Microbiology Laboratory

Lab 10: Streak Plate Method- Principle, Types, Methods, Uses

What is Streak Plate Method?

Streaking is a technique used in microbiology for the isolation of single colonies of microorganisms, either from a mixed species or from the same species. This technique is mostly applicable to bacteria but is also used for some yeasts. It is an old technique that has been in use since the time of Rober Koch. It was first demonstrated by Loeffler and Gaffky in Koch's laboratory. It is a mechanical isolation technique used in microbiology, commonly known as the "**streaking method**".

Objectives of Streak Plate Method

1. To obtain a pure culture of bacteria from a mixed culture
2. To obtain well-isolated colonies
3. To propagate bacteria

Principle of Streak Plate Method

The streak plate method is based on the principle of dilution. It can be described as a rapid qualitative isolation technique. The main criterion of isolation is to obtain a reduced number of colonies. In this technique, a loopful of culture is spread on an agar plate to get individual cells far apart enough from each other. The streaking method gradually dilutes the inoculum such that the bacterial cells can be counted as colony forming units (CFUs). The streak plate method is based on dilution during the process of **mechanical spreading of inoculum** over the surface of solidified culture media in order to obtain well-isolated colonies of the sample at the terminal streaks. First, let us understand the microbial culture. Microbial cultures are of two types:

1. **Mixed Culture:** two or more species of microorganism or bacteria are present in mixed culture.

2. **Pure culture**: single species of microorganism or bacteria is present in Pure culture. In nature, bacteria exist in mixed populations. Our aim is to isolate pure culture containing single bacteria from mixed culture of many bacteria. The streak plate method is widely used to isolate pure culture.

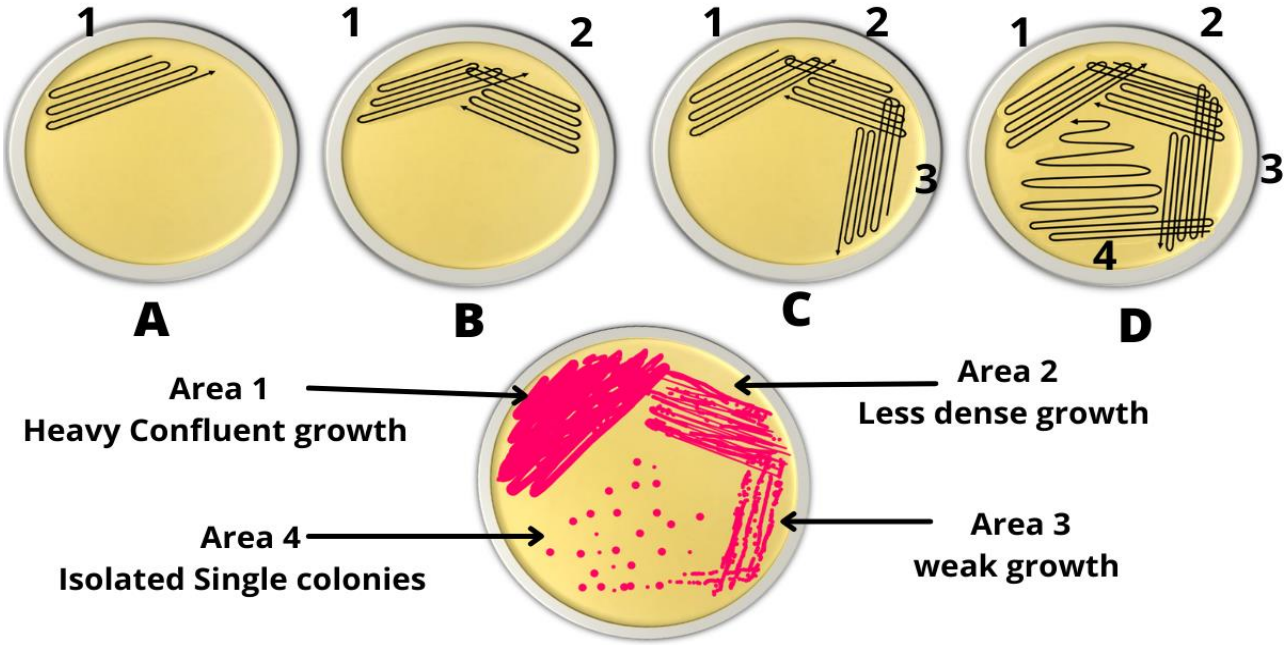
Types of Streak Plate Method

Based on the pattern of streaking, the streak plate method can be classified into 4 types: Quadrant Streaking, T-Streaking, Continuous Streaking, and Radiant Streaking.

