

# Aseptic technique and cell counting biology essay



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## **Introduction to Aseptic techniques**

In microbiological and biochemical engineering studies, one almost always deals with a pure culture or a mixture of known cultures, except perhaps in waste water treatment studies. Unless aseptic culture techniques are followed strictly, an originally pure culture will definitely become contaminated with other unwanted species. The use of a contaminated culture with unknown microorganisms will only lead to incredible results that are of little value. Thus, isolation and maintenance of a pure culture is of utmost importance in many microbiological studies. It is especially important to work with a well-characterised strain if the microorganism is used for a food preparation, as well as in antibiotic production where the product is to be taken internally.

The need for a clean working environment in biological engineering studies is a must and cleanliness is the prerequisite for any meaningful work. The environment we live in is full of microorganisms capable of surviving in any condition.

When working with microorganisms it is desirable to work with a pure culture. A pure culture is composed of only one kind of microorganism. Occasionally a mixed culture is used. In a mixed culture there are two or more organisms that have distinct characteristics and can be separated easily. In either situation the organisms can be identified. When unwanted organisms are introduced into the culture they are known as contaminants.

Aseptic technique is a method that prevents the introduction of unwanted organisms into an environment. An example of using aseptic techniques is

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when growing bacteria; aseptic techniques are carried out to prevent the contamination of the culture. When working with microbial cultures aseptic technique is used to prevent introducing additional organisms into the culture.

Microorganisms are everywhere in the environment. When dealing with microbial cultures it is necessary to handle them in such a way that environmental organisms do not get introduced into the culture.

Microorganisms may be found on surfaces and floating in air currents. They may fall from objects suspended over a culture or swim in fluids. Aseptic technique prevents environmental organisms from entering a culture.

Doors and windows are kept closed in the laboratory to prevent air currents which may cause microorganisms from surfaces to become airborne.

Once these microbes are airborne they are more likely to get into cultures.

Agar plates are held in a manner that minimizes the exposure of the surface to the environment. When removing lids from tubes, lids are held in the hand and not placed on the countertop during the transfer of materials from one tube to another.

## **Introduction and aims of this report**

This report was based on two lab sessions; the reason for undertaking two lab sessions was to allow time for the culture to grow. In these sessions the objective was to learn basic aseptic techniques that are required in a lab, and also to learn how to count cells effectively using different methods. Another thing which was trying to be achieved was to be able to grow a

colony of bacteria from a single cell by doing streak plating and serial dilution.

## **Overview of practices and uses of aseptic techniques**

The laboratory session involved learning about the aseptic techniques. These aseptic techniques are important in a lab because they help keep the lab sterile, and sterility is vital in a lab because it allows the scientist to study and grow the bacteria they require accurately. Sterility is also important in preventing bacteria that are not required from replicating and growing on the sterile growth medium or the agar plate.

There were a few aseptic techniques we had to follow while working with bacteria's and sterile growth medium. To prevent the growth medium from being contaminated by air borne bacteria and other free floating matter, a Bunsen burner was set up near where the growth medium and bacteria samples were to be used. The Bunsen burner created a convection current that killed and destroyed most of the air borne bacteria and other free floating matter near the work station. This reduced the chance of the growth medium and bacteria samples from being contaminated.

The Bunsen burner was also set up to allow the use of another technique called flaming. This technique involves passing through the flame of the burner anything that has come in to contact of any bacteria or anything that is about to come in to contact of the bacteria sample. The items that are flamed are lab equipment such as bacteriological loops, glass pipette and bottle or flask necks. The items must reach a temperature of over 100 oC for it to be sterilized.

Another aseptic technique is called manipulation. In this technique the smallest finger is used to remove the lid of the bottle containing the bacteria; this allows the rest of the fingers to pick up anything else that is required. This technique also ensures the lid of the bottle is not placed down onto the bench where it is liable to contamination and thus contaminating the culture of bacteria in the bottle.

