An aseptic technique

Introduction

Aseptic means to be free from microorganisms. Aseptic Technique is the procedure that is performed under sterile condition to prevent the growth of other microorganisms on the growth medium such as the Petri dishes containing the nutrient agar or the pure culture. If the growth medium or the pure culture is contaminated with microorganisms from the environment, it will results in confusion and inaccurate data. Hence, it is important to reduce the risks of these microorganisms to come in contact with the experimental materials.

In addition, by maintaining a clean environment when transferring the culture of microorganism onto the nutrient agar is part of the aseptic technique. This is usually done by disinfecting the table before and after working with microorganism using alcohol. Flaming the experimental materials such as bacteriological loops, bottle or flask necks can help in sterilizing. It must be done for several seconds so as to raise the temperature to kill the contaminants; however, the bacteriological loop must be cooled for a while before it is used to pick up the microorganism as picking up microorganism with a hot tool will kill the cells. When removing caps from bottles, it is important to keep the cap in the hand as by putting them on the table, it will be contaminated. Flaming is also required before replacing the cap onto the bottle. It is important to handle open tubes at an angle so that airborne and other microorganisms will not fall into the tube and cause contamination. During streaking, it is important to keep the lids of the Petri dish over it to prevent contamination. Lastly, try to avoid breathing,

coughing, sneezing and talking while transferring the culture so as to reduce the risk on contaminating.

Apart from this general aseptic technique, there also several other methods to ensure that destruction of living microorganisms in materials and apparatus. One of the methods is by using dry heat which is sterilising using naked flame or hot air. Sterilising materials or apparatus by a naked flame is usually heated to redness and allowed to cool. They are usually made of metal. Exposure to hot air helps to destroy microorganisms in glass and porcelain apparatus.

The other method is sterilizing using moist heat which can be used in three different ways which are heating in water or steam at 100oC, heating in steam under pressure and discontinue heating at low temperature. The different ways are employed according to the different materials or apparatus used.

The last method is by using chemical, it can be either in liquid or gaseous state. They are often used in the disposal of contaminated materials and apparatus after a laboratory session.

Microorganisms worked with in a lab should not be released into the environment as these strains may contain genetic markers such as antibiotic resistance. Therefore, they must be discarded properly.

In addition, aseptic technique is not only applied in laboratory, it is also applied in clinical and surgical setting.

Aims

There are two aims in this experiment. The first aim is to show that a large number of microorganisms exist on the surface of our hands. The second aim is carry out the aseptic technique properly by transferring pure culture and inoculating them onto an agar plate.

Materials and Methods

Bacteria on skin

Please refer to the Laboratory Manual unless otherwise stated of changes made.

Streak Plate

Please refer to the Laboratory Manual unless otherwise stated of changes made.

A disposable sterile bacteriological loop is use instead of the metal sterile bacteriological loop so no heating is required.

Discussion

Bacteria on skin

The human skin’s surface do carry a large number of microorganism and that by washing hands, individual can reduce the number of microorganism noticeably. However, even after a hand wash, microorganisms are still present on the surface.

Streak Plate

By employing the streaking technique on an agar plate correctly, a single colony can be obtained. Furthermore, it can be used to separate colonies of mixed culture. Hence, this pure colony can be picked up and to be grown in

large quantity. From the result above, it can be observed that single colonies of the S. aureus are found. Due to the colour and morphology, it can be noted that the S. aureus is of a pure culture.

Conclusion

Aseptic technique is a basic laboratory technique that must be employed especially during Microbiology laboratory session so as to prevent any contamination and affecting the accuracy of the result. Since microorganism can replicated rapidly, disposal of contaminants must be done properly so as to protect both the equipments and the health of individuals.

B- Gram Staining

Introduction

Gram stain is also known as differential stain in which it will divide bacteria into two large groups, mainly Gram Positive and Gram Negative. This difference is due to the chemical and physical structure of the cell wall called peptidoglycan. During solvent treatment, if the peptidoglycan is able to retain the crystal violet dye, the bacteria will be group as Gram Positive bacteria. However, if it is not able to retain the crystal violet, the bacteria will be group as Gram Negative bacteria and that it will be stained pink.

Gram Positive bacteria has a thicker peptidoglycan (50-90% cell wall) as compared to the Gram Negative bacteria (10% cell wall). In addition, the Gram Negative bacteria has another layer which is make up of liposaccharides and proteins and is separated from the cell wall by the periplasm.

