

The Streak Plate Protocol

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Information

History

The modern streak plate procedure has evolved from attempts by Robert Koch and other early microbiologists to obtain pure bacterial cultures in order to study them, as detailed in an 1881 paper authored by Koch (5). Slices of sterilized potatoes became the first solid media employed on which to grow bacteria. This process was a procedure that worked only for a few organisms and only until the bacteria decomposed the potato surface. A search for other materials led to experimentation with the suitability of gelatin and agar-agar as solidifying agents. Gelatin was difficult to prepare and difficult to use at room temperature, let alone at the higher temperature of an incubator, and many bacteria digest the protein. Agar, because of its characteristics of melting only when boiled, rarely being digested by bacteria, and providing a substance in which other nutrients could be dissolved, proved to be a suitable material on which to grow bacteria. Agar was originally called agar-agar and is derived from seaweed. The agar that we use today is the same substance as agar-agar, but it has been processed by the manufacturer. Agar, as purchased 100 years ago, required filtering before it was clear enough to use in media (12). In the early eras of microbiology, making media was an extensive process of preparing the extracts of meat or other nutrient sources, as well as purifying and filtering the gelatin or agar. Before the invention of the autoclave, sterilizing the media properly was also time consuming. The 1939 edition of *An Introduction to Laboratory Technique in Bacteriology*, an early microbiology lab manual, contains extensive instruction for students to prepare their own media from "scratch" (7) for use in the lab. Before R. J. Petri invented the petri dish, flat plates of glass covered by glass lids were most commonly used to culture organisms in gelatin.

Even after agar was adopted and solid media were available, the streak plate was not commonly used. Historically, microbiologists most frequently used pour plates to isolate organisms for pure cultures. A pure culture was made from an isolated colony, represented only one species or strain, and traditionally arose through the growth of a single cell. Colonies are considered isolated if they are not touching any other colony. Isolated colonies were identified and transferred by streaking onto a new agar or gelatin plate using a sterile needle, a process called "picking colonies." More rarely, a researcher would try to isolate organisms directly on the surface of a gelatin or agar plate. A typical description of the streaking process was given by Huber Williams, revised

by Meade Bolton in *A Manual of Bacteriology* published in 1908 (11). "...the isolation of bacteria may sometimes be effected by drawing a platinum wire containing material to be examined rapidly over the surface of a petri dish containing solid gelatin or agar; or over the surface of the slanted culture medium in a test tube; or by drawing it over the surface of the medium in one test tube, then without sterilizing, over the surface of another, perhaps over several in succession."

Bacteriology textbooks and lab manuals from the early and mid 20th century did not mention the streak plate nor did they have our typical "isolation streak" exercise. For instance, isolation by streaking is absent from Buchanan and Buchanan, 1938 (2) and from Sherwood, Billings and Clawson's manual published in 1952 (10). During a literature search to pinpoint the first appearance of our modern streak plate, several papers published in the 1940s were found to mention streak plates. However, these did not describe the process or illustrate the results, and from the context, most probably referred to the process of picking colonies and creating a pure culture in fresh media.

An early version of our modern isolation streak is found in Levine's *An Introduction to Laboratory Technique in Bacteriology* published in 1939 (7) and a similar version from 1954, in *Salle's Laboratory Manual on Fundamental Principles of Bacteriology*, 4th ed. (9). In that process, the student picked up organisms on a needle or loop and then either stabbed into the agar or spread the loopful of the culture at the upper end of the petri dish to thin it out. Then a series of strokes 1/4-inch apart was made over the rest of the plate. Dr. Salle noted that the first streaks would contain too many organisms but that the last streaks should give isolated colonies. He suggested that a second plate be inoculated without flaming the wire loop first, to give a better chance of obtaining isolated colonies. This process dilutes the bacteria as the plate is streaked, similar to the dilution observed in a modern streak plate.