The last but the most important aseptic technique is who someone prevents bacteria from themselves contaminating the lab and the equipment. Every person carries a large amount of bacteria inside and the outside of the body. When working with bacteria in a lab, we had to wearing a lab coat, this prevent bacteria from our clothes and bodies spreading out in the lab. Also we had to be careful that we don't cough or sneeze on the growth medium, as this would lead to the growth of the bacteria released by the body. Also after performing the experiment it was vital that hands were washed with antibacterial soap to help prevent cross contamination. If hands are not washed correctly and if bacteria are still left on hands they are able to multiply at an exponential rate and can cause bacterial infections.

The first part of the experiment was to see the different variation and amount of bacteria on hands pre wash and after wash. This was done by placing the fingers in a Petri dish with nutrient agar. Nutrient agar is a microbiological growth medium commonly used for the routine cultivation of bacteria. The dish was separated in two and was labelled with one side of dish having prints from pre washed fingers and the other side after wash. The dish was then placed into incubation at 37 degrees as it is the optimum temperature where bacteria are able to multiply at an exponential rate

depending on some factors an example being the amount of food available or space.

The next part of the experiment consisted of doing a streak plate. This was done using the bacteria *Staphylococcus aureus*. Small sample of the bacteria SA was taken and put on a sterile loop and streak an agar medium. An example of the streak plate which was carried out is shown on the diagram below:

Diagram to show procedure of streak plating

1. Flame the loop and wire and streak a loopful of broth as at A in the diagram.
2. Reflame the loop and cool it.
3. Streak as at B to spread the original bacteria over more of the agar.
4. Reflame the loop and cool it.
5. Streak as at C, D E and F following same procedure after each streak as quoted above.
6. Label the plate and incubate it inverted.

The next part of the first session was to do serial dilution. This allows you to determine the number of cells in a bacterial culture. Since bacterial cell numbers are usually very high in the original sample, plating out this sample in an undiluted fashion would just lead to the creation of a bacterial lawn (a

smear of many, many individual bacteria colonies that are all growing next to or on top of one another).

Bacterial cell numbers need to be reduced, which is done by repeatedly diluting the amount of bacteria in the sample. A small amount of bacteria sample is mixed with a diluent solution (such sterile broth), and then successive dilutions are made. A small amount of each of the diluted bacteria samples is then spread onto an agar plate. The numbers of bacteria colonies that grow on each plate are counted. By working backwards using multiplication with the "dilution factor" (the number of times that you have diluted the bacteria sample with the diluent solution), we were able to make a determination of the numbers of bacteria in the original sample. After the dilutions were created 100  $\mu$ l of each dilution was transferred to an agar plate using a pipette, it was then spread around the agar plate with a spreader. These six agar plates were then put into incubation at 37 °C for 24 hours. When spreading the bacterial lawn the plate with the dilution level 10<sup>-5</sup> was done first and then the others 10<sup>-4</sup>, 10<sup>-3</sup>, 10<sup>-2</sup>. This is because the spreader which was used was plastic so the lower concentrated bacterium was spread first as the plastic spreader could not be flamed to kill the bacteria. If this aseptic technique was not used and the highest concentration of bacteria was used first it would have meant that the bacterial dishes would have become contaminated and also single colonies of bacteria would not be gained. If a glass spreader was used then it could have done in ascending order as the glass could be flamed by placing ethanol on the surface killing the bacteria on the glass spreader before doing the next part of the serial dilution.

The final part of the first lab sessions was to prepare smears of bacteria for gram staining. Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by colouring these cells pink or purple. Gram positive bacteria stain purple due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain pink, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decolouring process.

Gram staining involves three processes: staining with a water-soluble dye called crystal violet, decolourisation, and counterstaining, usually with safranin. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolourisation process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process. The process involves three steps:

1. Cells are stained with crystal violet dye. Next, a Gram's iodine solution (iodine and potassium iodide) is added to form a complex between the crystal violet and iodine. This complex is a larger molecule than the original crystal violet stain and iodine and is insoluble in water.
2. A decolouriser such as ethyl alcohol or acetone is added to the sample, which dehydrates the peptidoglycan layer, shrinking and tightening it. The



large crystal violet-iodine complex is not able to penetrate this tightened peptidoglycan layer, and is thus trapped in the cell in Gram positive bacteria. Conversely, the outer membrane of Gram negative bacteria is degraded and the thinner peptidoglycan layer of Gram negative cells is unable to retain the crystal violet-iodine complex and the colour is lost.

