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- (b) Gram negative: *Helicobacter pylori* (pathogenic); *E. coli* (generally non - pathogenic); *Azotobacter vinelandii* (non pathogenic).



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# EXPERIMENT 6

## ISOLATION OF PURE CULTURES OF BACTERIA BY STREAK PLATE METHOD

### Structure

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|----------------------------|------------------|
| 6.1 Introduction           | 6.4 Protocol     |
| Expected Learning Outcomes | 6.5 Observations |
| 6.2 Principle              | 6.6 Results      |
| 6.3 Requirements           | 6.7 Precautions  |

### 6.1 INTRODUCTION

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Microorganisms exist in nature as a mixture of many different kinds of organisms whose composition varies from one environmental niche to another. Traditionally microbiologists have studied the characteristics (cultural, morphological or biochemical) of a particular microbe only after obtaining a pure culture. This has led to development of techniques for isolation of pure cultures. To begin with, discrete colonies on semi solid agar plates are isolated using streak- plate, spread plate or pour plate method and then cells are picked from individual colonies for isolation of pure cultures. A lot of information has been gathered since then for microbes that could be isolated from mixed populations. But detailed analysis of molecular processes and their use as reactors in recombinant DNA technology has been limited to a handful of microbial systems.

We all recognise that microbes are extremely versatile and are found in every possible environment. But many of them could not be cultured and maintained in laboratory conditions by usual techniques, making it almost impossible to study them in isolation. With the development of metagenomic approach (molecular tool to analyse DNA from an environmental community) the prerequisite condition of having pure cultures to initiate investigations is no longer there. This has greatly complimented the earlier techniques and has yielded information about a large number new microbes and novel metabolic pathways.

In this exercise you shall learn to isolate pure cultures of microorganisms from a mixed population using four-quadrant streak plate method.

## Expected Learning Outcomes

After studying this experiment, you should be able to:

- ❖ Perform streak plate technique to dilute bacterial cultures;
- ❖ Isolate pure cultures from discrete colonies; and
- ❖ Identify some of the isolated organisms from their cultural morphology.

## 6.2 PRINCIPLE

The isolation of microorganisms (most often bacteria) by streaking a loopful of culture on agar surface spread in Petri dishes was first developed by Loeffler and Gaffky in Koch's laboratory. It is a rapid qualitative method requiring minimal skills. In essence streaking brings about dilution of the initial inoculum such that individual discrete colonies can be formed, allowing isolation and identification of different species of bacteria from a given environmental sample. Each colony represents a single organism that has multiplied many times. A pure culture of bacteria can be also be streaked to get individual colonies which can be picked and used for conducting various studies.

Many variations of streak plate methods are available that are essentially based on the number of sectors in which the agar plate is streaked. These include continuous streaking, T-streak (three-sector streak) and four quadrant method (Fig. 6.1).

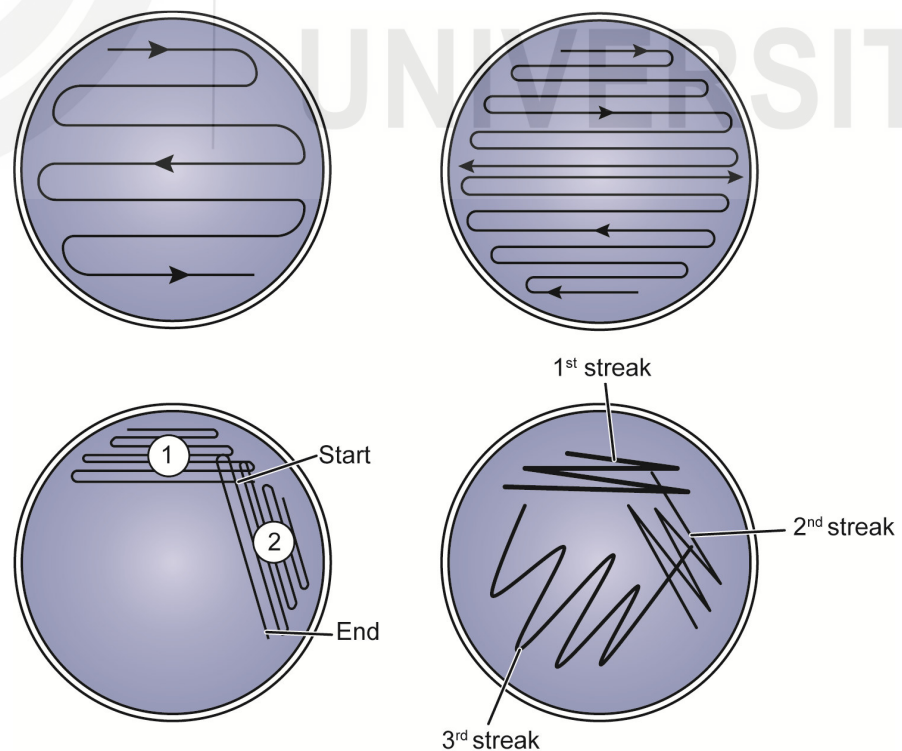


Fig. 6.1:Streaking methods.

This method cannot be used in case of anaerobic organisms and only viable organisms in the mixed population can multiply to form visible colonies.

## 6.3 REQUIREMENTS

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- ❖ Sterilised semi solid nutrient agar plates (or some other nutrient medium)
- ❖ Inoculating loop
- ❖ Ethyl alcohol (95%)
- ❖ 500ml beaker
- ❖ Burner / spirit lamp
- ❖ Fresh nutrient broth cultures from environmental sources.
- ❖ Glass marking pencil
- ❖ Liquid broth
- ❖ Agar slants / broth culture tubes.

