4.1661	4.3727	4.4117	4.597	4.7197	4.9057	4.8834
578.5	3.8499	4.0091	4.1305	4.3288	4.3297	4.5556	4.7015	4.8501	4.8263
579	3.8094	3.9663	4.0875	4.2913	4.2708	4.5057	4.6982	4.7752	4.7649
579.5	3.7773	3.949	4.0508	4.2404	4.2195	4.4563	4.6931	4.6871	4.6968

Appendix – Spectrometric data for sample (S1) (Continued)

Em. WL (nm)	S1 - Sample without ampicillin								
	3h	5h	7h	9h	11h	13h	15h	18h	21h
580	3.7488	3.9406	4.0118	4.1936	4.1853	4.4132	4.6796	4.5979	4.6321
580.5	3.7252	3.9165	3.98	4.1475	4.162	4.3681	4.6463	4.5378	4.5777
581	3.7057	3.8752	3.9471	4.1193	4.1453	4.308	4.5848	4.4853	4.545
581.5	3.6726	3.8364	3.9186	4.0882	4.1166	4.249	4.5045	4.4396	4.5154
582	3.6428	3.7772	3.8794	4.0578	4.0757	4.206	4.4178	4.4051	4.4903
582.5	3.6106	3.7133	3.8378	4.0394	4.0391	4.1532	4.3341	4.3542	4.4749
583	3.5694	3.6709	3.8042	4.0025	3.9868	4.1104	4.262	4.3004	4.4532
583.5	3.5197	3.655	3.773	3.9546	3.9416	4.0804	4.2102	4.2358	4.4114
584	3.4707	3.634	3.744	3.8961	3.9064	4.0652	4.1672	4.1887	4.3443
584.5	3.4212	3.603	3.7219	3.836	3.8789	4.0353	4.1256	4.152	4.276
585	3.3638	3.5825	3.7073	3.7888	3.8302	4.0007	4.0864	4.1125	4.2073
585.5	3.3121	3.546	3.6833	3.7444	3.7787	3.958	4.0494	4.0821	4.1381
586	3.2705	3.4849	3.6485	3.7056	3.7325	3.8823	4.0124	4.0447	4.0838
586.5	3.2382	3.4319	3.6056	3.6725	3.6742	3.8083	3.9782	4.0104	4.0512
587	3.2151	3.406	3.5526	3.6334	3.607	3.7493	3.9507	3.9663	4.0356
587.5	3.1919	3.3871	3.4824	3.5873	3.5525	3.7122	3.9243	3.9442	4.0114
588	3.1688	3.3652	3.4099	3.5271	3.5163	3.6861	3.8956	3.9378	3.9825
588.5	3.1448	3.347	3.3472	3.478	3.4714	3.672	3.8517	3.9257	3.948
589	3.1298	3.3234	3.3031	3.4435	3.4417	3.6479	3.8042	3.897	3.899
589.5	3.1097	3.2733	3.273	3.4087	3.4177	3.5982	3.7528	3.8583	3.8514
590	3.0903	3.2136	3.2614	3.3918	3.3917	3.5354	3.7094	3.8196	3.7929
590.5	3.0754	3.1594	3.2592	3.3814	3.3527	3.4837	3.6627	3.7603	3.7456
591	3.0466	3.1142	3.2462	3.3694	3.3029	3.4475	3.6103	3.7087	3.7026
591.5	3.0008	3.0876	3.2182	3.3369	3.2708	3.4215	3.5746	3.6731	3.6635
592	2.9468	3.0674	3.1743	3.2956	3.2459	3.4115	3.5384	3.6487	3.6229
592.5	2.8959	3.0514	3.1266	3.26	3.236	3.395	3.4996	3.6145	3.5852
593	2.8382	3.0133	3.0734	3.2074	3.2343	3.3595	3.4548	3.5788	3.5648
593.5	2.788	2.9621	3.0171	3.1635	3.2284	3.3065	3.4244	3.5529	3.5239
594	2.7587	2.8939	2.9604	3.1234	3.2144	3.257	3.3904	3.5156	3.4761