3. A counter stain, such as the weakly water soluble safranin, is added to the sample, staining it pink. Since the safranin is lighter than crystal violet, it does not disrupt the purple coloration in Gram positive cells. However, the decolourised Gram negative cells are stained pink.

(The descriptive methods are shown in the handbook for all experiments.)

### **Results for the gram staining**

After following the method as stated in the handbook we examined the slides under a microscope using the oil immersion objective of 100x. We then noted the shape of the bacteria that could be seen and the colour being purple (Gram positive) or pink (Gram negative). Below are the traces of the bacteria which could be seen under the microscope.

**Figure 1 – Staph aureus – gram positive (purple)****Description of what could be seen:**

- Cocci shaped bacteria
- Irregular clusters of bacterial cells

**Figure 2 – Bacillus cereus – gram positive (purple)****Description of what could be seen:**

- Rod shaped bacterial cell
- Singular bacteria

**Figure 3 – Saccharomyces cerevisiae – gram positive (purple)****Description of what could be seen:**

- Cocci shaped bacteria
- Clusters of bacteria closely packed
- Smooth

**Figure 4 – E-coli – gram negative (pink)****Description of what could be seen:**

- Rod shaped bacteria cells
- Linked (string like, filamentous)

**Results of the experiments**

After 24 hours the agar plates with the bacteria were ready to be viewed.

Firstly the agar dishes with the hand prints were viewed. Below is a diagram of the agar dish and the bacteria which was present:

There are different bacteria which were present while observing the dish, they were the following

A - The first bacteria which were seen and labelled as A were the largest of the three visible colonies which are circle in shape and yellow in colour with smooth edges, they can be seen to have a slightly humped surface.

B - These are slightly smaller in size than the ones described above and are also circle in shape but are white in colour, again the edges are smooth and the surface is humped.

C- These bacterium were only seen after washing hands they had no specific shape and were a lighter colour which was not very clear. They were flat with rough edges.

Before wash

After wash

The next dish which was observed was the streak plating dish, this had been left to incubate for 24 hours also. The results are shown on the picture below:

A sample of *Staphylococcus aureus* was inoculated onto an agar plate using the streak plate method.

From this diagram it can be seen that part 1 shows a higher concentration of bacteria. Parts 2, 3 have fewer bacteria but still there are very few single colonies. Part 4 shows many different single colonies of *Staphylococcus aureus* and are easily visible.

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The final dish which was viewed after 24 hours of incubation was the dish containing *Staphylococcus aureus* where it had been diluted to  $10^{-5}$ . These results can be seen below where there is diagram of the Petri dish including the bacterium.

From counting the colonies on the dish the figure which was calculated was 486. The amount of colonies calculated was still quite high as the preferred number of colonies would have been from 30-300. This may have been achieved if the serial dilution was carried further.

To calculate the amount of cells in this agar plate first the following was done:

$$0.1 \text{ ml of solution} = 4.86 \times 10^{-2} \text{ (486)}$$

$$1 \text{ ml of solution} = 4.86 \times 10^{-3}$$

so as it was the serial dilution of  $10^{-5}$  the calculation was then multiplied by 5 to give the final answer number of bacterial cells =  $4.86 \times 10^{-8}$

The last part of the lab session was to count cells using an Improved Neubauer Counting Chamber also sometimes known as a haemocytometer. The main objective of this session was to be able to calculate the total cells in the given sample. Below is a diagram of a haemocytometer with the slide placed over it:

The haemocytometer contains 9 large squares under the microscope at the 40X lens. The area of the square can be measured at  $1 \text{ mm}^2$ . The way to distinguish these squares from one another is by the triple dense lines. Within

each large square there are smaller grids which can be used to help during counting. Also when the counting the bacteria cells there was a set way to do this as shown below in the diagram:-

### Bacterial cells

So as can be seen in the diagram if the bacteria cells are at placed on the edge of the small squares then they will not be recorded. The orange lines representing cells will not be recorded as they are the end of the grid.