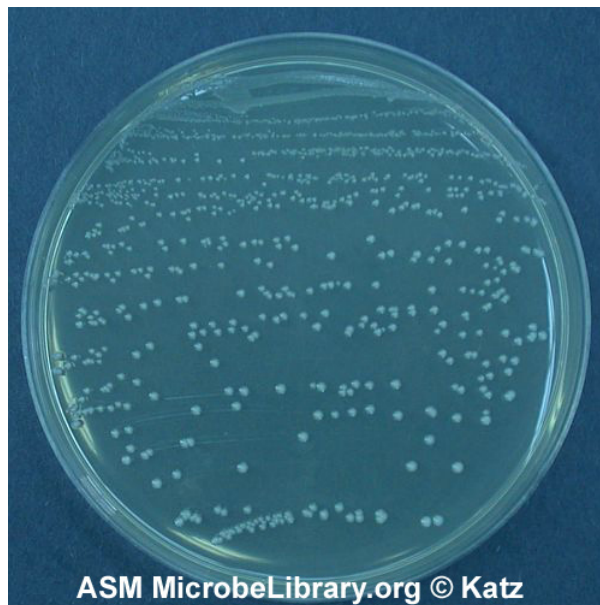


FIG. 1. An example of the one-directional streak pattern as described in the lab manuals by Levine and Salle (7, 9). The plate illustrated is a 100-mm petri dish.

In 1958, in the first edition of *Laboratory Exercises in Microbiology*, Pelczar and Reid (8) presented a streak plate exercise. It utilized a 4-quadrant streak pattern, and the procedure described using both a loop and a needle in the streak and all streaks were in the same direction, rather than both back and forth.

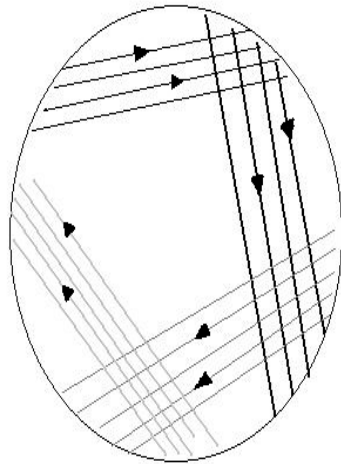


FIG. 2. A drawing representing the streak pattern recommended by Pelczar and Reid (8). All strokes of the loop or needle are done in a single forward direction, rather than in a back-and-forth pattern, as indicated by the arrowhead directions. The initial sector is at the top of the plate, followed clockwise by sectors 2, 3, and 4.

The earliest appearance of the three sector streak pattern (called the T streak) commonly used today may be the 1961 photos published in Finegold and Sweeney (4). An illustration detailing how to perform this streak is in the 1968 edition of the *Manual of BBL Products and Laboratory Procedures* (1). In addition to the T streak, the BBL Manual illustrates two other streak patterns, neither of which is the simple monodirectional streak pattern used earlier in the century.

Today, there are two most commonly used streak patterns, a three sector T streak and a four quadrant streak. Microbiology lab manuals since the 1970s have presented an isolation streak exercise. Lab manual editions published between 1970 and 2000 illustrated and described several streak pattern variations. However, today, almost all published microbiology lab manuals illustrate at least the T streak.

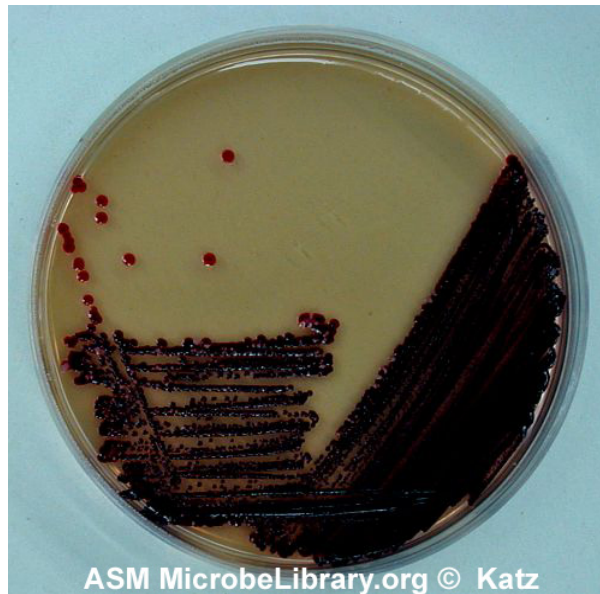


FIG. 3. A three sector T streak of *Serratia marcescens* grown on trypticase soy agar. This illustrates a streak plate which has many isolated colonies.