594.5	2.7451	2.8227	2.9098	3.0969	3.172	3.2125	3.3469	3.4763	3.4263
595	2.7327	2.7642	2.8639	3.064	3.119	3.1816	3.312	3.4412	3.3859
595.5	2.7156	2.7225	2.8226	3.0345	3.0554	3.1624	3.2675	3.4076	3.3425
596	2.698	2.7029	2.804	3.0245	2.9917	3.1334	3.2222	3.3649	3.2991
596.5	2.6722	2.7001	2.8039	2.999	2.9398	3.0991	3.1733	3.31	3.2825

Appendix – Spectrometric data for sample (SA)

Em. WL (nm)	SA - Sample with ampicillin 3h	5h	7h	9h	11h	13h	15h	18h	21h
500	5.337	5.415	6.596	6.805	7.844	8.284	8.397	9.229	9.349
500.5	5.3043	5.6134	6.5975	6.7996	7.7741	8.205	8.5126	9.32	9.5679
501	5.3018	5.7785	6.6099	6.8381	7.7494	8.1548	8.6243	9.3749	9.7408
501.5	5.331	5.9098	6.6418	6.8755	7.7702	8.1667	8.7074	9.4076	9.8634
502	5.4101	6.0022	6.7252	6.9281	7.8266	8.2427	8.768	9.418	9.9346
502.5	5.5074	6.0677	6.8177	6.9953	7.9065	8.3328	8.7926	9.4157	9.9416
503	5.6183	6.1094	6.942	7.0911	7.9921	8.3913	8.7916	9.4123	9.9223
503.5	5.7144	6.163	7.0758	7.1698	8.0669	8.4353	8.7766	9.4557	9.9338
504	5.8013	6.238	7.18	7.2291	8.1089	8.458	8.7696	9.5189	9.959
504.5	5.8942	6.31	7.2455	7.3199	8.1609	8.4688	8.7837	9.5915	10
505	5.9795	6.3857	7.2847	7.3898	8.2213	8.4881	8.8122	9.6587	10.076
505.5	6.0626	6.4528	7.3007	7.4331	8.2915	8.5253	8.8719	9.7374	10.177
506	6.1284	6.5113	7.2772	7.4604	8.3609	8.5848	8.9598	9.8096	10.255
506.5	6.2028	6.5532	7.2549	7.5021	8.4174	8.6355	9.0512	9.8631	10.291
507	6.2698	6.6017	7.275	7.5539	8.4771	8.6785	9.1278	9.9036	10.338
507.5	6.3225	6.6661	7.3088	7.6028	8.5167	8.7155	9.1896	9.9188	10.363
508	6.3792	6.7272	7.3729	7.6991	8.5487	8.7485	9.2355	9.9315	10.377
508.5	6.4202	6.8016	7.461	7.8042	8.5905	8.7858	9.2593	9.9134	10.421
509	6.4531	6.8769	7.5616	7.8902	8.6559	8.8234	9.2727	9.9161	10.493
509.5	6.4931	6.9546	7.6612	7.9497	8.735	8.8716	9.3007	9.9442	10.578
510	6.5579	7.0406	7.7318	8.0008	8.801	8.9231	9.3513	10.009	10.628
510.5	6.6452	7.1384	7.8084	8.0562	8.8826	8.9714	9.4204	10.089	10.689
511	6.7318	7.2439	7.8597	8.0996	8.9686	9.0183	9.5036	10.162	10.729
511.5	6.8132	7.3275	7.881	8.1385	9.0181	9.0601	9.5914	10.216	10.751
512	6.8684	7.3802	7.8964	8.1784	9.0373	9.0946	9.6767	10.24	10.774
512.5	6.8938	7.405	7.9161	8.2158	9.0417	9.1239	9.732	10.25	10.779
513	6.9129	7.4005	7.9485	8.2439	9.0612	9.1555	9.7367	10.273	10.788
513.5	6.9348	7.3875	7.9661	8.2815	9.0726	9.2102	9.7182	10.325	10.783
