# COMPONENTS

Culture media are formulated to create appropriate environments for specific microorganisms. Some common media constituents include:

## Amino-Nitrogen

Peptones, hydrolysates, infusions, and extracts are the main sources of nitrogen in culture media, and are essential for microorganism reproduction and metabolism.

## Growth Factors

Sheep, horse and rabbit blood, serum, and vitamins are added to support or enhance microorganism growth.

## Energy Source

Carbohydrates and alcohols are added as carbon and energy sources, which stimulate growth of microorganisms. Carbohydrates also are used to aid in microorganism identification and differentiation.

## Buffering Agents

Phosphates, acetates, and citrates maintain the pH in culture media.

## Minerals

Phosphate, sulfate, magnesium, calcium, manganese, and iron salts provide trace elements needed for organism growth.

## Selective Agents

Antimicrobials, dyes, and bile salts are used to restrict the growth of certain organisms, while permitting the growth of others.

## Indicator Dyes

Dyes, such as phenol red and bromthymol blue, are used in the preparation of differential and selective culture media.

## Gelling Agents

Agar and gelatin are added to a liquid medium to change the consistency to a solid or semisolid medium.

# INOCULATION

## Streak Method for Agar Plates

The streak plate primarily is used for isolating microorganisms in pure cultures from specimens or samples containing mixed flora. Obtaining isolated colonies on plates allows colonial morphology and hemolytic reactions to be examined, and biochemical/serological testing to be performed.

1. With a sterile inoculating loop, streak a loopful of the sample across the surface of an agar plate. The four-quadrant streak is the most common, and accomplished by streaking and rotating the plate in four sections, one quarter at time, slightly overlapping the original streak area. The fourth quadrant contains the greatest dilution of microorganisms, and usually provides isolated colonies for further testing.
2. Incubate plates under favorable growth conditions.
3. Examine plates for isolated colonies.

## Spread Plate Technique

The spread plate technique is used for enumerating microorganisms.

1. Drop 0.1 mL aliquots from serial dilutions onto the surface of an agar plate.
2. Aseptically spread inoculum across the surface using a bent glass rod or sterile inoculating loop. By spreading the suspension over the plate, a dilution gradient is established to provide isolated colonies.
3. Incubate plates agar inverted in appropriate conditions.
4. Count colonies and calculate the number of microorganisms in the original suspension.

## Pour Plate Technique

The pour plate technique also is used for enumeration of microorganisms in a particular sample. In this technique, test samples or suspensions of microorganisms are mixed with molten agar (45–50°C). The agar is allowed to solidify, trapping the bacteria at separate discrete positions within the matrix of the medium. Although the medium holds bacteria in place, it is soft enough to permit growth of bacteria and the formation of discrete isolated colonies.

1. Perform serial dilution of sample.
2. Aseptically pipette microorganism dilutions into labeled petri dishes.
3. Add melted agar that has been cooled to approximately 44–45°C.
4. Mix well by slightly rotating plate with bacteria and agar mixture.
5. Allow the agar to solidify, trapping bacteria at separate discrete positions within the medium.
6. Incubate plates in a favorable environment.
7. Count the number of colonies and calculate the number of microorganisms in the original sample.

## Streak/Stab Method for Agar Tubes

Tubed media may be in the form of solid agar slants, semisolids, or broths. Depending on the type of medium used and the purpose of the inoculation, use an inoculating loop or needle.

1. For agar slants, place the loop at the base of the tube surface and draw it up the agar surface while moving it from side to side.
2. For semisolid media, insert the loop into the medium to approximately one-fourth of its depth. If testing motility, use an inoculating needle and stab it in the center of the agar tube to the bottom. Carefully draw the needle out, keeping it straight.

## Inoculation of Broth Media

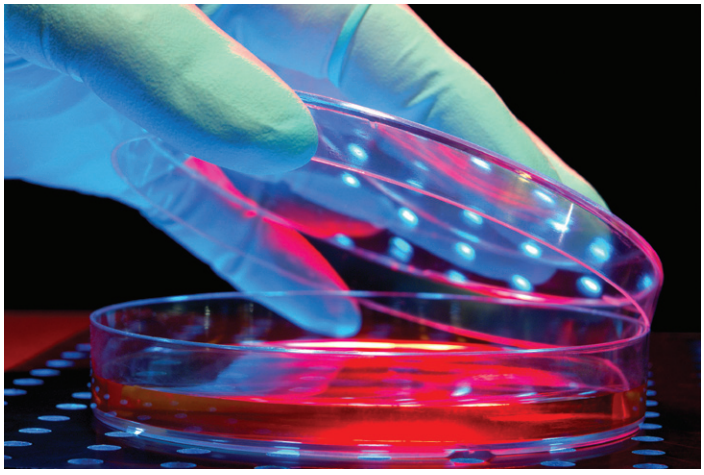
Broth media generally are used for enrichments, general cultivation and sterility testing.

1. Aseptically inoculate the appropriate broth media with the sample or specimen using a sterile pipette, syringes or forceps.
2. Incubate inoculated broth at the appropriate atmospheric conditions, temperature, and time.
3. Examine broth for any signs of growth including turbidity with or without gas bubbles, "puff-ball" appearance, hemolysis (in blood cultures), pellicle formation, and precipitate on the bottom of the tube or bottle.

## Membrane Filtration Method

The membrane filtration method is used to test large volume of liquid samples, including water and filterable beverages.

1. Pass the sample through a sterile membrane filter enclosed in a filtration assembly and attached to a vacuum source.
2. After filtering the sample, carefully remove the filter with sterile forceps and apply it to the surface of an agar plate or pad saturated with a broth medium. Avoid trapping air bubbles by using a rolling action. (The media used depends on the type of microorganism being tested.)
3. Invert plates and incubate under appropriate conditions.
4. Count colonies and calculate the most probable number.



## TROUBLESHOOTING GUIDE

| | Deteriorated medium | Improperly washed glassware | Impure water | Overheating | Repeated remelting | Incorrect dilution | Incomplete mixing/ Incorrect weighing |
|---|---|---|---|---|---|---|---|
| Incorrect pH range | X | X | X | X | X | | X |
| Nontypical precipitate | X | X | X | X | | | X |
| Abnormal medium color | X | X | X | X | | | X |
| Incomplete solubility | | | | | | | X |
| Carmelization or darkening | X | | | X | X | | X |
| Toxicity | | X | X | | | | |
| Trace substances | | X | X | | | | |
| Medium will not solidify | | | | X | | X | X |
| Loss of nutrients/properties | X | | X | X | X | X | X |

Improper sterilization, poor technique in adding enrichments and pouring plates, and improper boiling of medium are also sources of contamination.