

# [Pure culture methods and gram staining](https://assignbuster.com/pure-culture-methods-and-gram-staining/)

Individual and well separated colonies are crucial when working with bacterial cultures. Pure culture method involves the step of taking a small and well isolated colony and transferring it to a sterile growth medium in a suitable culture vessel (Ryan & Ray, 2010). There are three types of commonly used methods to produce pure cultures. They are the streak plate, spread plate and pour plate. All these methods require the use of aseptic technique.

Streak plate is suitable for the direct plating of media that contains a large number of cells. To prepare a streak plate, a colony or a loopful of bacteria culture will be taken using a sterile inoculating loop and then the sample will be streaked several times over the surface of the solid growth media. Single cells are obtained along the streak as each streak represents a dilution process. The bacteria will grow into a separate colony and to be used for pure culture after being incubated at optimum condition (Carter & Wise, 2004).

Another alternative to obtain pure culture is the spread plate method where a small volume of suspension is spread evenly over the surface of an agar plate. The bacteria colonies will be evenly distributed over the surface of the agar plate after incubation. Larger colonies are the colonies that are well isolated.

Pure culture can also be done by using the pour plate method. The diluted sample is suspended in agar growth at about 50 â°c. The mixture is then poured into a suitable vessel and incubated. The bacteria colonies will grow throughout the agar plate. The colonies within the agar will be very tiny and difficult to see and count as compared to those which grow on the surface of the agar.

## Objectives:

The purpose of conducting this experiment was to understand the principles and aim of obtaining pure cultures, to compare the separation of bacterial colonies using streak plate, pour plate and spread plate techniques and to observe and compare the colonial features of isolated bacterial colonies on solid nutrient media as well as the morphological cellular features of isolated bacterial colonies using the gram stain method. In addition, the experiment explained about the principles of gram stain method and allowed the interpretation of each of the steps in gram staining.

## Method:

Refer to lab manual page 32-36.

## Results:

In this experiment, mixture of Bacillus cereus, Escherichia coli and Staphylococcus aureus was used to prepare pure culture using the three methods which are the streak plate, spread plate and pour plate. The plates were incubated and the colony morphology of the plates was observed. The cellular morphology of the bacteria was determined using the gram stain method. The results were recorded and tabulated in a table.

Table 1: colony morphology and cellular morphology of different organisms.

Organism

Colony morphology

Cellular morphology

Gram stain

Shape

Arrangement

Bacillus cereus

Irregular, opaque and cream in colour, flat

Positive (purple colour)

Rod

Chain

Escherichia coli

Circular, center, smooth, translucent, white (small), raised (Cham &Tan, Group 1).

Negative

(pink colour)

Rod

Cluster

Staphylococcus aureus

Circular, smooth, some yellow, some white, convex.

Positive (purple colour)

Cocci

Cluster

## Discussion:

1) Bacillus cereus was obtained from the big bacteria colony. When observed from the agar plate, it appeared to be irregular in shape, flat, opaque and cream in colour. It is a gram positive bacteria. So when gram stain was performed, the bacteria appeared purple in colour under the microscope. Bacillus cereus is of rod shape and the arrangement is in the form of chain. It has Î²- capsule and is non motile. For Escherichia coli, it was obtained from the medium colony. When observed from the agar plate, it appeared to be circular in shape and mostly was located at the center of the agar. The bacteria colony appeared to be smooth, translucent, and some were white in colour (Cham & Tan, Group 1). Escherichia coli is a gram negative bacteria. Therefore, it was pink in colour under the microscope. It has rod shape and its arrangement is in cluster form. It is motile and is a type of non spore forming bacteria. Staphylococcus aureus was from the small bacteria colony. It is circular, smooth, convex and some are yellow in colour while some are white in colour. It is a gram positive bacteria where it showed purple colour when under the microscope. The shape for Staphylococcus aureus is cocci and it is in cluster form. It is a non spore forming bacteria and is non motile. The cells seen in the original mixture matched with those obtained in the pure culture.

Streak plate method is suitable for the direct plating of media that contains a large number of cells and as for spread plate method, a small volume of suspension is spread evenly over the surface of an agar plate. On the other hand, bacteria are suspended and grow throughout the agar plate for pour plate method. 2) Spread plate will be more suitable to produce well isolated single colonies from a broth culture that contains a mixture of organisms as the bacteria will be on the surface or the agar and not suspended. This way, the isolated colony can be counted and also be obtained easily compared to streak plate and pour plate method. When the bacteria is taken from a mixed broth culture, the colonies will grow and overlapping each other after being incubated. When the bacteria is incubated using pour plate method, the bacteria grown will be smaller and it will be harder to obtain a well isolated colony as they grow throughout the media.

Agar has a higher melting temperature than gelatin. Gelatin will melt and becomes soft when bacteria is being incubated on gelatin media at their optimum temperature which is 37 â°c. 3) Agar is used in preference to gelatin for making solid media as agar will remain solid at the optimum temperature for the growth of most bacteria. Therefore, the property of agar makes it to be more effective than gelatin as a solidifying agent (Muir & Ritchie, 1953).

4) Bacteria that are well separated grow as isolated colonies without affecting the growth process as these bacteria cell do not merge or overlap with other bacteria cells in compete for sources. The growth of isolated bacteria will not be limited by the depletion of the nutrients (Cruickshank, 2005)

5) Individual colony does not always consist of one type of bacterium as the bacteria could be from a mixed culture of different organisms. Therefore, an individual colony does not always originate from a single parent cell.

Before staining process, bacteria culture has to be dried and fixed. Heat fixation is convenient and it retains the cell wall structure. 6) Smear preparation has to be heat fixed through the Bunsen burner flame to kill the bacteria and make them adhere to the slide. Besides, it will also allow the cells to stain better during the staining process.

A good smear preparation will allow a better microscopic view of the bacteria cellular features. Correct techniques in preparation of smear are important. 7) If the whole colony is used to make the smear, the smear will be too thick. Thick smear will cause the difficulties in distinguishing individual cell. In addition, the stain might not be completely washed out. If the bacteria used is gram negative, the cells will appear purple in colour giving gram positive results which is wrong. If the bacteria is gram positive, the intensity of purple colour will be so high that it will not be easy to differentiate the cellular features of the cell.

8) Cell wall determines the shape of a cell. Cells that lose their cell wall will change its shape. However, changing the shape of the cells will not affect the reaction to gram stain. Hence, there will be no association between cell shape and reaction to gram stain

The cell wall structure of gram positive bacteria is different from the cell wall structure of gram negative bacteria. The cell wall of gram positive bacteria has a thick peptidoglycan layer while the peptidoglycan layer in the cell wall of gram negative bacteria is thinner. The thick peptidoglycan layer of the cell wall of gram positive bacteria retain the crystal violet dye and stain purple even after decolourisation but for gram negative bacteria, it is decolourised by the decolourising agent. Gram negative bacteria retain the second dye and stain pink. 9) The cell wall structure of a bacterial cell is important to determine its gram reaction.

10) The third step which is the decolourisation step of the gram staining procedure is important to determine the outcome of the result. The time taken should not be too long or too short as over-decolourising will cause the cell not able to retain the crystal violet dye and appear colourless. If the decolourising time is too short, the gram negative cells will remain purple giving a gram positive result.

## Conclusion:

In conclusion, pure culture can be obtained by using several methods which are the streak plate method, spread plate method and pour plate method. Using the pure culture, the separation of bacterial colonies, the colonial features and cellular features can be compared. Aseptic technique should be followed all the time to prevent introducing new contaminants to the environment.