

LAB 05: Cultivation of Bacteria

Culture Media, Inoculation, and Isolation

Pre-Read: Tortora textbook:

- Culture Media (pg. 157-162)
- Obtaining Pure Cultures (pg. 162-163)

Supplemental Material: Video Clips & Virtual Lab

Primary Learning Objectives: Upon successful completion of this laboratory, students should be able to:

1. Understand the importance of proper specimen collection and transport to the laboratory.
2. Explain the five principle steps used by microbiologists to process specimens.
3. Differentiate between the various types of culture media.
4. Explain purpose of streak-plate culture technique (aka **streaking for isolation**).
5. Define the following terms: selective media, differential media, reducing media.

INTRODUCTION

The ability to isolate pathogens from a clinical specimen submitted for culture is only as good as the collection technique and timely transport of the specimen to the lab. Body fluids should be collected using aseptic technique and submitted in sterile containers (Figure 5.1).



Figure 5.1 Example of Sterile Collection Containers: Cups, Vials, and Syringes

Swabs collected from body areas may also be submitted for culture (Figure 5.2). However, some microbes are sensitive to desiccation (drying) and exposure to oxygen (anaerobes) and require a special transport medium to preserve the specimen's integrity prior to being processed for microbial culture.



Figure 5.2 Cultiplate Transport System

Once a specimen is received in the laboratory for microbial culture, microbiologists employ the five principle “I” steps to process it:

1. *Inoculation* – placing the specimen on an artificial culture medium to initiate growth.
2. *Isolation* – spatially separating microbial growth into distinct colonies.
3. *Incubation* – placing the culture medium in environmental conditions to facilitate growth.
4. *Inspection* – examining culture for presence of microbial growth.
5. *Identification* – performing specific tests to ID any pathogenic microbe(s) isolated.

These five steps will be described in detail after first discussing different types of culture media.

Selective, Differential, and Reducing Culture Media

Many different types of media are available for growing bacteria. You are already familiar with **general purpose media** (NA, NAYE, TSA; see Lab 01) that are rich with nutrients that allow the growth of many types of organisms. Other kinds of media contain chemicals that cause differences in bacterial growth.

To promote the growth of **fastidious** microbes (those that have very specific nutritional requirements), general purpose media may be supplemented with other nutrient sources to create what is called **enriched general purpose media**. For example, 5% sheep blood may be incorporated in growth media to help the organisms grow. For example, blood agar plates (BAP) and chocolate agar plates (CAP).

Selective media contain chemicals that inhibit or halt the growth of some organisms. Dyes, bile salts, alcohols, acids, or antibiotics may be added to either inhibit or halt the growth of some species. When using these media, results are evaluated on the basis of whether or not an organism grows on that medium. Some organisms have enzyme systems that allow them to deal with the selective agents. Thus, selective media are useful for: (1) isolating a particular group of organisms from a mixed culture and/or (2) helping to characterize a pure culture. You must keep in mind two things when using selective media: First, a selective medium isn't necessarily perfectly selective—if given enough time, even organisms that are inhibited may reproduce enough to form visible colonies. Second, even those organisms that are supposed to grow may form smaller colonies or may grow more slowly than they would on a general purpose medium. There are many kinds of selective media.

Differential media contain chemicals that produce visible differences among different groups of organisms when grown on/in that medium. These differences are unrelated to how well the organisms grow on the medium; all organisms will grow well on differential media. Differential culture media allow dissimilar bacteria to be visually distinguished from each other.

Visible differences may take the form of differences in colony color, morphology, or changes in the appearance of the culture medium itself. Some differential media incorporate indicator dyes and result in a change of coloration of bacterial colonies (on solid media) or a change in the coloration of a broth medium based on bacterial metabolic characteristics. There are many kinds of differential media. They are useful for helping to characterize and identify bacterial species.

Lastly, **reducing media** promote the growth of anaerobic bacteria, because low or no oxygen conditions are required to culture these organisms. They are tricky to prepare because of the difficulty of removing oxygen, a ubiquitous gas, from the media.

It is important to note that a culture medium can be classified into more than one category. For example, MacConkey agar is both selective and differential (see Figure 5.4), and blood agar is both an enriched general purpose medium and a differential medium (see Figure 5.5).

IMPORTANT NOTE: some of the culture media discussed below (e.g. MacConkey) can be used to test bacteria for the ability to ferment a sugar(s). Fermentation is a metabolic process in which microbes are able to convert sugars into end products that include acids, alcohols and/or gases.

