

NRM Nutritional Requirements and Media

Learning Objectives

The student will

- Use aseptic techniques in the safe inoculation of various forms of media.
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques, instruments, microbial growth, media types and forms when making observations.
- Correctly perform various inoculation techniques.

Background/Theory

Organisms can be identified according to the source of carbon they use for metabolism as well as their energy source. The prefixes auto- (“self”) and hetero- (“other”) refer to the origins of the carbon sources various organisms can use. Organisms that convert inorganic carbon dioxide (CO₂) into organic carbon compounds are **autotrophs**. Plants and cyanobacteria are well-known examples of autotrophs. Conversely, **heterotrophs** rely on more complex organic carbon compounds as nutrients; these are provided to them initially by autotrophs. Many organisms, ranging from humans to many prokaryotes, including the well-studied *Escherichia coli*, are heterotrophic. (OpenStax CNX, 2018)

This exercise investigates the nutritional requirements of some heterotrophic bacteria. Heterotrophs need an organic source of carbon for ATP generation and for anabolic (growth) activities. Carbohydrates, proteins and fats are all organic compounds. In addition, nitrogen is needed for building amino acids which form proteins. Nitrogen can come from organic sources (proteins, for example) or inorganic sources.

Some heterotrophs can manufacture many complex molecules from inorganic and small organic molecules. Others rely on many large, more complex molecules being available in the environment. The more ready-made, complex organic compounds an organism requires of the environment, the more **fastidious** it is.

In this course you will encounter many types of growth media. Most of the types, TSA/TSB (Tryptic Soy Agar/Broth) and NA/NB (Nutrient Agar/Broth) for example, are **all purpose or general media**. They contain a wide variety of complex carbon, nitrogen and sugar compounds. You will often see terms like beef extract, peptone, tryptone, soytone in the list of ingredients. These components are slurries of animal and plant tissue that have been partially hydrolyzed (broken down) so that the components are more readily available. These media contain many different complex molecules, however the exact amount and types of each is unknown. These media will support a wide range of **nonfastidious** microbes with differing nutritional requirements.

Some media are **enriched** referring to extra ingredients, such as sheep blood, yeast extract or animal digests, which contain additional nutrition to support the growth of **fastidious** organisms. Because we do not know the exact composition of these plant and animal extracts, both enriched media and all-purpose media are considered **complex** or **undefined**.

In contrast, a **minimal medium** contains specific compounds providing simple forms of carbon, nitrogen and sugar. This type of medium is termed **chemically defined** because the exact chemical composition is known. If an organism is to grow in this medium, it must be able to synthesize all the complex macromolecules it needs. For example, it will need to have the enzyme systems that can build all the necessary amino acids from scratch. You know a medium is chemically defined if there is a specific chemical formula for each and every ingredient. (The exception to this rule is agar. Agar is a

solidifying agent and plays no role in the nutrition of the organism. You can ignore it when determining whether a medium is chemically defined or not.)

In this exercise, you will observe how microbes grow in three types of media, minimal, all-purpose and enriched. Note the recipes for each medium. Can you classify each?

Brain Heart Infusion Broth (BHI)

Brain heart infusion from (solids) 6g/L
Peptic digest of Animal Tissue 6g/L
Pancreatic digest of Gelatin 14.5g/L
Dextrose 3g/L
Sodium Chloride 5g/L
Disodium Phosphate 2.5g/L
Distilled water to bring volume to 1.0 L

Glucose Salts Broth (GSB)

Glucose 5.0 g/L
Sodium chloride 5.0 g/L
Magnesium sulfate 0.2 g/L
Ammonium dihydrogen phosphate 1.0 g/L
Dipotassium phosphate 1.0 g/L
Distilled water to bring volume to 1.0 L

Tryptic Soy Broth (TSB)

Tryptone 17g/L
Soytone 3g/L
Sodium Chloride 5g/L
Dipotassium Phosphate 2.5g/L
Distilled water to bring volume to 1.0 L