514	6.9792	7.3872	7.9921	8.3536	9.1002	9.2869	9.6949	10.383	10.796
514.5	7.0481	7.4256	8.0359	8.461	9.1717	9.3763	9.6523	10.464	10.803
515	7.1371	7.4955	8.0706	8.5531	9.2431	9.4637	9.6289	10.516	10.81
515.5	7.2208	7.5664	8.1124	8.6159	9.3115	9.5176	9.6336	10.54	10.839
516	7.2855	7.6376	8.1655	8.6461	9.366	9.5174	9.6664	10.549	10.849
516.5	7.3339	7.6879	8.2348	8.6333	9.4077	9.4788	9.6918	10.568	10.865
517	7.3706	7.7251	8.2748	8.5939	9.4164	9.4224	9.7197	10.583	10.886
517.5	7.399	7.7456	8.2999	8.5559	9.402	9.3776	9.7736	10.589	10.915
518	7.4209	7.7798	8.3368	8.5655	9.3889	9.3546	9.8153	10.626	10.926
518.5	7.4358	7.8313	8.3464	8.6008	9.3678	9.3706	9.8742	10.645	10.921
519	7.4471	7.8596	8.3407	8.6514	9.3627	9.4142	9.9273	10.662	10.923
519.5	7.4673	7.8785	8.3708	8.7271	9.3698	9.4525	9.9655	10.669	10.905

Appendix – Spectrometric data for sample (SA) (Continued)

Em. WL (nm)	SA - Sample with ampicillin								
	3h	5h	7h	9h	11h	13h	15h	18h	21h
520	7.4908	7.8836	8.4431	8.8076	9.3945	9.4695	9.9714	10.693	10.886
520.5	7.5249	7.8744	8.509	8.8757	9.4223	9.4713	9.9332	10.714	10.88
521	7.576	7.8464	8.569	8.9204	9.4626	9.4627	9.8823	10.712	10.872
521.5	7.6279	7.8315	8.6229	8.922	9.532	9.436	9.8274	10.678	10.872
522	7.695	7.8581	8.6404	8.9075	9.5679	9.4332	9.8037	10.614	10.885
522.5	7.7576	7.8988	8.6049	8.877	9.5835	9.4539	9.8121	10.558	10.914
523	7.7943	7.9395	8.5643	8.8627	9.5796	9.4884	9.8252	10.518	10.931
523.5	7.8035	7.9688	8.5397	8.8726	9.554	9.5295	9.8486	10.517	10.949
524	7.7911	8.0068	8.53	8.8934	9.5026	9.5683	9.8627	10.54	10.968
524.5	7.7636	8.0182	8.522	8.9428	9.4202	9.5872	9.8556	10.586	10.965
525	7.7128	8.0007	8.5319	8.9602	9.3918	9.5524	9.8358	10.62	10.938
525.5	7.689	7.983	8.5475	8.9674	9.3771	9.504	9.8065	10.623	10.903
526	7.6923	7.9739	8.537	8.9573	9.3925	9.4428	9.7733	10.602	10.871
526.5	7.6849	7.9641	8.5296	8.9463	9.4053	9.3928	9.704	10.566	10.823
527	7.6748	7.9411	8.5134	8.9475	9.4348	9.3689	9.6463	10.523	10.77
527.5	7.6636	7.949	8.5285	8.9395	9.4706	9.382	9.6039	10.488	10.727
528	7.6535	7.968	8.5353	8.9489	9.4459	9.4204	9.5692	10.461	10.676
528.5	7.6361	7.9836	8.5272	8.9257	9.4128	9.4363	9.5625	10.438	10.615
529	7.6431	7.9996	8.5242	8.8949	9.373	9.4206	9.5845	10.406	10.565
529.5	7.6795	8.0214	8.5179	8.8524	9.3212	9.3811	9.6258	10.367	10.539
530	7.7217	8.0489	8.5175	8.8182	9.252	9.312	9.6264	10.327	10.51