## 6.4 PROTOCOL

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### (A) Isolation of discrete colonies from a given mixed culture

The four streak quadrant method is a discontinuous streaking method involving four individual streaks. As the name suggests in this method the Petri plate is divided into four quadrants / areas (1-4). The expected result is shown in Fig. 6.2. The steps for four-quadrant streak plate is as follows:

- ❖ Sterilize the inoculating loop by heating it in the blue flame of the Bunsen burner until it is red hot. Allow it to cool.
- ❖ For convenience invert the agar plate and divide it into four sectors with a glass marker.
- ❖ Hold the agar plate in the left hand between the fingers and the thumb close to the flame.
- ❖ Loosen the cap / cotton wool plug of the tube containing the inoculum.
- ❖ Remove the cap of the test tube with the little finger of the right hand. Flame the rim of the tube and work faster and closer to the flame.
- ❖ Aseptically pick inoculum by dipping the cooled loop into the bacterial suspension or from a colony on solid medium.
- ❖ Open the lid of the Petri plate and streak the first quadrant.
- ❖ Reflame the loop and allow it to cool. You can even touch the surface of uninoculated agar medium.
- ❖ Rotate the plate 90°.
- ❖ Now streak the second quadrant, starting from the edge of the first streak.

- ❖ Repeat the preceding three steps for quadrant 3.
- ❖ Without re-flaming the loop, turn the dish 90°.
- ❖ Streak area 4 with inoculum from the corner of area 3.
- ❖ Close the lid of the Petri plate and invert it.
- ❖ Incubate the plates overnight at 37°C for 48hrs at 25°C.
- ❖ Observe the plates and record.

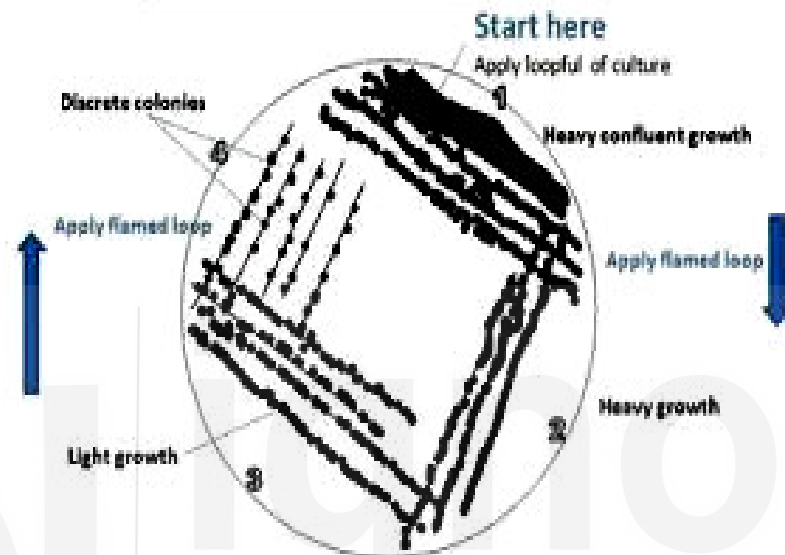


Fig. 6.2: Four streak quadrant method. (from: [microbeonline.com](http://microbeonline.com))

(Observe the progressive dilution of the culture from area 1 to area 4.)

### (B) Isolation of pure cultures

- ❖ The next step is to pick one by one aseptically cells from discrete well separated colony from streaked plates and transfer them to separate nutrient agar slant / broth.
- ❖ Incubate the cultures overnight at 25°C.
- ❖ Each of these slant / broth cultures has descended from a single bacterial species. They are pure / stock cultures.
- ❖ Store the cultures. Depending on the storage conditions, the time between sub culturing would vary.

## 6.5 OBSERVATIONS

**Observe the streaked plated next morning and report:**

(a) The cultural morphology of the colonies. Your instructor would help you to analyse.

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(b) Assess the quality of your plate; either draw it or take pictures.

## 6.6 RESULTS

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- (a) Compare the distribution and amount of growth in the four quadrants. Based on your observations discuss your results
- (b) If you have not obtained the expected dilution of the culture by four quadrant streak plate method, can you suggest possible reasons for your failure?
- (c) Indicate the names of the isolated organisms.
- (i)-----
- (ii)-----
- (iii)-----

## 6.7 PRECAUTIONS

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- ❖ Best results are obtained when the loop is flamed between streaks. This will further reduce the number of microorganisms as we move from one area to the next.
- ❖ Always cool the flamed inoculation loop before picking the inoculum.
- ❖ Invert the streaked plates during incubation to prevent condensed water droplets from falling on agar surface. This may spread the colonies and even cause contamination.
- ❖ Perform the experiment using aseptic techniques to prevent contamination.
- ❖ Label the plates. It should carry complete information about the organism, date of experiment, your name, etc
- ❖ Apply light pressure while streaking to avoid digging into agar.
- ❖ Take care not to let the loop touch any of the previously streaked areas.

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### SAQ 1

1. Indicate two applications of streak plate method.
  2. What is the purpose of flaming the inoculation loop between streaks in different areas?
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## ANSWERS

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1. (a) It allows isolation of specific microorganisms from mixed cultures.
- (b) Bacterial cultures can be stored (short term) in an inverted position and after covering the Petri plates.















## SUGGESTED READINGS

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