Culture Transfer Instruments, Techniques, Isolation and Maintenance of Pure Cultures

Culture Transfer Instruments

A ***pipette*** is an instrument often used to transfer aliquots of culture, to prepare serial dilutions of microorganisms, and to dispense chemical reagents. Contamination problems are avoided by storing sterile pipettes in a pipette can. Clean, sterile pipettes are placed tip first in the pipette can be autoclaved and dried by setting the autoclave on the "fast exhaust and dry" cycle. Pipettes can also be sterilized in a dry- heat oven. After a pipette has been used, it should immediately be placed tip down in a container of a disinfectant such as 3 to 5% Lysol and completely immersed.

Inoculating needles *and* ***loops*** are used to aseptically transfer microorganisms from broth, slant, or agar cultures to other media. Both may consist of handles, a shaft, and a turret, which holds a nickel chromium or platinum wire. Before using either, the end of the wire must be sterilized by passing it slowly through the tip of the flame from a Bunsen burner or into a Bacti–Cinerator (figure 2). When done correctly, all parts of the wire will turn red with heat. The needle or loop should then be used before it becomes contaminated. After you have finished using an inoculating loop or needle, it should be thoroughly flame-sterilized.

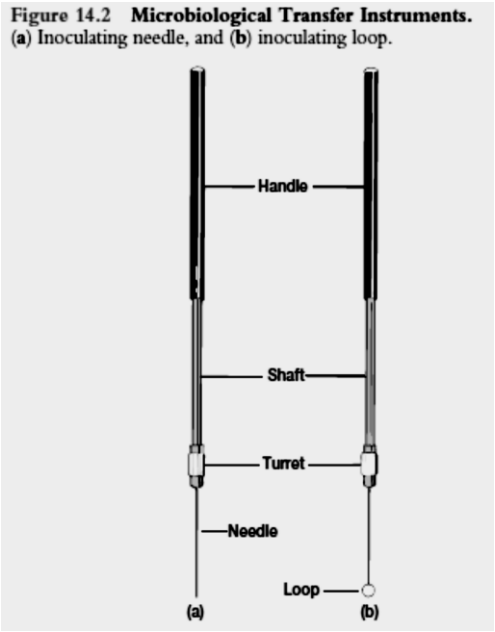
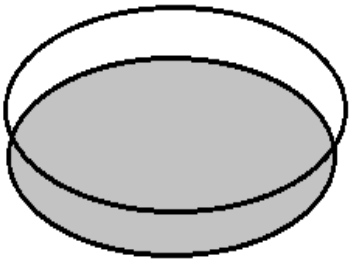


Figure 14.2 Microbiological Transfer Instruments.
(**a**) Inoculating needle, and (**b**) inoculating loop.

a Bacti–Cinerator (figure 2)

In1887 Julius Petri, a German bacteriologist, adapts two plates to form a container for holding media and culturing microbes.



Culture

To cultivate, or **culture,** microorganisms, one bring in a little sample (the inoculum) into a container of nutrient medium**,** which provides a suitable growth environment this process is called ***inoculation*.** The visible growth that later appears in or on the medium is known as a ***culture***. The samples being cultured are different depend on the purpose of the analysis. Clinical specimens for determining the cause of an infectious disease are obtained from body fluids (blood, cerebrospinal fluid), discharges (sputum, urine, feces), or tissue. In addition to nearly any natural material such as; soil, water, sewage, foods, air.

Note*: prepare media and store in refrigerator and use it in the time of need and be careful to avoid contamination*.

Principal Culture techniques

1. Prepare the bench by disinfecting its surface with the available disinfectant in the laboratory.
2. Label the bottom surface of a sterile Petri plate or the slant with your name and date. Use a china marking pen.
3. flame the inoculating loop or needle over a Bunsen burner until the wire becomes red-hot. Cool the hot loop in the broth culture until it stops "hissing."
4. Pass the sterile inoculating loop in suspected sample. Inoculate the Petri plate or slant by **streaking** as in next figure (**3**) by loop or needle .or **stabbing** the agar deep tube.
5. If you used the test tube (slant) ,you should flame the neck of the tube and then capped but not closed strictly ??.if you used Petri plate ,you should cover the plate directly after streaking
6. Incubate the slant or Petri plate in the incubator at 37 C° for 24-84 hours .the Petri plates incubated in inverted position.