3 dense line separating each large square

The way the cells were counted was to identify which squares were going to be used to observe and calculate the number of bacteria present. The way this was done was there were 9 squares and only 5 squares were chosen as shown in the diagram below:

Squares 1, 3, 5, 7 and 9 were the squares which were used to count the bacteria.

Once the haemocytometer was set and the proposed samples were placed into the counting chambers and then placed under a microscope to view (extended method is described in the module handbook). The cell count was done for two different cell suspensions whole blood (ovine) and brewer's yeast (*Saccharomyces cerevisiae*) the samples were not diluted. They were then counted and the results are shown in the table below.

## **Table to show cell count from haemocytometer for the whole blood**

Square Number

Number Of Cells Present

1

50

2

48

3

47

4

48

5

44

**Total**

**237**

To calculate the total cell count in the neat solution a calculation was needed. Firstly the average number of cells was needed to be worked out.

The sum was  $237/5 = 47.4$ . To work out the cell number it was multiplied by  $1 \times 10^{-4} = 4.74 \times 10^{-5}$  So then finally to work out the cell yield the number of

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cells/ml was used which was  $4.7 \times 10^{-5}$  and was multiplied by the total volume of 10ml and thus the yield calculated was  $4.74 \times 10^{-6}$ .

### **Table to show cell count from haemocytometer for the Brewer's yeast**

Square Number

Number Of Cells Present

1

22

2

20

3

15

4

9

5

15

**total****81**

The same steps were taken to work out the cell number and yield for the Brewer's yeast.

Cell number =  $1.62 \times 10^{-5}$

Cell Yield =  $1.62 \times 10^{-6}$

**Table to show cell number and cell yield of both samples****Brewer's yeast****Whole blood****Cell number**

$4.74 \times 10^{-4}$

$1.62 \times 10^{-5}$

**Cell yield**

$4.74 \times 10^{-6}$

$1.62 \times 10^{-6}$

**Discussion of results**

In this part of the report I will refer to the results obtained and assess if they were accurate or not to the study of aseptic techniques. Firstly the practical which involved looking at bacteria on the skin showed that after washing there was fewer bacteria but another form of bacterial cells started to grow.

The reason for this could have been that when closing the taps I may have used my hands. The taps in the lab are made to be closed by the wrist so the <https://assignbuster.com/aseptic-technique-and-cell-counting-biology-essay/>



bacteria is unable to come in contact with the surface of your hands. The reason for this is because if you wash your hands and then close the taps with your hands again then you are just collecting the bacteria off the taps again. In some cases the amount of microbacterium on the skin can increase after washing, this is because by covering the skin with water you are making conditions for microorganisms more favourable and thus more will grow. It is therefore understandable that the growth of microorganisms will depend upon the chemical composition of the skin, for example if it is dry or whether it has a low pH. Most microorganisms that are present on the skin are located near hair follicles or sweat glands this is because they provide the nutrients and the correct environment for there growth.

Also another reason for the bacteria still being there after washing hands on the agar dish was because of the process of washing hands. The process of washing hands should be done surgically as there is not such thing as part sterile. So to make sure that all germs and bacteria are washed away from skin the procedure of hand washing should be followed correctly.

There are over 100 different types of bacteria on hands. The most common types of bacteria found on hands are familiar household names:

Propionobacterium (the bacteria responsible for acne), strep, and staph (of which the infamous methicillin resistant staph aureus, MRSA is a subtype).

Not all these bacteria are harmful as skin infections do not arise because you have bacteria on your skin. Rather, they arise because the type of bacteria on infected skin is not healthy bacteria but aggressive pathogenic bacteria.

## **Streak plating discussion**

From looking at the results obtained from the streak plating it can be seen that the streak plate was not very accurate as the intended result was not achieved. The aim of this experiment was to try and gain single colonies but the problem with the streak plate which I had carried out was that there was not enough room for the single colonies to advance. This was because the initial inoculation streaks were too thick and so took up too much space thus leaving little space in the middle of the plate for single colonies. It is therefore required that the initial streaks are made thinner and cover, as a rough estimate, the outer 2cm of the agar plate thus, leaving plentiful space at the centre of the plate for single colonies to grow. The problem with this procedure is that each colony may not represent the progeny from one cell, as two or more cells which are very close together could appear as one colony. Another problem which may have caused difficulty achieving single colonies may have been the concentration of the bacteria. If the bacteria were diluted it may have helped to achieve single colonies.

