

# Sterilization and aseptic technique biology essay



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This experiment was done to learn proper way of using aseptic technique and sterilization by isolating pure culture of bacterial. Afterwards, the bacterial cells in a sample and their optical density were determined.

First of all, various sterilization methods were introduced. Sterilization is important in a sense that it ensures there is absolutely no contamination in the glassware or apparatus used in the lab. Different sterilization methods are used for different materials. One of them is autoclaving. Autoclaving machine uses high-pressure steam to sterilize and therefore, heat resistant plastics, glass or solutions can be sterilized by autoclaving. As the temperature of the steam is above 100 oC, the organisms cannot survive. Second sterilization method is radiation. As heat sensitive plastics does not have resistance to heat, autoclaving cannot be used and these are often sterilized by using radiation such as UV, gamma-ray or X-ray. The last method is filter sterilization. Some solutions are heat labile, and to sterilize these kind of solutions, filter sterilization can be used. This technique uses the fact that microorganism is around 5micrometer by 1micrometer, and if the filter has a smaller diameter, microorganisms cannot pass through the filter.(1)

In part B, aseptic technique is learned. This technique prevents any kind of contamination while handling the glassware or transferring. To be more specific, it prevents any contaminant to be introduced in the area of interest. The first step of this technique involves wiping the lab bench with 70% ethanol, which would kill most microorganisms. Then, Bunsen burner is turned on, and the movement of the air goes upwards. Therefore, it minimizes the chance of microorganisms landing on the media of interest. In

addition, briefly heating glass tube mouths and minimizing the time of opening lids minimizes contamination.(1)

Using aseptic technique, streaking technique was used to isolate single colonies. To do this, a pure culture of the target microorganism is taken. Then, with an inocular loop, which is flamed with Bunsen burner until red hot, it cooled down. Afterwards, take a bit of pure culture with the loop and streak lines in the medium. The streaking lines should not cross each other to avoid too much diluting.

After streaking, colonies are grown. To count the number of cells, viable cell count method is used. Viable count is only useable with singles colonies and not bacterial lawns. Therefore, in order for cell to have single colonies, appropriate dilution of the bacteria is necessary. The dilution helps for spreading of the cells on the agar. For this, serial dilution, which was introduced last project can be useful. Then, the number of viable cells can be obtained by counting the number of colonies that have developed multiplied by the respective dilution factor. (2)

## **Material and Methods:**

All procedures are performed according to the BIOL 368 lab manual (Concordia Biology Department 2013) except for the following modifications: for the contamination part, we used shoe, finger, E. coli, and E. coli with 70% ethanol.

Results:

## Colony isolation by streaking

First of all, the color of the bacteria in all the plates are thick beige colored. In streak 1, extremely small and many colonies were observed. The size of the colonies were very small, they were circular, opaque and smooth. There are 123 colonies. Streak 2 shows chain of bacterial formation, but the number of the colonies is decreased from streak 1. The number of colonies were 60. They were larger than the colonies in streak 1, opaque, circular and smooth as well. In streak 3, single colonies are observed. None of them was huge, but they were larger than the colonies from streak 2. They were opaque, circular and smooth as well. About 9 colonies were observed. In the 4th streak, no single colony was observed. As a result, single colonies of a pure E. coli strain was successfully isolated.

## Viable count

Table 1. Raw data of viable count of my group.

Dilution

10-4

10-5

10-6

Number of colony

Too many

Too many

252

Viable count (cfu/ml)

—

—

2. 52 x 10<sup>9</sup>

Sample calculation:

Viable count at 10<sup>-6</sup> dilution:

Since the plate, -6, has 252 colonies which is in the range of 100-300, I picked the plate to calculate cfu/ml.

$$252 \times 10 / 10^{-6} = 2.52 \times 10^2 \times 10^7 = 2.52 \times 10^9 \text{ cfu/ml}$$