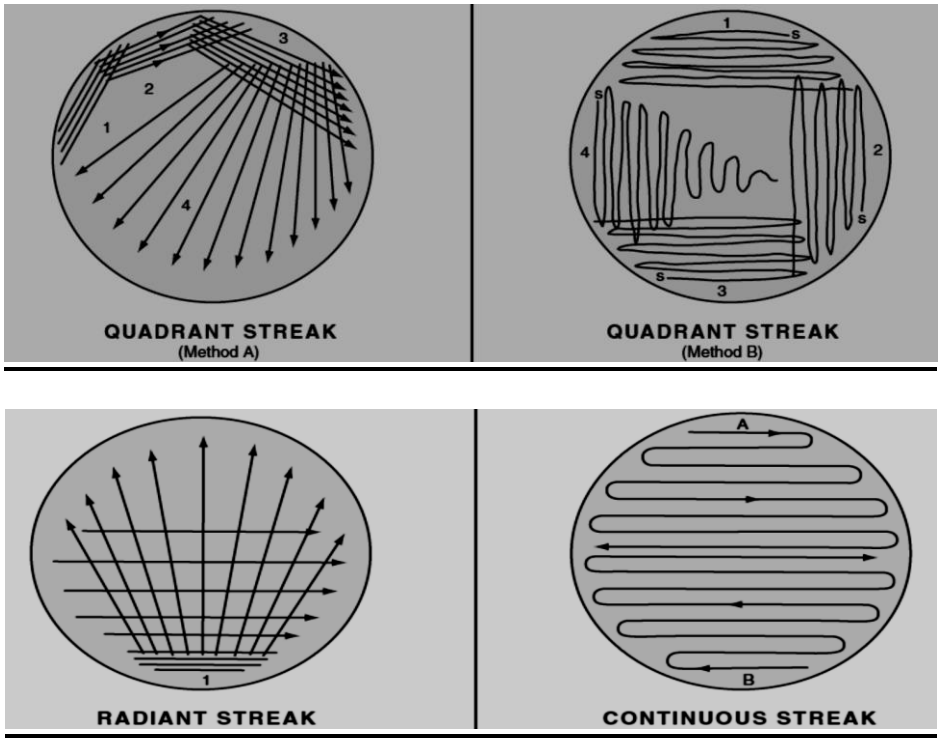
Culturing methods:

1. *Streaking*
2. *Stabbing*
3. *Spread plate technique.*

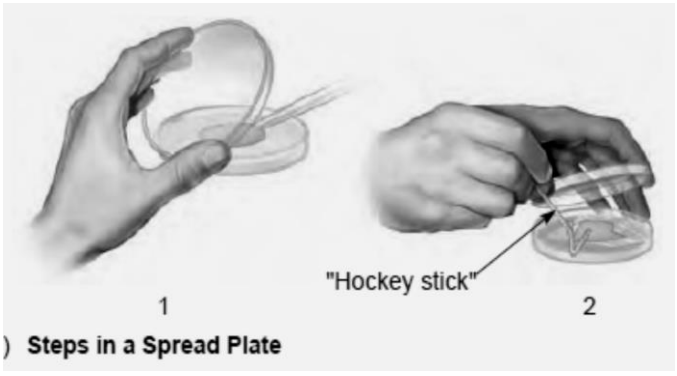
Note:

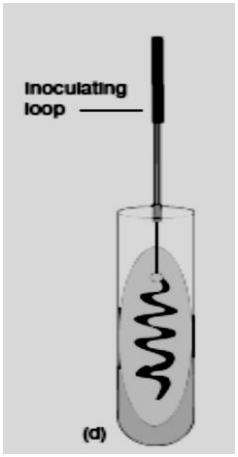
Incubation the plate in an inverted position because Failure to cool agar media prior to pouring into the plate will result in condensation of moisture on the cover. Any moisture on the cover is undesirable because if it drops down on the colonies, the organisms of one colony can spread to other colonies, failing isolation technique.

streaking methods (figure 3)

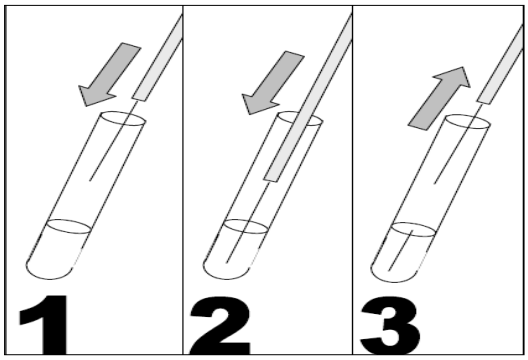


Spread plate technique, a small volume of liquid, a diluted sample is pipetted onto the surface of the medium and spread around evenly by a sterile spreading tool (sometimes called a "hockey stick").