In gram staining, there are four basic steps which include flooding the heat fixed smear with crystal violet stain, following by the addition of iodine solution to form complex, adding of alcohol for decolourisation and counterstaining with safranin.

After flooding the peptidoglycan with crystal violet stain, the dye will enter the cells and all cells will turn purple. With the addition of iodine, a crystal violet-iodine complex will be form such that it will not be able to exit the cells easily. By decolourizing the cell with alcohol, the peptidoglycan of the Gram Negative bacteria will break down because the alcohol will dissolves the liposaccharides layer and hence, with the removal of the layer, the crystal violet-iodine complex will run off which will results in the loss of the crystal violet stain and the cells turn colourless. On the other hand, the alcohol will dehydrate the Gram Positive bacteria’s peptidoglycan, closing the pores as the peptidoglycan shrinks. As a result, the crystal violet-iodine complex will not be able to run off as the “ exits” will be blocked and they remained stained. By counterstaining with safranin, the Gram Negative cell will turn pink and the Gram Positive cells will remain violet.

With gram staining, one is able to differentiate if the culture is a pure or a mixed, the morphological details of the bacteria and the arrangement of the bacteria.

Aims

The aim is to prepare smears for staining, observe the morphological details of the bacteria and to be able to differentiate between Gram Positive and Gram Negative bacteria.

Materials and Methods

Preparation of Smears for staining

Please refer to the Laboratory Manual unless otherwise stated of changes made.

A disposable sterile bacteriological loop is use instead of the metal sterile bacteriological loop so no heating is required.

Gram Staining Method

Please refer to the Laboratory Manual unless otherwise stated of changes made.

Discussion

According to the result observed, Bacillus subtilis is rod shaped (bacillus). They are stained purple which suggests that they are Gram Positive bacteria. They are arranged in singles. Although, endospore cannot be observe in this experiment, they can also be found on Bacillus subtilis. The endospore enables the bacteria to tolerate harsh environmental condition such as high temperature. Bacillus subtilis can also be known as a single bacillus bacterium.

Escherichia coli is stained pink and thereby, it is a Gram Negative bacterium. The cells are also rod shaped but they do not have any particular cell arrangement. They are found in singles, pairs and even clusters.

Proteus vulgaris is also stained pink and hence, a Gram Negative bacterium. Its morphology rod shaped and is arranged in singles. They can also be known as a single bacillus bacterium.

Staphylococcus aureus is a Gram Positive bacterium as it is stained dark purple after gram staining. It has a spherical shaped, otherwise known cocci and they are usually arranged in grape-like clusters. Therefore, they are known as a staphylococci bacterium.

There were no differences in the shape and colour observed for each of the bacteria, hence, they can be known as a pure culture.

Conclusion

The Gram staining method is a useful tool used in most laboratories as it helps individual to visualise the bacteria accurately and effectively such as the shape, arrangement and even whether the culture is a pure or mixed.

However, it should be noted that not all bacteria will give a gram reaction as some of them are gram variable, otherwise known as gram indeterminate. Therefore, they will give a mix of pink and purple cells after gram staining. For some of the Gram Positive bacteria, their peptidoglycan breaks easily during cell division, hence, after staining, they will give pink cells instead of purple. In addition, the duration of a culture can also affect the gram stain.

C- Cell Counting

Introduction

Cells counting is the accurate and precise counting of cells. They are usually carried out manually or electronically. By counting cells manually, a counting chamber, otherwise known as the haemocytometer is used. The counting chamber is used to determine the number of cells per unit volume of a suspension. On the other hand, a coulter counter is used to count cells electronically.

There are two approaches to count the number of cells, mainly total cell counts and the viable counts. Total cell counts are counted directly using the microscope and that both living and dead cells are counted. This is normally accompanied by the use of the counting chamber or coulter counter. Another approach is the viable counts which only count the living cells. The small volume of culture, otherwise known as the dilution of the culture is applied to the surface of an agar plate. After incubating, the colonies are counted, normally colonies between 30-300 are chosen to be used for the calculation of concentration of the given sample. The units given is colony forming units (CFU) per ml.