Figure 5.4 MacConkey Agar selectively allows = Gram-negative organisms to grow while inhibiting the growth of Gram-positive organisms, and differentially distinguishes lactose fermenters (pink) from non-lactose fermenters (not pink).



Figure 5.5 Blood Agar Plate and the differential hemolytic patterns of *Streptococcus* spp.

Alpha (α): Partial hemolysis
Beta (β): Complete hemolysis
Gamma (γ): No hemolysis



In summary, a variety of agar media may be inoculated to identify and isolate bacteria. As a general rule, if a bacterium grows on selective media, it will also grow on non-selective media.

Agar Type	Comments
Tryptic Soy Agar (TSA) Nutrient Agar (NA) Nutrient Agar Yeast Extract (NAYE)	General purpose media: Promotes growth of most organisms (Gram-positive & Gram-negative bacteria) that are non-fastidious
TSA with 5% Sheep Blood, aka Blood Agar Plate (BAP) Chocolate Agar Plate (CAP)	Enriched general purpose medium: Promotes the growth of organisms that grow on TSA & NA plus <i>Streptococcus species</i> Enriched general purpose medium: Promotes the growth of organisms that grow on BAP plus fastidious bacteria such as <i>Haemophilus species</i> and <i>Neisseria gonorrhoeae</i>
MacConkey (MAC) Eosin Methylene Blue (EMB) Hektoen Agar (HE)	Selective media used to isolate Gram-negative bacilli; MAC, EMB and HE also differential on basis of lactose fermentation ability.
Phenyl Ethyl Alcohol (PEA)	Selective medium used to isolate Gram-positive bacteria
Mannitol Salt (MSA)	Selective medium used to distinguish <i>Staphylococcus aureus</i> from other <i>Staphylococcus species</i> . Also differential on basis of mannitol fermentation ability.

Now we can get back to explaining the five principle “I” steps that microbiologists use to process a specimen: **Inoculation – Isolation – Incubation – Inspection – Identification.**

1. Inoculation (also see Lab 01): It is essential that the microbiologists use proper aseptic techniques when inoculating appropriate culture media that facilitates bacterial growth. This reduces accidental contamination and isolation of unwanted environmental microbes, which are **ubiquitous**. Figure 5.6 demonstrates a petri dish containing an agar medium that was exposed to air for only a short period of time and allowed to grow overnight.



Figure 5.6 Culture of Environmental Microbes from Air.

2. Isolation: In the 1850s, Louis Pasteur developed the concept of the **pure culture**. This was an important intellectual step forward because the ability to work with pure cultures allowed Pasteur (and subsequent microbiologists) to study individual bacterial species. Pasteur's method for developing pure cultures—repeatedly diluting broth cultures until he thought he had individual species—was very inefficient.

In the 1880s, Robert Koch developed the **streak-plate method** of inoculating an agar plate (aka **streaking for isolation**). The main goal of this procedure was to obtain isolated colonies on a solid medium. Remember that a colony is a population of millions of cells that are identical and are descended from a single parent cell by binary fission. An **isolated colony** is one that is not touching any other colonies on an agar plate. Koch picked cells from a single isolated colony to inoculate another sterile medium to produce a pure culture. This process presented a powerful new way to develop pure cultures. Streaking for isolation is now a standard procedure in all microbiology labs.

Clinically, specimens submitted for culture may contain more than one type of bacteria. In order to separate bacterial species on agar plates and obtain isolated colonies, microbiologists use the streak-plate culture technique.

In the initial inoculation when using the streak-plate method, many bacterial cells may be deposited on the plate, resulting in confluent growth. However, spatial separation (dilution) of bacterial cells is achieved by streaking adjacent areas (quadrants) of the agar. Sterilizing the inoculation loop between streak quadrants facilitates the separation of bacterial cells. Theoretically, the last (or sometimes earlier) quadrant should have individual bacterial cells that form **isolated colonies** after incubation. See Figure 5.7.

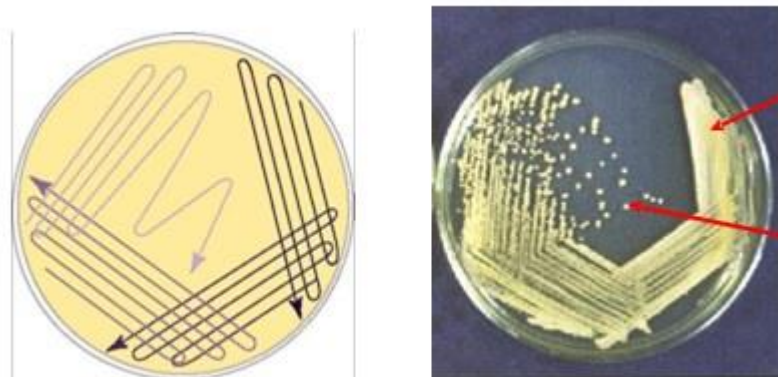


Figure 5.7 Left: The pattern of streaking used in the streak-plate technique. Right: Streak-plate after incubation; upper arrow = confluent growth, lower arrow = isolated colony.