1. Quadrant Streaking

It is the most commonly used and the most preferred method where four equal-sized sections of the agar plate are streaked. It is also referred to as the "four-quadrant streak" or "four sectors" or "four-way streak" method.

In this method, each plate is divided into four equal sectors and each adjacent sector is streaked sequentially. The sector which is streaked first is called the first sector or the first quadrant, and it has the highest concentration of inoculum. Gradually the second, third, and fourth quadrants will have diluted inoculum. By the time the fourth quadrant is streaked, the inoculum is highly diluted giving rise to isolated colonies following the incubation.

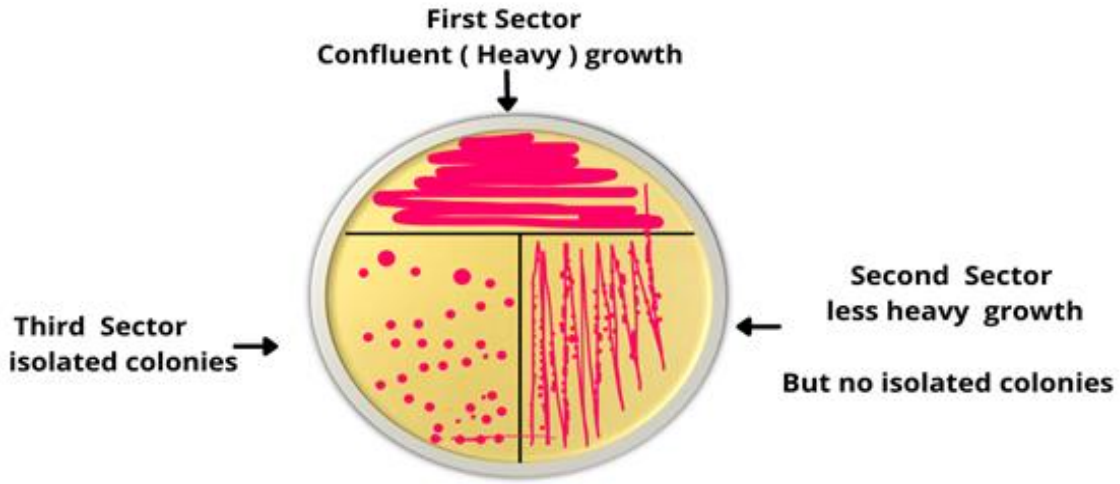


2. T-Streaking

It is another method of streaking where the agar Petri plate is divided into three sections and each section is streaked. Hence, this method is also known as the "three-sector streak" method.

The media is divided into three sections by drawing a letter "T" and each adjacent section is streaked sequentially. By the time the final section is being streaked, the inoculum is diluted to the point to give rise to isolated colonies following the incubation. Mostly discontinuous fashion of streaking is followed; however, a continuous fashion can also be used in the very dilute specimen.

As in quadrant streaking, it is difficult to culture two or more samples in a single 10 cm plate using this method.



3. Continuous Streaking

It is another commonly followed method where an inoculum is evenly distributed in a single continuous movement from starting point to the center of the plate. There is no need to divide the plate and sterilize the loop during the process. It is easy and quick; however, the problem is that we can use it only if the inoculum is either very diluted or we just have to propagate pure culture rather than isolate one.

We can divide the 10 cm Petri plate into different sections (mostly 2 to 6), and in each section, we can streak different specimens following this method. Hence, it is used in the clinical laboratory to culture urine, sputum, pus, etc. if multiple samples have arrived at a single time. This will allow us to save media and get maximum output using a minimum resource.

4. Radiant Streaking

It is another method of streaking where the inoculum is first streaked at one edge and spread in vertical lines above the edge. Finally, the vertical lines are cross streaked diagonally. This method is suitable to propagate pure culture, and also in the case of a dilute specimen.