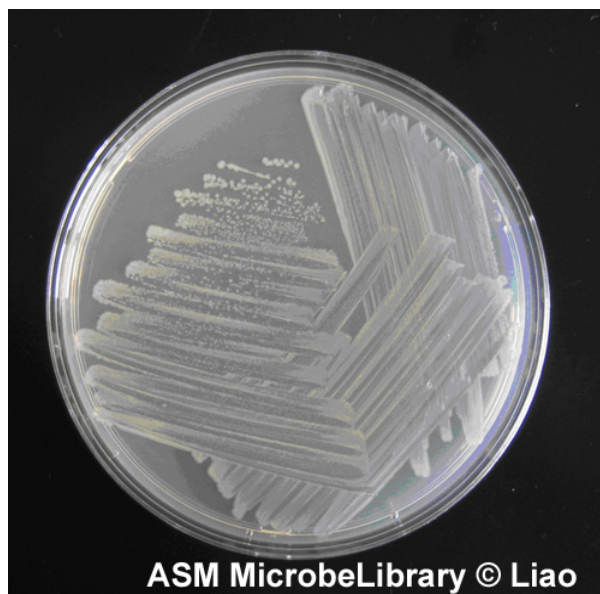


FIG. 4. This plate illustrates a streak plate which did not achieve isolation, and which would not be considered a good streak plate example. This photograph is by Dr. Min-Ken Liao, Furman University.

Purpose

The purpose of the streak plate is to obtain isolated colonies from an inoculum by creating areas of increasing dilution on a single plate. Isolated colonies represent a clone of cells, being derived from a single precursor cell. When culture media is inoculated using a single isolated colony, the resulting culture grows from that single clone. Historically, most microbiology research and microbial characterization has been done with pure cultures.

Theory

One bacterial cell will create a colony as it multiplies. The streak process is intended to create a region where the bacteria are so dilute that when each bacterium touches the surface of the agar, it is far enough away from other cells so that an isolated colony can develop. In this manner, spreading an inoculum with multiple organisms will result in isolation of the different organisms.

PROTOCOL

Mesophilic bacteria are generally streaked onto media solidified with 1.5% agar or agarose. Gelatin can be used if a high enough concentration of gelatin protein or a low enough incubation temperature is used. Thermophiles and hyperthermophiles can also be streaked onto growth media solidified with agar substitutes, such as Gelrite and guar gum.

One-hundred-mm-diameter petri dishes are the most commonly used size of plate for streaking. The agar surface of the plate should be dry without visible moisture such as condensation drops. Traditionally, inoculated petri dishes are incubated with the agar side up to prevent condensed moisture from falling onto the agar surface, which would ruin the isolation by allowing bacteria to move across the moist surface creating areas of confluent growth instead.

The inoculum for a streak plate could come from any type of source, for example clinical specimen, sedimented urine, environmental swab, broth, or solid culture. The two most common streak patterns are the three sector T streak and the four sector quadrant streak.

In a streak plate, dilution is achieved by first spreading the specimen over the agar surface of one sector. If a cotton swab or disposable loop

or needle was used to inoculate the first sector, it is now discarded into an appropriate container, while reusable loops, usually with nichrome or platinum wire (24 gauge), are flamed to incinerate any organisms on the loop. When cooled, the sterile loop is streaked through the initial sector and organisms are carried into the second sector where they are spread using a zig-zag movement. In a similar manner, the organisms present on the loop are incinerated after the second sector is streaked, and the third sector is streaked. For a four quadrant plate, the process is carried an extra step.

Detailed procedure for a Three Sector Streak, the T Streak:
Reference J. Lammert, Techniques in Microbiology, A Student Handbook (6)

Materials:

- Specimen to be streaked; this protocol is written for a test tube culture
- Transfer loop (usually nichrome, a nickel-chromium alloy, or platinum; it may also be a single-use disposable plastic loop, which would be discarded between sectors rather than resterilized)
- Bunsen burner
- Sterile petri dish with appropriate bacterial media, such as trypticase soy agar
- Labeling pen
- Sterile cotton swabs (if necessary to remove condensation from the agar surface and from around the inner rim of the petri dish)

A. Label a petri dish.

Petri dishes are labeled on the bottom rather than on the lid. In order to preserve area to observe the plate after it has incubated, write close to the edge of the bottom of the plate. Labels usually include the organism name, type of agar, date, and the plater's name or initials. Using sterile cotton swabs, remove any visible water on the agar in the plate or around the inner rim of the petri plate. Observe the plate and mentally divide it into three sectors, a "T." The area above the "T" will become the first sector streaked. The plate will then be turned clockwise (if you are right handed) with the agar side up. The second sector will then be at the top for streaking and then the plate is turned again so that the third sector can be streaked.