Table 2. Raw data for viable count for all section

Dilutions

Colony count

Group 1

Group 2

Group 3

Group 4

Group 5

Group 6

Group 7

Group 8

Group 9

Group 10

Section 1

10-4

lawn

lawn

lawn

lawn

lawn

lawn

lawn

lawn

lawn

lawn

10-5

360

lawn

1848

lawn

1028

2168

696

ã€€

3040

1646

10-6

78

287

441

270

234

347

363

ã€€

300

306

Section 2

10-4

920

> 300

Lawn

Lawn

Lawn

> 300

> 300

Lawn

Lawn

Lawn

10-5

249

> 300



590

> 300

Lawn

> 300

> 300

406

> 300

189

10-6

23

231

189

269

384

222

265

154

180

108

Section 3

10-4

too many

> 1000

too many

too many

too many

too many

too many

too many

too many

too many

10-5

too many

> 1000

too many

too many

too many

too many

too many

too many

544

too many

10-6

194

420

258

252

295

217

240

197

79

224

Table 3. Viable count for all section data (for 10-6 dilution)

Section

Group

Number of colonies

Cfu/ml

01

1

78

7. 8. E+08

2

287

2. 87. E+09

3

441

4. 41 E+09

4

270

2. 70 E+09

5

234

2. 34. E+09

6

347

3. 47E+09

7

363

3. 63E+09

8

—

—

9

300

3. 00E+09

10

306

3. 06E+09

Max

441

4. 41E+09

Min

78

7. 8 E+08

Average

291. 8

2. 92 E+09

Standard Dev.

94. 40

2. 67E+08

02

1

23

2. 3. E+08

2

231

2. 31. E+09

3

189

1. 89. E+09

4

269

2. 69. E+09

5

384

3. 84. E+09

6

222

2. 22. E+09

7

265

2. 65. E+09

8

154

1. 54. E+09

9

180

1. 80. E+09

10

108

1. 08. E+09

Max

384

3. 84. E+09

Min

23



2. 3. E+08

Average

202. 5

2. 02. E+09

Standard dev.

93. 09

9. 31E+08

03

1

194

1. 94. E+09

2

420

4. 20. E+09

3

258

2. 58. E+09

4

252

2. 52. E+09

5

295

2. 95. E+09

6

217

2. 17. E+09

7

240

2. 40. E+09

8

197

1. 97. E+09

9

79

7. 9. E+08

10

224

2. 24. E+09

Max

420

4. 20. E+09

Min

79

7. 9. E+08

Average

237. 6

2. 38. E+09

Standard Dev.

81. 55

8. 16. E+08

Max

441

4. 41. E+09

Min

23

2. 3. E+08

Average

242. 3

2. 42. E+09

Standard Deviation

96. 75

1. 15E+09

Sample calculation for STD DEV. (section 1):= 94. 40

## **Bacterial cell count by optical density**

Table 4. Cell density for My Group:

OD600 of diluted cultures (Au)

E. Coli Count of diluted Culture (cells/ml)

Original Culture (cells/ml)

Me

0. 427

2.  $18 \times 10^8$

2.  $18 \times 10^9$

Partner

0. 436

2.  $14 \times 10^8$

2.  $14 \times 10^9$

E. Coli Count of diluted Culture:  $0.202 \times (5 \times 10^8) = 1.01 \times 10^8$

Original Culture:  $1.01 \times 10^8 \times 10 = 1.01 \times 10^9$

Table 5. Raw OD600 values for all sections (unit: Au)

Group

Section 1 (1)

Section 1 (2)

Section 2 (1)

Section 2(2)

Section 3(1)

Section 3 (2)

1

0.389

0.383

0.359

0.371

0.358

0.365

2

0.368

0.369

0.247

0.447

0.345

0.408

3

0.364

0.343

0. 344

0. 360

0. 323

0. 335

4

0. 374

0. 374

0. 390

0. 338

0. 427

0. 436

5

0. 415

0. 430

0. 386

0. 368

0. 320

0. 247

6

0. 359

0. 357

0. 302

0. 350

0. 379

0. 352

7

0. 347

0. 372

0. 354

0. 369

0. 391

0. 364

8



—

—

0. 362

0. 361

0. 358

0. 328

9

0. 370

—

0. 35

1. 018

0. 350

0. 341

10

0. 737

0. 367

0. 368

0. 353

0. 413

0. 322

(> 1. 96 therefore outlier)

Table 6. Diluted Cell Density for all sections (unit: cells/ml)

Group

Section 1 (1)

Section 1 (2)

Section 2 (1)

Section 2(2)

Section 3(1)

Section 3 (2)