There are other modified forms of streaking like:

5. Semi-quantitative Streaking

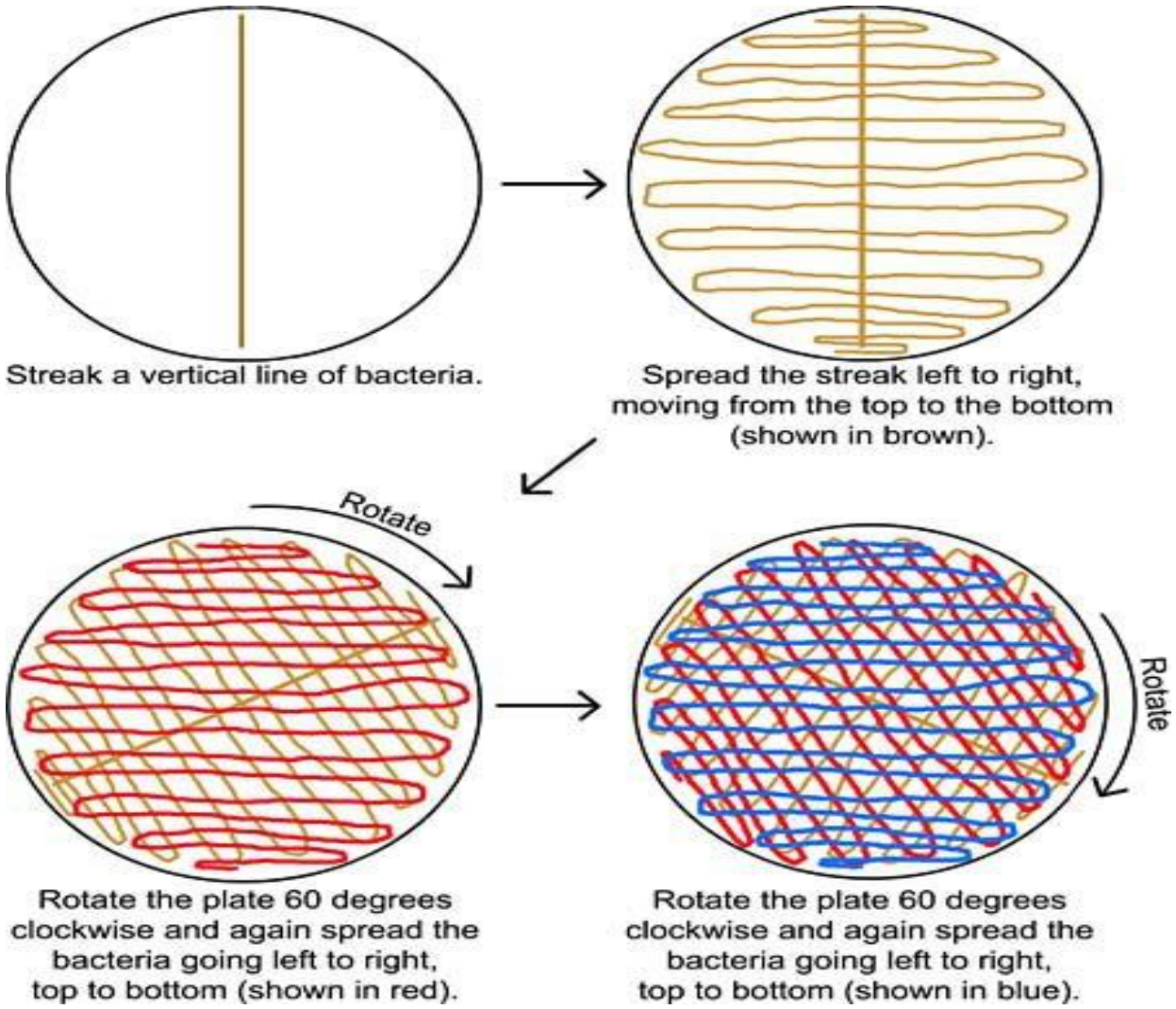
It is routinely followed in urine culture. It is a modified form of continuous streaking. In this method, a calibrated loop (usually a loop of 1 or 2µl) is used to streak a certain volume of the liquid specimen. A loopful of the specimen is streaked in a horizontal line in the middle of the Petri plate, and the specimen is spread all over the plate in a single continuous back and forth movement. This method allows us to approximately quantify the viable load (in a range, not an exact number) as well as get the pure culture in a single go.