530.5	7.7516	8.0447	8.4988	8.8138	9.199	9.2296	9.6047	10.278	10.479
531	7.7665	8.0316	8.5143	8.8268	9.1952	9.1591	9.5604	10.23	10.44
531.5	7.7658	8.0172	8.5196	8.8469	9.1942	9.1093	9.4948	10.156	10.41
532	7.748	7.9877	8.4932	8.8475	9.1908	9.0919	9.4135	10.09	10.36
532.5	7.7318	7.9375	8.4572	8.8445	9.2095	9.0777	9.3528	10.031	10.306
533	7.7157	7.8684	8.4141	8.822	9.2276	9.0781	9.3083	9.9799	10.256
533.5	7.7008	7.7961	8.3832	8.7866	9.2143	9.065	9.2611	9.9498	10.212
534	7.6943	7.7429	8.3231	8.7417	9.192	9.0234	9.2221	9.915	10.168
534.5	7.6626	7.6904	8.2895	8.722	9.1594	8.9647	9.1954	9.8964	10.124
535	7.6189	7.6675	8.2666	8.71	9.1155	8.8853	9.1852	9.8728	10.097
535.5	7.5527	7.6607	8.2301	8.6841	9.046	8.8231	9.1752	9.8396	10.059
536	7.487	7.6603	8.1852	8.6567	8.9784	8.7587	9.173	9.7835	10.021
536.5	7.4334	7.6582	8.1556	8.6179	8.9028	8.7334	9.1663	9.7175	9.9715
537	7.3955	7.6465	8.1583	8.5739	8.8326	8.7222	9.1602	9.649	9.9324
537.5	7.391	7.636	8.1451	8.5214	8.7828	8.7047	9.1353	9.5856	9.9027
538	7.3806	7.5976	8.1386	8.4798	8.7459	8.6817	9.0917	9.5357	9.8675
538.5	7.3691	7.5649	8.1262	8.4445	8.7251	8.6339	9.0565	9.5183	9.8357
539	7.3217	7.5341	8.092	8.4104	8.7086	8.5762	9.0247	9.5396	9.7961
539.5	7.2354	7.4966	8.0196	8.3694	8.6951	8.493	8.9779	9.5554	9.7587

Appendix – Spectrometric data for sample (SA) (Continued)

Em. WL (nm)	SA - Sample with ampicillin								
	3h	5h	7h	9h	11h	13h	15h	18h	21h
540	7.1359	7.4515	7.9403	8.3164	8.6764	8.4257	8.9205	9.5633	9.7096
540.5	7.0407	7.4008	7.8917	8.2578	8.6459	8.3742	8.8658	9.5355	9.6542
541	6.986	7.3641	7.8532	8.1942	8.5994	8.3373	8.7991	9.4876	9.6004
541.5	6.9676	7.3162	7.8272	8.1357	8.5266	8.3099	8.7321	9.3968	9.548
542	6.9915	7.2893	7.8187	8.0943	8.4439	8.2767	8.6656	9.3051	9.4703
542.5	7.0261	7.2757	7.8118	8.0648	8.3603	8.2507	8.6229	9.257	9.3804
543	7.049	7.2613	7.7834	8.0332	8.2916	8.2094	8.5773	9.2155	9.284
543.5	7.0414	7.234	7.7316	7.9905	8.2381	8.1564	8.5308	9.1712	9.1865
544	7.007	7.1864	7.6506	7.9388	8.2107	8.0964	8.4848	9.0952	9.0951
544.5	6.9631	7.1419	7.5572	7.8843	8.1927	8.0361	8.4373	9.0152	9.0365
545	6.9078	7.0795	7.4655	7.8148	8.1766	7.9825	8.3783	8.9137	9.0106
545.5	6.8578	7.0225	7.384	7.765	8.1563	7.9291	8.3093	8.7909	8.9841
546	6.8088	6.9738	7.3231	7.7421	8.1227	7.8951	8.2431	8.6903	8.9556
546.5	6.7749	6.9481	7.2767	7.7263	8.0688	7.8625	8.1457	8.6277	8.9186