3. Incubation: Recall from Lab 01 that while culture media contain the chemical nutrients necessary for *in vitro* growth, microbiologists must additionally provide the appropriate physical

environment to cultivate microbes. Physical factors that need to be considered when incubating inoculated culture media include O₂ and CO₂ availability, temperature, humidity, and time. Obligate anaerobes must be incubated under conditions devoid of free oxygen, which may be accomplished by placing reducing media agar plates in an anaerobic jar system. Humidity is easily provided by placing a container of water in the incubator.



Figure 5.8. Left: Constant temperature incubator. Right: an anaerobic jar system used for excluding oxygen from cultures during incubation.

4. Inspection: There are obvious visible colony morphological variations between different species of bacteria. Microbiologists can describe the appearance of the colonies by a variety of descriptors, i.e.: size, shape, margin, elevation, etc. Refer to Lab 01 for a review of the appropriate terms.

5. Identification: Once relevant microbes are isolated in pure culture further studies are required such as staining (Laboratory 4), antibiotic susceptibility testing on pathogenic organisms (Laboratory 6), and biochemical testing (Laboratories 7-9).

In summary, every specimen received in a clinical microbiology laboratory for culture has a defined processing protocol that includes:

- Assessment of the quality of specimen submitted
- Type of culture media that needs to be inoculated
- Prescribed incubation environment
- Recommended length of incubation
- Appropriate biochemical tests to be performed for identification

Noteworthy are the limitations of culturing microbes *in vitro*. Viruses and some bacteria that are obligate intracellular parasites (e.g. *Mycobacterium leprae*, *Rickettsia spp.*, and *Chlamydia spp.*) along with spirochetes (e.g. *Treponema pallidum*) cannot be grown on artificial media and require other methods for clinical diagnosis.

Overview of Terms

Differential media – contain chemicals that produce visible differences among different groups of organisms when grown on/in that medium.

Enriched (enrichment) media – growth media that have been supplemented with specific nutrient sources to facilitate the growth of fastidious microbes.

Fastidious microbes – have very specific nutrient requirements that may not be provided for by using a general growth medium.

Fermentation – a metabolic process performed by certain microbes in which they are able to convert sugars into end products that can include acids, alcohols and/or gases.

Isolated colony – a colony that is not touching any other colonies on an agar plate.

Reducing media – type of media used to cultivate obligate (strict) anaerobes.

Selective media – type of media used to inhibit or halt the growth of certain bacteria.

Spread-plate – a technique used by microbiologists to obtain confluent growth (a lawn of continuous growth).

Streak-plate method (aka streaking for isolation) – a technique used by microbiologists to isolate bacterial colonies; useful for separating a mixture of species in an original sample into individual colonies; each colony type can then be used to generate pure cultures.

Next – please complete the Laboratory Exercise Worksheet (found in a separate file)

References

Barnett, M. (1997). *Microbiology: Laboratory exercises* (2nd ed). McGraw-Hill: Boston, MA.

Microbe Online. *Bacterial culture media: classification, types and uses*. Retrieved June 2017. <http://microbeonline.com/types-of-bacteriological-culture-medium/>

Microbe World. *Culture media*. Retrieved June 2017. <http://www.microbeworld.org/careers/tools-of-the-trade/culture-equipment/culture-media>

Owlcation. *Types of culture media*. Retrieved June 2017. <https://owlcation.com/stem/Types-Of-Culture-Media>

Pearson Education. (2006). *Colonial morphology*. Retrieved September 2009. http://www2.bc.cc.ca.us/bio16/images/06-07a_bacprop_1.jpg

Robertson, Leslie. Bacterial cultivation (culturing). Retrieved June 2017. <http://slideplayer.com/slide/10384388/>

Environmental microbiology laboratory: Pure culture techniques. Retrieved September 2009.
<http://www.personal.psu.edu/faculty/k/h/khb4/enve301/301labs/lab4pureculture.html>

Tortora, G. (2016). *Microbiology: An introduction* (12th ed.). Pearson: San Francisco, CA.

Todar's Online Textbook of bacteriology. Retrieved September 2009. <http://www.textbookofbacteriology.net>

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