6. Zigzag Streaking

It is another form of continuous streaking where a loopful of the specimen is streaked all over the plate in a zigzag pattern in a single continuous movement. It is commonly done to propagate the pure culture and culture them in large quantities.

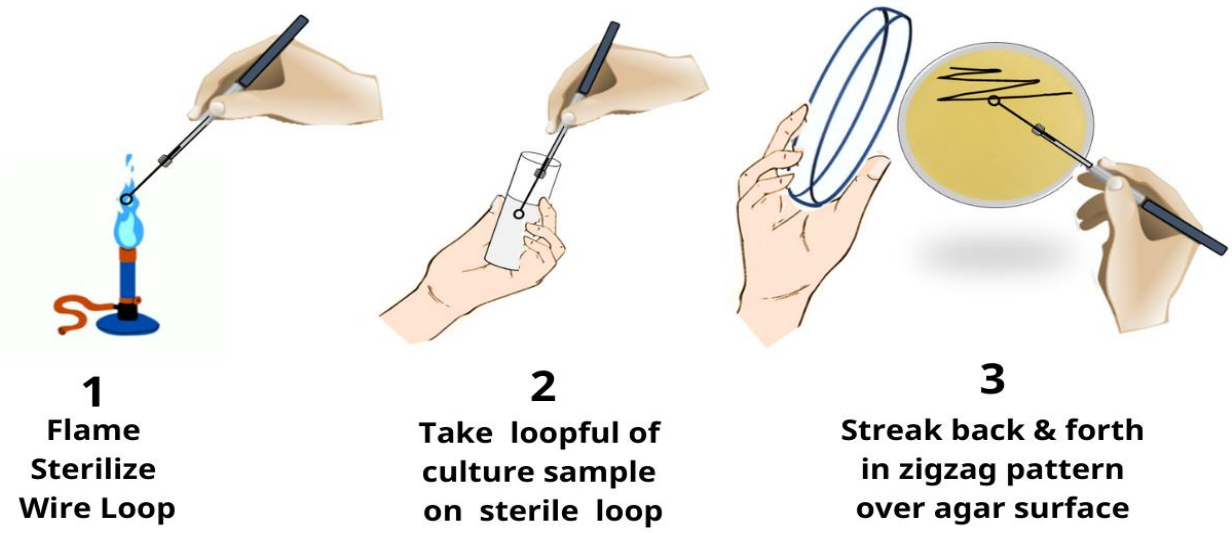
7. Lawn Culture method or Carpet culture method

is employed when large amount of growth is required and used for Antibiotic sensitivity test, on each agar plate, streak the bacteria by first making a vertical line, then spreading this left to right (and top to bottom), rotating the plate 60 degrees clockwise and again spreading the bacteria left to right (and top to bottom), and then rotating the plate another 60 degrees clockwise and spreading the bacteria again. *Note:* The bacteria should all be the same color on your plates. Color has been added in this diagram to help clarify the procedure. Uses for antibiotic susceptibility testing: It is useful for antibiotic susceptibility testing by disk diffusion method and Bacteriophage typing.



Materials Required: The following apparatus is required for the streak plate technique.