547	6.7332	6.9221	7.2582	7.7044	7.9919	7.8262	8.0441	8.5602	8.8364
547.5	6.6734	6.867	7.24	7.6825	7.908	7.7853	7.9446	8.4836	8.717
548	6.6184	6.817	7.2043	7.6542	7.8206	7.7303	7.8692	8.4115	8.591
548.5	6.5546	6.7639	7.1594	7.5891	7.7357	7.6652	7.8034	8.3417	8.4725
549	6.4886	6.7056	7.1105	7.5088	7.652	7.583	7.7569	8.2834	8.3748
549.5	6.414	6.6267	7.0497	7.4224	7.5931	7.5186	7.7396	8.2162	8.3107
550	6.3616	6.5681	6.9748	7.3488	7.5504	7.46	7.7044	8.188	8.306
550.5	6.3117	6.5082	6.9192	7.2899	7.508	7.4019	7.6586	8.1467	8.3043
551	6.2588	6.4249	6.8824	7.2428	7.4648	7.3594	7.5995	8.0842	8.2912
551.5	6.2086	6.353	6.8404	7.213	7.42	7.3287	7.5481	8.0029	8.2617
552	6.1687	6.2965	6.7839	7.1783	7.366	7.2911	7.4757	7.9141	8.2058
552.5	6.1214	6.2503	6.7334	7.1238	7.2912	7.2325	7.392	7.8424	8.1228
553	6.0676	6.1897	6.6838	7.0326	7.219	7.175	7.3322	7.7517	8.0088
553.5	6.0216	6.1594	6.6161	6.9359	7.1573	7.1171	7.2721	7.6959	7.8969
554	5.9707	6.1275	6.5403	6.8489	7.106	7.05	7.2082	7.652	7.7838
554.5	5.9484	6.0626	6.48	6.7885	7.0624	6.9725	7.1551	7.6007	7.6858
555	5.9175	6.005	6.4308	6.7615	7.0314	6.8968	7.1108	7.5348	7.5912
555.5	5.8913	5.939	6.3606	6.7572	7.0035	6.8204	7.0593	7.4607	7.5143
556	5.8378	5.8779	6.2928	6.7521	6.9669	6.7446	6.9886	7.397	7.4548
556.5	5.776	5.8073	6.2297	6.717	6.9239	6.6656	6.8988	7.3217	7.401
557	5.6993	5.7716	6.1669	6.6574	6.8585	6.6089	6.8042	7.2573	7.3546
557.5	5.6104	5.7599	6.1012	6.5774	6.7648	6.5616	6.699	7.225	7.3111
558	5.5594	5.7221	6.0376	6.4906	6.6585	6.5003	6.6066	7.1985	7.2785
558.5	5.5225	5.6977	5.9852	6.4047	6.5511	6.4242	6.536	7.1576	7.2234
559	5.5113	5.6599	5.9375	6.3413	6.4524	6.3298	6.4705	7.1272	7.1465
559.5	5.4886	5.6188	5.8899	6.2749	6.3722	6.247	6.4197	7.0737	7.0621

Appendix – Spectrometric data for sample (SA) (Continued)

Em. WL (nm)	SA - Sample with ampicillin 3h	5h	7h	9h	11h	13h	15h	18h	21h
560	5.4707	5.549	5.8423	6.2162	6.3213	6.1619	6.377	6.9792	6.9611
560.5	5.4369	5.5035	5.7913	6.1619	6.2842	6.0965	6.3373	6.8597	6.8486
561	5.3828	5.4859	5.7461	6.1147	6.2448	6.0549	6.2828	6.754	6.7413
561.5	5.3282	5.4521	5.6982	6.0677	6.2022	6.0315	6.2264	6.6623	6.6765
562	5.2764	5.4292	5.6347	6.0187	6.1592	6.0121	6.1705	6.5623	6.6357
562.5	5.2362	5.391	5.5711	5.9927	6.1115	5.9716	6.1024	6.5014	6.5925