streaking slant



stabbing method

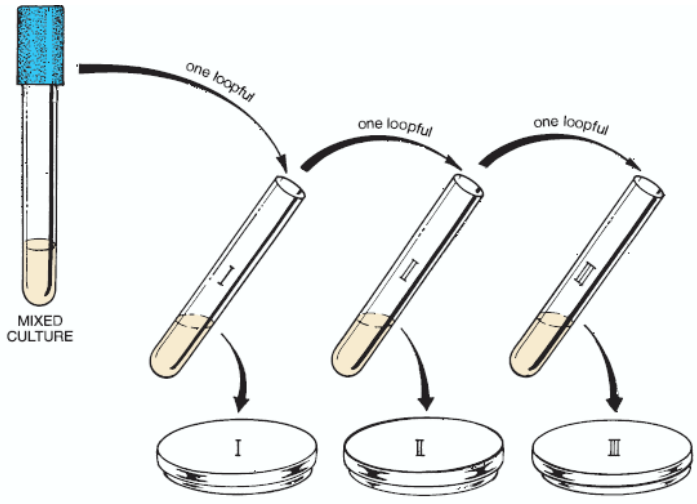
Isolation of pure culture

when we try to culture samples, we soon discover that bacteria is mixed and called a **mixed culture (**is a container that holds two or more easily differentiated species of microorganisms), and we need to isolate one species .**isolation** mean that a single bacterial cell is separated from other cells and provided enough space on a nutrient surface, it will grow into a discrete colony return to single known species or type of microorganism and called **pure culture**. A standard method for preparing a pure culture is to use a **subculture** technique to make a second-level cultures, selected colony may be picked up with an *inoculating needle* and transferred to separate culture tubes, Where possible, bacteria from the center of a colony are transferred, *because the center is less likely to be contaminated than the edges represents the growth of a single species of microorganism and is called a* ***pure or stock culture***.

Microorganisms are transferred from one culture medium to another by subculturing, using specific procedures and aseptic technique. (Asepsis means free from sepsis [a toxic condition resulting from the presence of microorganisms.]. Since microorganisms are always present in the laboratory, if aseptic technique is not followed, there is a good possibility that external Contamination will result and will interfere with the results. Proper aseptic technique also protects the laboratory worker from contamination with the culture.

Pure culture methods:

1. **Streak plates method**. Streaking method achieved as in figure in page 2.
2. **Pour Plate Method**. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies upon plating .One advantage of this method is that it requires somewhat less skill than that required for a good streak plate; a disadvantage, it requires more media, tubes, and plates.



Pure culture maintenance

One of the more important problems in a microbiology laboratory is the maintenance of pure stock cultures over a long period. Ideally, one should employ a technique that minimizes the need for subculturing the microorganism. This is achieved by storing the microorganism in a state of dormancy either by refrigeration or desiccation.

- **Short periods maintenance** (generally between one to three months) of aerobic bacteria can often be achieved by storing slant cultures in the refrigerator at °4 to 10°C. The use of screw-cap tubes for these slants will minimize desiccation during storage.
- **long periods maintenance** is achieved by sealing them with sterile mineral oil in order to prevent moisture loss. The white mineral oil used can be sterilized by heating at 110°C for 1 hour in a drying oven. After an agar slant culture has grown, the slant surface is aseptically covered with the sterile oil about 1/4 inch above the top of the slant then stored at the normal storage temperature.

  In many cases, long periods maintenance of cultures does not even require mineral oil. *E. coli* and many other members of the family *Enterobacteriaceae*، *Pseudomonas aeruginosa*, *staphylococci*, can often be successfully stored for years at room temperature with the following procedure. Stab inoculates screw-cap deeps containing nutrient agar or 0.7% agar in distilled water. Incubate overnight at optimal temperature. Finally, screw down the caps tightly and seal the tubes with tape or paraffin.Store the cultures in a safe place at room temperature.
- **lyophilization (freeze-drying).** This eliminates the need for periodic transfers and reduces the chance of mutations occurring in the stock culture. In lyophilization, the bacterial culture is suspended in a sterile solution of some protective medium such as milk, serum, or 3% lactose. Small amounts of the thick suspension are transferred to vials and then quickly frozen in a dry-ice/alcohol mixture and dried under vacuum while still frozen, and the vial sealed. These sealed, desiccated cultures may often be stored for years.