1. Bunsen Burner
2. Nichrome Wire Loop
3. Sterile Nutrient Agar plates
4. Mixed Culture of bacteria

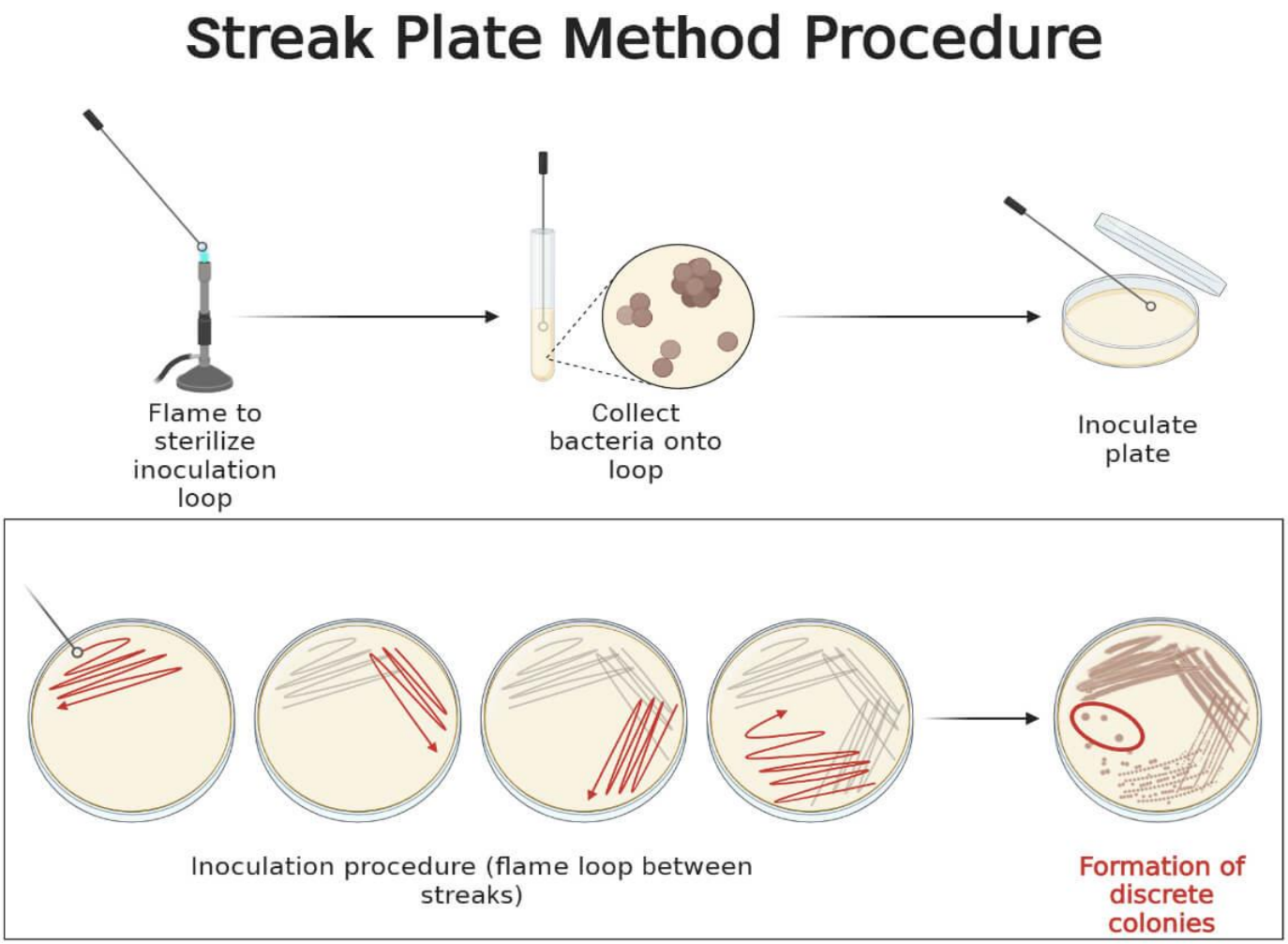


Procedure or Protocol of Streak Plate Method

The general procedure of the streak plate method can be summarized as:

1. Arrange all the requirements, put on the PPE, sterilize the work surface, and allow all the samples and media to come to room temperature if were refrigerated.
2. If the sample is very concentrated then dilution can be helpful to get the isolated colonies. (But it is not compulsory as the sample will be diluted during the streaking process.)
3. Sterilize the inoculating loop by flaming and allow it to cool. Pick a small portion of the isolated colony. (if the sample is in the suspension, then take a loopful of the sample)

   The inoculating procedure is different according to the method of streaking, let us deal with each type.



Applications of Streak Plate Method:

1. Used to obtain a pure culture from the mixed culture in order to perform morphological, biochemical, and molecular tests to identify and for other applications.
2. Used to define the specimen as pure or mixed species.
3. Used to study colony characters of bacteria.
4. Used to produce a colony of genetically identical individuals
5. Used in inoculation of clinical specimens in diagnostic laboratories to grow isolated colonies of pathogen
6. Used in urine culture to isolate pathogens and semi-quantify the uropathogens to determine the significance of the infection. A calibrated loop is used for this purpose.