563	5.1857	5.3527	5.5101	5.9475	6.0536	5.9302	6.0395	6.4628	6.565
563.5	5.1149	5.2754	5.4554	5.887	5.9967	5.8776	5.9898	6.4156	6.536
564	5.0233	5.1968	5.4072	5.8129	5.9485	5.8319	5.954	6.3521	6.4931
564.5	4.9334	5.1106	5.3617	5.7292	5.8826	5.7897	5.918	6.2765	6.4225
565	4.845	5.0028	5.3149	5.6338	5.8097	5.7458	5.8917	6.2041	6.3628
565.5	4.7681	4.9089	5.2442	5.5325	5.742	5.7035	5.8724	6.1198	6.2983
566	4.7121	4.8287	5.1609	5.4652	5.6873	5.639	5.8364	6.0599	6.2025
566.5	4.687	4.7764	5.0726	5.4098	5.6308	5.5663	5.7814	6.0169	6.1246
567	4.6606	4.7204	5.0066	5.3709	5.5834	5.464	5.7158	5.973	6.0649
567.5	4.6252	4.6912	4.9695	5.3574	5.5476	5.3602	5.6539	5.9379	6.0182
568	4.5894	4.6651	4.9427	5.3443	5.5035	5.2604	5.5901	5.8919	5.9665
568.5	4.5475	4.6166	4.9271	5.3208	5.4508	5.1694	5.5374	5.8373	5.9429
569	4.4921	4.5575	4.9226	5.2847	5.3969	5.1097	5.4906	5.7797	5.9228
569.5	4.4275	4.5113	4.9024	5.2369	5.3311	5.0747	5.4489	5.7322	5.8707
570	4.3802	4.4784	4.8503	5.1733	5.2465	5.0707	5.407	5.6841	5.8187
570.5	4.339	4.4423	4.7862	5.0929	5.1507	5.0582	5.3521	5.6248	5.7643
571	4.3057	4.4289	4.7367	5.0249	5.0515	5.045	5.3003	5.5775	5.7138
571.5	4.2797	4.4247	4.6883	4.9472	4.95	5.0235	5.2557	5.5312	5.6565
572	4.2603	4.4051	4.6287	4.8953	4.864	4.9894	5.2233	5.4797	5.6074
572.5	4.2278	4.3603	4.5889	4.8731	4.8103	4.9448	5.1898	5.4175	5.5669
573	4.1787	4.3065	4.5506	4.8554	4.7731	4.8889	5.1618	5.3587	5.497
573.5	4.1225	4.2434	4.496	4.8371	4.7572	4.8345	5.122	5.2927	5.423
574	4.0606	4.1715	4.4372	4.8064	4.7528	4.7788	5.053	5.2093	5.3461
574.5	4.0075	4.1143	4.3886	4.7675	4.74	4.7049	4.9706	5.1244	5.2874
575	3.9656	4.0779	4.3438	4.6783	4.7177	4.6321	4.8895	5.0475	5.2343
575.5	3.9419	4.0558	4.2832	4.5918	4.6759	4.5736	4.8272	4.9942	5.1807
576	3.9153	4.0304	4.2409	4.5221	4.6332	4.5357	4.776	4.9574	5.137
576.5	3.8882	4.0019	4.2093	4.4597	4.5737	4.5035	4.744	4.9267	5.0922
577	3.8605	3.9609	4.1703	4.4174	4.5269	4.4583	4.7197	4.8987	5.0391
577.5	3.8231	3.9139	4.1234	4.3807	4.4777	4.4185	4.68	4.8794	4.973
578	3.7839	3.8713	4.0751	4.3617	4.429	4.351	4.6291	4.8647	4.9232
578.5	3.7496	3.8384	4.0384	4.3099	4.3893	4.2729	4.5598	4.8364	4.882
579	3.7142	3.7966	3.9992	4.2519	4.3518	4.2025	4.4829	4.8053	4.8298
579.5	3.6706	3.7554	3.9752	4.1908	4.3132	4.1526	4.415	4.7613	4.7769

Appendix – Spectrometric data for sample (SA) (Continued)