The single colonies which were achieved were all similar to one another this shows that the bacterium which was present in them colonies was the same bacteria. This was achieved as the inoculating loop was sterilised each time so only the bacterium which was being used grew on the agar dish.

## **Gram Staining**

In this part of the practical there were four different bacteria which were tested by using the gram staining process to see if they were gram positive or gram negative. The first bacterium was *Staphylococcus aureus*, *Bacillus cereus* and *Saccharomyces cerevisiae* which were gram positive bacterium

as after testing the bacteria under the microscope it showed that it was stained purple. The SA under the microscope was seen like a bunch of grapes as its names suggest as Staphyle in Greek terms meant grapes. Staphylococcus aureus is a bacterium, frequently living on the skin or in the nose of a healthy person that can cause illnesses ranging from minor skin infections and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis and septicemia.

The Bacillus cereus is a facultative anaerobic bacterium associated with food poisoning in humans. The food poisoning is a result of ingesting toxins produced by the bacteria. B. cereus is widespread in the soil and the food industry in such foods as herbs, spices, milk, and vegetables. Transmission of this disease results not only from contaminated foods, but also from improper food handling/storage and improper cooling of cooked food. The bacteria seen under the microscope and as can be seen in the diagram (figure 2) shows the bacteria as rod shaped bacteria which do not cluster together and are separated around the slide, in different directions and are not in any particular order as they all in different angles.

The Saccharomyces cerevisiae is also known better as yeast which could be used for baking or used while making alcohol. These cells were seen under the microscope as single cells which were rounded shaped cells and were closely packed together in groups.

The last bacterium was the E. coli which was stained pink as this was a gram negative bacterium. This bacterium is found in animals and birds in the lower intestines it helps with the digestion of food. If E. coli is ingested it will cause

the small intestine to become inflamed. People can contract an E. coli infection by drinking contaminated water, eating fruit or vegetables that have been watered with contaminated water, drinking unpasteurised milk, or eating undercooked ground meat.

In Gram-positive cells, peptidoglycan makes up as much as 90% of the thick cell wall; more than 20 layers of this polymer stacked together. These peptidoglycan layers are the outermost cell wall structure of Gram positive cells, whereas in Gram negative cells, the thinner peptidoglycan component is covered by an external lipopolysaccharide (LPS) membrane.

### **Serial dilution – agar dish 10-5**

This practical was done to see if individual colonies were able to be produced so the cells could be counted. The main aim was to reach from 30 -300 individual colonies. The amount of colonies which I produced in my agar dish was calculated and counted at 486. The method used was to try and calculate the number of cells in 1 ml solution of SA. This could only be done by serial dilution as it would be too difficult to count the cells if the bacterium solution was not diluted. The other dishes had too many colonies to count just by using the naked eye because it looked like a bacteria lawn. To try and achieve a better result and fewer colonies the experiment could have gone further and instead of having a concentration of  $10^{-5}$  the solution could have been diluted further. By diluting down the solution it also allows the bacterium to grow in optimum conditions as they do not have problems such as less space or food.

## **Cell counting using a haemocytometer**

In this part of the practical two solutions were supplied and cells were counted by the use of a haemocytometer and a microscope. While counting the bacteria it may have been misjudged as some bacteria may not have been counted this is one reason why the numbers recorded were quite low as the solution was not diluted as well. Also when counting the bacteria it's a total cell count so it is the living and dead bacteria so the results are not as accurate if only doing a live cell count. A better idea would be to do a serial dilution when doing a live cell count as only the living cells will grow into individual colonies. For the whole blood the amount of cells which were calculated was 237 and in the brewer's yeast there were 81 cells. A reason for the brewer's yeast having less amount of cells maybe that the cells form flocks of cells so it may be hard to visually see separate cells so when counting them a few cells may be counted as one cell. Also when counting these cells the same person was used to count the cells in both solutions the reason for this being as different people have different judgments and by using the same person it will help gain fair and more accurate results.