B. Sterilize the transfer loop before obtaining a specimen.

In order to streak a specimen from a culture tube, metal transfer loops are first flamed so that the entire wire is red-hot. The incineration and flaming process is described below in the Tips section. When flaming, the wire loop is held in the light blue area of a bunsen burner just above the tip of inner flame of the flame until it is red-hot. If a hot incinerator is available, the loop may be sterilized by holding it inside the incinerator for 5 to 7 seconds. Once sterile, the loop is allowed to cool by holding it still. Do not wave it around to cool it or blow on it. When manipulating bacteria, transfer loops are usually held like a pencil. If plastic disposable loops are being utilized, they are removed from the packaging to avoid contamination and after being used, are discarded into an appropriate container. A new loop is recommended for each sector of an isolation streak plate.

C. Open the culture and collect a sample of specimen using the sterile loop.

Isolation can be obtained from any of a variety of specimens. This protocol describes the use of a mixed broth culture, where the culture contains several different bacterial species or strains. The specimen streaked on a plate could come in a variety of forms, such as solid samples, liquid samples, and cotton or foam swabs. Material containing possibly infectious agents should be handled appropriately in the lab (see biosafety references below), only by students with appropriate levels of skill and expertise.

Remove the test tube cap. It is recommended that the cap be kept in your right hand (the hand holding the sterile loop). Curl the little finger of your right hand around the cap to hold it or hold it between the little finger and third finger from the back. See the illustration. Modern test tube caps extend over the top of the test tube, keeping the rim of the test tube sterile while the rim of the cap has not been exposed to the bacteria. The cap can also be placed on the disinfected table, if the test tube is held at an angle so that air contamination does not fall down into the tube and the test tube cap is set with the sterile rim on the table.

Insert the loop into the culture tube and remove a loopful of broth.

Replace the cap of the test tube and put it back into the test tube rack.

D. Streak the plate.

Inoculating the agar means that the lid will have to be opened. Minimize the amount of agar and the length of time the agar is exposed to the environment during the streak process.

1) Streak the first sector.

Raise the petri dish lid to insert the loop. Touch the loop to the agar area on the opposite side of the dish, the first sector. Bacteria on the loop will be transferred to the agar. Spread the bacteria in the first sector of the petri dish by moving the loop in a back and forth manner across the dish, a zig-zag motion. Make the loop movements close together and cover the entire first region. The loop should glide over the surface of the agar; take care not to dig into the agar.

2) Between sectors.

Remove the loop from the petri dish and obtain a sterile loop before continuing to the second sector. Either incinerate the material on the loop or obtain a sterile loop if using plastic disposable loops. The loop must be cool before streaking can continue. Metal loops can be touched to an uninoculated area of agar to test whether they are adequately cooled. If the loop is cool, there will be no sizzling or hissing and the agar will not be melted to form a brand. If a brand is formed, avoid that area when continuing with the streaking process.

3) Streak the second sector.

Open the petri dish and insert the loop. Touch the cooled loop to the first sector once, invisibly drawing a few of the bacteria from the first sector into the second sector. The second sector is streaked less heavily than the first sector, again using a zig-zag motion.

4) Obtain a sterile loop for the third sector (see 2, above).

5) Streak the third sector.

Open the petri dish and insert the loop. Touch the cooled loop (if the loop has been flamed) once into the second sector and draw bacteria from the second sector into the third sector. Streak the third sector with a zig-zag motion. This last sector has the widest gap between the rows of streaking, placing the bacteria a little further apart than in the

previous two sectors. Watch closely to avoid touching the first sector as the streak is completed.

6) Final step.

Flame the loop to incinerate any bacteria that are left on the loop. Allow the loop to cool before placing it near anything that is flammable. Invert the petri dish so that the agar side is up and incubate the plates.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the [ASM Curriculum Recommendations: Introductory Course in Microbiology](#) and the [Guidelines for Biosafety in Teaching Laboratories](#).

COMMENTS AND TIPS

A. Alternate streak patterns and different culture media

A variety of alternate streak patterns exist. Some are used for specific inocula, such as a urine specimen. The patterns also differ in the number of sectors as well as in the number of times the loop is sterilized. The four quadrant streak pattern would be recommended for use when large amounts of bacteria are expected in the inoculum. The extra sector will provide additional dilution and increase the probability of isolated colonies on the plate. The four quadrant streak plate is described in a variety of references, e.g., in Cappuccino and Sherman's *Microbiology, A Laboratory Manual*, 8th ed. (3).