The haemocytometer is a modified glass slides with two count chamber of known area. Each chamber grid is composed of nine squares which are known as subgrid, each square is 1mm2. Within each large square, there are further sub divisions that help in counting. When the coverslip is placed over the grooves of the slide, there will be a thickness of 0. 1mm. Hence, the volume is 0. 1mm3 or 1 x 10-4ml. Therefore, the cell concentration will be calculated as the number of cells multiply by 1 x 104ml and again, multiplying the dilution factor.

Since cells are very small and they can be observed in a very high number, the suspensions should be diluted enough so that the cells are able to distribute uniformly in the counting chamber.

Aims

There are two aims in this experiment. Firstly, to be able to determine the cell count in different biological species and secondly, to be able to determine the viable count of a live bacteria, Staphylococcus aureus.

Materials and Methods

Cell Counting using Counting Chamber

Please refer to the Laboratory Manual unless otherwise stated of changes made.

Serial Dilution is carried out before the sample is loaded into the Neubauer Manual Counting Chamber. Normal saline (0. 9% NaCl) is used to dilute the blood and broth medium is used to dilute the brewer's yeast (Saccharomyces cervisiae). Both blood and brewer's yeast are dilute in the ratio of 1: 10 and 1: 100. The 1: 10 dilution is prepared by diluting the 10µL of whole blood or yeast with 90µL normal saline or broth medium respectively. The 1: 100 dilution is prepared by extracting 10µL from the respective sample from 1: 10 and adding 90µL of normal saline and yeast into the respective sample.

Cell Counting of Live Bacteria, S. aureus (after Serial Dilution)

Please refer to the Laboratory Manual unless otherwise stated of changes made.

Two changes were made in Step 1 and Step 12 respectively.

Only three nutrient agar plates , 10-3, 10-4 and 10-5 were labelled and culture in these dilutions were spread on the respective agar plates. A new spreader and pipette tip were used everything a different dilution culture

was spread on the agar plate. Count the number of colonies on the three different agar plates. Choose the agar plate with colonies between 30-300 to calculate the concentration of the original sample.

Discussion

Using the counter chamber, individual is able to give a quick assessment on the number of cells given that all the procedure on preparing and loading the sample onto it. One of it is that suspension/sample is not mixed before loading. This is due to the fact that cells tend to settle at the bottom of the tube and hence, while pipetting the sample out from the tube, individual do not have the actual or accurate number of cells. Therefore, to get a uniform suspension for a more accurate result, mixing the tube before pipetting is recommended. In addition, it can also help in reducing the clumping of cells.

Furthermore, improper filling of chambers can lead to inaccurate volume of suspension in the chamber and leading to inaccurate cell concentration. Improper filling of chamber includes overfilling or under filling of sample.

Moreover, there must be a consistency in counting cells which is in contact with the boundary lines (ie. the three lines just outside the grid) or when the cells are clump together. Individual will have to determine which cells to count and which not to count especially a cell which is situated on a border such as if a cell has half of its area outside the border, individual do not count those cells.

The other method of cell counting is the viable count where a single cell will give rise to a colony which is visible to the naked eyes on the agar plate. Therefore, by counting the number of colonies on the agar plate, individual is

able calculate the cell concentration. However, only plates which have 30 to 300 colonies are used to calculate the cell concentration.

In the result for viable count of S. aureus, the plate chosen was 10-5 because there was 82 colonies in one quadrant which is equivalent to 328 (82 x 4) colonies on the agar plate. Although, the number of colonies (328) exceed the number of colonies of 300 that we were supposed to chose, this 10-5 dilution plate has the closest number to 300. However, we should dilute even further because a single colony can have clumps or chain of cells in it and hence, resulting in inaccurate number of colonies/cells in which the actual number of cells should actually be more than the calculated number of cells.

The advantage of viable cell counting is that the organism counted will be a positive one (ie. S. aureus) instead of any other organism as if there is contaminant, the morphology or colour will be different.

Another disadvantage of viable cell counting, other than cells that clump together or have chains which will form a single colony, is that organism will only grow in condition which is suitable for their growth on the agar plate.

Cell counting usually is accompanied by serial dilution as it is impossible to count the number of cells if the concentration is too high as it will lead to a very high number of cells.

Conclusion

There are several other methods, other than using counting chamber and viable cell count, to count cells in a suspension. However, they are the least expensive and is able to give accurate result in a very short period of time.

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