1

1. 95E+08

1. 92E+08

1. 80E+08

1. 51E+08

1. 79E+08

1. 90E+08

2

1. 84E+08

1. 85E+08

1. 86E+08

1. 75E+08

1. 83E+08

1. 76E+08

3

1. 82E+08

1. 72E+08

1. 24E+08

1. 77E+08

1. 73E+08

1. 96E+08

4

1. 87E+08

1. 87E+08

2. 24E+08

1. 85E+08

2. 04E+08

1. 82E+08

5

2. 08E+08

2. 15E+08

1. 72E+08

1. 81E+08

1. 62E+08

1. 79E+08

6

1. 80E+08

1. 79E+08

1. 80E+08

1. 81E+08

1. 68E+08

1. 64E+08

7

1. 74E+08

1. 86E+08

1. 95E+08

1. 75E+08

2. 14E+08

1. 75E+08

8

1. 69E+08

5. 09E+08

2. 18E+08

1. 71E+08

9

1. 85E+08

1. 93E+08

1. 84E+08

1. 60E+08

2. 07E+08

10

1. 87E+08

1. 84E+08

1. 84E+08

1. 77E+08

1. 24E+08

1. 61E+08

Average

1. 87E+08

1. 95E+08

1. 79E+08

Min

1. 72E+08

1. 24E+08

1. 24E+08

Max

2. 15E+08

5. 09E+08

2. 18E+08

Standard Deviation

1. 05E+07

7. 43E+07

2. 13E+07

Sample calculation:

Section 1 group1 student 1: Cell Density =  $0.389 \times (5 \times 10^8) = 1.95 \times 10^7$

Section 1 Average:

Average =  $((1.95 + 1.84 + 1.82 + 1.87 + 2.08 + 1.80 + 1.74 + 1.85 + 1.87 + 1.92 + 1.85 + 1.72 + 1.87 + 2.15 + 1.79 + 1.86 + 1.84) \times 10^8) / 17 = 1.87 \times 10^8$

Table 7. Diluted Cell Density for all sections, class analysis

Class (cells/ml)

Average

1. 87E+08

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Minimum

1. 24E+08

Maximum

5. 09E+08

Standard Deviation

4. 66E+07

### **Part III. The ubiquity of microorganisms**

Table 8. The ubiquity of microorganisms

Place

Observation on TSA plate

Observation on Malt

Shoe

Irregular – orange, red, beige

opaque

Some are large, some are small

Some are smooth some are cracked

10colonies

None



Dirty finger

White and yellow all small colonies (4)

opaque

None

E. coli

Few circular, smooth, flat, beige colonies (lawn)

None

E. coli with 70% ethanol

None

None

Discussion:

The objective of the experiment is to learn aseptic technique, sterilization, and streaking. Part A involved isolating single colonies by streaking, part B involved viable cell count, part C involved bacterial cell count by optical density and lastly part D involved ubiquity of microorganisms.

In part A, a pure E. Coli sample was used to form single colonies by streaking. Four streaks were done in different parts of the plate. As described in the results, 1st streak results in forming smallest and very crowded colonies (123 colonies). The space between the colonies were either very small or even adjacent to each other. The 2nd streak forms a larger and less

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crowded or less population of colonies (60 colonies). Colonies were found to be further apart from streak 1, but they were adjacent to other colonies, so single colonies were not observed. In the 3rd streak finally, isolated single colonies were observed. This is due to the dilution. As in the streak 1, we have least diluted E. Coli pure sample. Therefore, streak 1 has the biggest cell density, where more colonies would grow. In the streak 2, we streak through the streak 1 once, and so, it is diluted. Then, in the third streak as well, it is even more diluted. This is why we have lesser and lesser colonies in the 2nd and 3rd colonies. Colonies all seem to have same opaque shape with beige color, but they differ in sizes. 1st streak ones have the smallest and 3rd streak ones have the largest. This is due to the fact that as the number of the colonies are bigger and crowded, there are less space to grow, so it tends to be smaller where as in 3rd streak, isolated colonies have more space where they can grow bigger.