Sometimes, cultures will be streaked on enrichment media or various selective and differential media. For instance, a culture which is expected to have a gram-negative pathogen will be streaked on a MacConkey agar plate, which inhibits the growth of gram-positive organisms.

B. Incinerating material on transfer loops—flaming

Reusable microbiological loops and needles are sterilized by flaming. A Bunsen burner is traditionally used for this process. Most microbiology manuals show the microbiologist positioned with his/her hand above the burner, with the loop placed into the flame. To avoid possible contact with the flame, the microbiologist might consider placing his/her hand

below the flame with the loop/needle above the hand in the flame. The flame of the Bunsen burner should be adjusted to blue, with the darker blue cone of cooler air visible in the center of the flame. The loop or needle should be placed into the hotter part of the flame and kept there until it glows red. There is a possible aerosolization hazard if the loop or needle contains liquid or a bacterial clump. These loops and needles should be placed into the heat slowly so that the moisture evaporates rather than sputters.

If an incinerator such as a Bacti-Cinerator is used to sterilize the loop, the loop is to remain inside the incinerator for 5 to 7 seconds. When warmed up (which will take 5 minutes), the temperature inside the incinerator is 815°C. The incinerator will take 5 to 10 minutes to warm up to working temperature.

C. Several techniques decontaminate transfer loops between sectors of a streak plate: flame, dig into agar, flame once and rotate loop

A variety of methods exist for removing organisms from the loop between sectors. Beginning students are generally taught to sterilize the loop between each sector by incinerating and then cooling the loop. Clinical microbiologists practice a variety of methods. Some flame once after the initial sector and then rotate the loop so that the next sectors can be streaked with an unused side of the loop. Other laboratorians (as clinical microbiologists name themselves) stab the loop several times into the agar to clear the loop between sectors.

D. Isolated colony appearances

Isolated colonies can be described using the traditional colony descriptions. The Colony Morphology Atlas-Protocol project provides information about bacterial colony appearance and characteristic photographs. The appearance of an organism can vary. For instance, a colony of an organism growing in a crowded sector of the plate will not grow as large as the identical organism growing in isolation. The media composition, pH, and moistness, as well as the length of time and temperature can all affect the organism's appearance. Colonies selected for subculturing should be colonies which are isolated, i.e., there is no other colony visibly touching the colony.

Agar with a surface layer of water is not suitable for obtaining isolated colonies. Obvious water drops should be removed from the surface of the plate and from the rim of the plate by using sterile cotton

swabs. Plates should be incubated agar side up, to avoid condensation that would drop onto the growing colonies on the agar surface.

E. Flaming tube mouths

Many protocols suggest flaming the tube mouth before and after removing organisms from a tube. Flaming was important when test tubes were capped with a cotton plug. Flaming would still be appropriate if a foam plug were being used. If a screw cap, KimKap, or similar test tube cap is used, the open end of the tube remains sterile since the cap normally covers that area.

F. Rehearsing the streak procedure

Some instructors have students practice the streaking procedure on a piece of paper. The process helps the student visualize the completed product and practice the fine muscle movements that are required in successful streaking for isolation.

Students may also find that they can visualize the pattern better if they mark the back of the petri dish (for instance, a T streak divide the plate into three sectors).

Before learning to streak, students should have had the opportunity to work with 1.5% agar media. Ideally they will have also previously had the opportunity to practice using a loop on a plate to determine the best angle of approach and the amount of force required to glide the loop over the surface of the agar without gouging the surface.

G. Holding the plate while streaking

If possible, adequate lighting should be available to help the microbiologist follow the tracings of the loop on the agar. For most labs, this means that the petri dish should be held in one's hand while being streaked in order to reflect the light properly. Additionally, the length of time the petri dish lid is removed should be minimized in order to limit contamination. There are several ways to hold the petri dish. Beginning students may find that they obtain the best results leaving the plate on the lab bench and lifting the lid to work. Other students may find that they can place the plate upside down on the workbench and lift the agar containing bottom, hold it to streak and then quickly replace it into the lid. Yet other students may have the manual dexterity to manipulate the entire dish in their hand, raising the lid with thumb and two fingers while balancing the plate in the rest of their hand.

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