Em. WL (nm)	SA - Sample with ampicillin 3h	5h	7h	9h	11h	13h	15h	18h	21h
580	3.6261	3.709	3.96	4.1193	4.2702	4.1135	4.3565	4.6899	4.7243
580.5	3.5772	3.6547	3.9373	4.0566	4.2376	4.075	4.3147	4.5925	4.6625
581	3.5216	3.5938	3.9087	4.0031	4.199	4.0475	4.2758	4.5031	4.5965
581.5	3.4712	3.5419	3.862	3.9656	4.1512	4.0115	4.2352	4.429	4.5512
582	3.4327	3.5156	3.8091	3.9257	4.0959	3.9692	4.1913	4.3733	4.5242
582.5	3.3942	3.4965	3.7429	3.9008	4.0356	3.9287	4.141	4.3465	4.5002
583	3.3615	3.4777	3.6836	3.8841	3.9628	3.8976	4.1035	4.3324	4.4701
583.5	3.3323	3.4536	3.6289	3.8576	3.8882	3.8735	4.0631	4.3127	4.4339
584	3.3012	3.4265	3.5704	3.8254	3.8384	3.8535	4.0318	4.2738	4.3808
584.5	3.2605	3.3868	3.5256	3.7894	3.8024	3.8187	4.0075	4.2349	4.3204
585	3.2218	3.3331	3.4764	3.7369	3.7874	3.7796	3.9777	4.1937	4.2637
585.5	3.1897	3.2903	3.4313	3.6692	3.7791	3.7348	3.9323	4.1443	4.2148
586	3.1564	3.2515	3.3917	3.606	3.7744	3.6904	3.8721	4.1055	4.1779
586.5	3.1333	3.2048	3.3752	3.5529	3.7559	3.6487	3.81	4.0631	4.1473
587	3.1132	3.1606	3.3692	3.5071	3.7154	3.6133	3.7428	4.0164	4.1083
587.5	3.085	3.1311	3.3585	3.4655	3.6655	3.5949	3.6922	3.9598	4.0574
588	3.0471	3.1135	3.3529	3.4406	3.5943	3.578	3.6671	3.9019	3.9995
588.5	3.0017	3.0885	3.3404	3.4217	3.5278	3.558	3.6623	3.8415	3.9426
589	2.9552	3.0778	3.3065	3.411	3.4714	3.5397	3.6579	3.7731	3.8879
589.5	2.9084	3.0759	3.2635	3.4104	3.4352	3.4968	3.6457	3.7347	3.8387
590	2.8775	3.0493	3.2225	3.4131	3.4194	3.4283	3.6213	3.7141	3.8025
590.5	2.862	3.0056	3.1821	3.405	3.4182	3.343	3.5692	3.7112	3.763
591	2.8487	2.9485	3.1377	3.3727	3.432	3.2649	3.5056	3.7201	3.7264
591.5	2.8471	2.8909	3.0971	3.3231	3.4369	3.2109	3.4348	3.7129	3.6893
592	2.8385	2.8321	3.0722	3.246	3.4166	3.1677	3.3806	3.6877	3.6499
592.5	2.8201	2.7968	3.0372	3.176	3.369	3.1572	3.3334	3.624	3.6222
593	2.7863	2.7797	2.9912	3.1244	3.3047	3.1577	3.2913	3.5529	3.5965
593.5	2.7506	2.7623	2.9404	3.0933	3.2347	3.1609	3.2564	3.4774	3.5877
594	2.7163	2.7552	2.8845	3.0722	3.1789	3.1496	3.2181	3.4087	3.5705
594.5	2.6796	2.7482	2.8239	3.0466	3.1384	3.1259	3.1873	3.3647	3.5557
595	2.6545	2.7293	2.7644	3.0218	3.1175	3.1008	3.1466	3.3318	3.5335
595.5	2.6301	2.7002	2.7307	2.9788	3.0976	3.0633	3.1225	3.3143	3.4823
596	2.606	2.6709	2.7141	2.9418	3.0698	3.022	3.1035	3.2884	3.4262
596.5	2.5717	2.6347	2.7162	2.9146	3.0448	2.9745	3.0989	3.2566	3.3506