In part B, viable count was used to estimate the number of bacterial cells in the sample. Firstly, we prepared 4-fold, 5-fold and 6-fold diluted solutions of E. Coli and they were incubated at 37 degrees Celsius. As we can see in the table 2, 4-fold dilution and 5-folded dilution are too concentrated that bacterial lawn is observed where we cannot apply viable count: they have small viable count to work with and would result in high uncertainty (1). 6-folded dilution appears to be fine to apply viable count and therefore, we used 6-folded dilution to analyze. Looking at the all section data, most of them have the viable counts ranged between 30 and 300. In addition, the average viable count of our section is very close to the class average:  $2.38 \times 10^9$  to  $2.42 \times 10^9$  cfu/ml. Also, my group value is very close to the class

average as well:  $2.52 \times 10^9$  cfu/ml. This means that our result is pretty accurate compared to the class result. Speaking of the minimum and the maximum value, there is a high chance that the errors come from these as these values are furthest from the average. Section 1 has the maximum value which is  $4.41 \times 10^9$  cfu/ml and section 2 has the minimum value which is  $2.3 \times 10^8$  cfu/ml. The minimum value seem to be "okay" but maximum value seem to lie over 300 colonies, and therefore, the biggest error comes from that value. However, none of these biggest error comes from our section, and therefore, we can say that our section value has the least error.

Part C was done to take cell density by optical density. CAG12033 was taken and was diluted with LB broth. When analyzing, the group 9 student's value was 1.018, which appeared to be as an outlier. Grubb's test was done, and it was eventually an outlier, so we excluded it from further analysis. Speaking of the cell density, as there are more and more of cell in the solution, the density increases. As well, the result shows that as absorbance increases, the cell density increases. Comparing the class average to our section average, it is fairly close:  $1.79 \times 10^8$  and  $1.87 \times 10^8$  cells/ml. However, we have the class minimum value which is  $1.24 \times 10^8$  cells/ml so we have one of the largest errors. But this is not very far from the average value, which is  $1.87 \times 10^8$  cells/ml it is not the biggest error. The class maximum value however is very far from the class average value:  $5.09 \times 10^8$  cells/ml. This value is in the section 2 data. Section 1 has the best result over the class with closest average value to the class average having no minimum nor maximum values;  $1.87 \times 10^8$  cells/ml which is the same as class average.

Comparing my cell density value to the section value, I had 2.

$1.8 \times 10^9$  cells/ml, whereas the class average was  $1.87 \times 10^8$  cells/ml. I have a fairly close value and it can be considered that CAG12033 is diluted fairly correctly.

Now comparing viable count method to the cell density measured by spectrophotometer, they can be considered the same. The class average value for the cell density was  $1.87 \times 10^8$  cells/ml and the class average result for the viable count method was  $2.42 \times 10^9$  cfu/ml. They can be considered the same with the following reasons. First of all, for the optical density method, there is an assumption that there are  $5 \times 10^8$  cells/ml when the absorbance is 1 Au. This is an assumption and is not an accurate value. Secondly, there are experimental errors such as when diluting, the dilution was not done perfectly, where the error would increase as serial dilution was done in viable count part. As a result, factor of 10 difference is quite big, but within these assumptions and errors, they can be considered as similar.

Part D was done to see what contamination looks like and how it is. TSA and malt medium were used to contaminate. Different samples were taken with a sterilized rod and were streaked different parts of the plates on both TSA and malt. They were then incubated at 37 degrees Celsius if it is from internal body or incubated at 30 degrees otherwise. TSA is usually considered the best under neutral to slightly basic conditions and required high N for bacteria to grow. On the other hand, malt is best under acidic condition and high in C and N. Malt is best for fungi. First of all, the shoe was rubbed, and streaked on both TSA and Malt plates. A week later, all different kinds of bacteria were grown. Various colored and various sizes were observed:

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orange, red and beige. Some were really huge and flat, some were small, opaque and smooth. 10 colonies were observed. On malt, nothing grew. Due to the fact that nothing grew on malt, the colonies have to be bacteria. Another possibility is that malt plate was put in the 37 degrees Celsius which is inappropriate. In quarter of the plate, dirty finger was used to contaminate. 4 colonies of white and yellow were observed. They were all opaque. Nothing grew on malt. In another part of the plate, we put E. Coli sample. Circular, smooth, flat colonies were observed. There were a lot of colonies (bacterial lawn) grown. Again nothing grew on malt. Lastly, we put E. coli with 70% ethanol. Absolutely nothing grew on both malt and TSA. Overall, nothing grew on malt. It is maybe because there was no fungi, or the plates were incubated in the wrong temperature (37 degrees Celsius instead of 30 degrees Celsius). Also, we can say that 70% ethanol kills most of the bacteria or at least enough to prevent them to grow.