Experiment/Exercise

Materials per student pair

4 tubes BHI broth (label as you take from the media cart, looks like TSB)
4 tubes Glucose Salts Broth
4 tubes TSB (label as you collect from the media cart, looks like BHI)

Cultures

Fresh overnight broth cultures of similar turbidity
E. coli
Kokuria rosea
Staphylococcus epidermidis
Lactococcus lactus

Procedure Lab 1

1. Label each of the BHI tubes with your name, section, date, organism name (different for each tube) and medium type, BHI. (NOTE: BHI and TSB look exactly alike. Make sure you label these as you collect them from the media cart.)
2. Label the glucose salts broth tubes and the TSB tubes the same way.
3. Gently flick each parent culture to resuspend the cells as demonstrated on the Basic Aseptic Transfers video.

4. Aseptically inoculate each tube with one loopfull of the corresponding organism.
 - Make sure that the thin film of broth across the loop does not break. You want the amount transferred to each tube to be as uniform as possible.
 - Completely incinerate your loop between each inoculation. One never returns a loop to a parent culture for a second inoculation unless the loop has been resterilized, even though you may be going back into the same culture. I call this “double dipping” and it is not allowed.
 - You have four organisms and three types of media. This means you will have a total of 12 tubes for this exercise. You should make 6 of these inoculations and your partner should make 6 inoculations.
5. A set of uninoculated tubes will be incubated with the cultures for the class.
6. Place the tubes in the rack for your section to be incubated. They will be incubated for 24 hours at 37°C.

Procedure Lab 2

1. Place your culture tubes in a test tube rack and carry them back to your work area.
2. Gently flick each tube to resuspend any cells that have fallen to the bottom of the tube.
3. To determine the relative amount of growth (based on turbidity) in each broth, place all the tubes together in a rack. Arrange them in order from most turbid to least. Then assign each a number from 0 to 5 (5 represents the greatest amount of growth). Record each number in the corresponding row and column of the data table. Be sure to write in the full scientific name of each organism.
4. Observe the uninoculated tubes and assign a number representing the relative amount of growth as well.
5. After you make observations, dispose of your culture tubes on the disposal cart. Return the uninoculated tubes to the demonstration area so that other students can observe them.

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Lab Report: Nutritional Requirements and Media

Name _____

Lab Section _____

Data and Observations

Relative growth (0=no growth; 5= abundant growth)

Organism	BHI	TSB	GSB
Uninoculated			

Post Lab Questions

1. Classify each medium as all purpose, enriched or minimal and briefly describe your reason for classifying it that way. Classify each medium as defined or undefined and give your reasoning.

Medium	All purpose, enriched, minimal (reason)	Chemically defined or undefined? (reason)
Brain-Heart Infusion		
TSB		
Glucose salts		

2. What is the purpose of the uninoculated broth of each medium? What results are you expecting? If you observe growth in the uninoculated broths, how are your results impacted?

3. Based on your results, list the organisms in order from MOST fastidious to LEAST fastidious.

4. From the lists of media ingredients of each medium, list a source(s) of carbon and a source of nitrogen. For those that have multiple sources, list at least two.

Medium	Carbon source(s)	Nitrogen source(s)
BHI		
GS		
TSB		

5. To support heterotrophic life, must all media contain a carbon source and a nitrogen source?

6. *Haemophilus influenzae* must be grown on chocolate agar, which is blood agar treated with heat to release growth factors in the medium. *H. influenzae* is described as _____.

- a. an acidophile
- b. a thermophile
- c. an obligate anaerobe
- d. fastidious

(OpenStax CNX, 2018)

References

OpenStax CNX. (2018, Mar 19). OpenStax Microbiology <http://cnx.org/contents/e42bd376-624b-4c0f-972f-e0c57998e765@4.24>. Retrieved from <http://cnx.org/contents/e42bd376-624b-4c0f-972f-e0c57998e765@4.24>

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