**Microbiological Medium**

# Background on media:

Since bacteria were first discovered and studied, there has been a push to develop ways to cultivate the organisms in the laboratory. Microorganisms, like all organisms, have requirements for their growth and maintenance. It was therefore the chore of the early microbiologists to discover what those requirements were and to develop suitable media to allow for the growth of the various bacteria and other microorganisms the wanted to cultivate.

Over the years hundreds of different formulas for media have been developed to cultivate all manner of bacteria, fungi, algae, etc… Some of these media are simple in nature made up of known amounts of salts, sugars and other organic molecules like vitamins. These simple or defined media are fine for growing many organisms, but there are some bacteria in particular that have more demanding requirements. For these organisms, more complex media were developed using infusions of animal and plant parts and often supplemented with blood or serum. The list of ingredients for some of these media may sound a bit like a witch’s brew; brains of calves, the blood of sheep, and so forth...

Another important concept when dealing with media development is to determine if the medium needs to be a liquid (broth) or a solid (agar based). Broth media are most commonly used to cultivate pure cultures in the laboratory or to investigate a variety of biochemical reactions. Agar based media are often used to isolate organisms from a mixed

culture or contaminated sample.

Besides the general make up of the media, there are other considerations that one must look at. Agents such as salt or antibiotics can be added to a medium to give it a selective property. Selective media allow for the growth of some organisms, but inhibit the growth of others. These types of media are very useful when trying to isolate a bacterium from a mixed culture or contaminated sample.

Another consideration is the use of differentiation agents in the medium to determine different patterns of growth or metabolism. Differential medium usual have some form of indicator in them that allows for easy determination of the metabolic activity of the organism growing on it. These medium are useful in identifying unknown organisms.

Sometimes selective agents can be added to a differential medium to make is both selective and differential.

In this laboratory we will be looking at bacteriological media and examining examples of and uses for general lab media, selective media, and differential media.

The first place to start is with the actual preparation of the media. The absolute first step is to **READ THE INSTRUCTIONS** printed on the bottle of medium. The instructions will outline exactly what you need to do in order to properly make up the medium. There is usually a list of ingredients, steps to follow in the preparation, how the medium is to be sterilized, and how and

when any additives are to be added. Below is an example of the label from a bottle of media.



*Figure 1* – Label from a bottle of EMB agar

# Materials:

1. 1 plate plain agar
2. 1 plate M-9 agar with glucose
3. 1 plate nutrient agar
4. 1 plate blood agar
5. 1 plate MSA
6. 1plate EMB agar
7. 1 plate DNase agar
8. 2 tubes of TSI agar slants
9. 2 tubes of BHI broth
10. 2 tubes of urea broth
11. bacterial cultures:
	1. *S. aureus*
	2. *S. epidermidis*
	3. *E. coli*
	4. *P. vulgaris*
	5. *S. pyogenes*
	6. *P. aeruginosa*
	7. *S. typhimurium*
	8. Mixed culture
12. Gram stain reagents
13. glass microscope slides
14. wax pencils
15. Bunsen burner
16. inoculating loop
17. Kovac’s reagent

# Day One Protocol:

1) obtain all the necessary media and label each plate and tube with your initials

**Nutritional Requirements of Bacteria** In this section we will be inoculating several different media with 4 different bacteria and determining if they will grow on the different media. Each media has a different level of nutrients and each organism has a different set of nutritional requirements. To set up this section of the lab follow these steps:

1. using your wax pencil divide the following plates into 4 equal portions and label each quadrant with the initials of one of the organisms to be used – Pa, Spy, Se or Ec
	1. plain agar
	2. M-9 plus glucose agar
	3. nutrient agar
	4. blood agar

*Be sure to write on the bottom of the plates not the tops*

1. using aseptic technique, inoculate each plate in the appropriate quadrant with a small amount of the bacterial culture. The instructor will give specific instructions on how to perform the inoculation. *Be sure not to spread the organism beyond the bounds of the quadrant.*
2. incubate the plates at 37oC overnight

# Isolation of Bacteria from Mixed Cultures

In this section we will be using two selective media to isolate two different bacteria from a mixed culture.

1. inoculate a MSA and an EMB plate with a sample from the **Mixed culture.** The instructor will give specific instructions on how to perform the inoculation.
2. perform a Gram stain on the mixed culture and record your results below:
3. incubate the plates at 37oC overnight

# The use of differential media

In this section we will be looking at the differences in growth and reactions of several different bacteria on a number of different media. We will be looking for differences in carbohydrate fermentation, production of biological end products, and the production of enzymes.

**Triple Sugar Iron Agar** TSI media contains three sugars (glucose, lactose and sucrose) and an iron ingredient for detecting H2S

production - thus the name Triple Sugar Iron. If the sugar is fermented the medium will turn from orange-red or to yellow. If H2S is produced the medium will be blackened. If the sugars are not fermented, the media will turn a pink or red color.

1. Obtain two TSI agar slants and inoculate. It is important that the inoculation be done exactly as described below:
2. aseptically take a loopful of bacteria from the *E. coli* (Ec) culture and gently stab it into the butt of the

will give more details on how to properly inoculate these tubes.

1. repeat the process using the

*Salmonella* (St) culture.

1. incubate the tubes at 37oC overnight



***Figure 2*** – TSI tube. Note the color of the medium in the area of the slant and the butt. Be sure to record and blackening of the medium as this indicates the production of hydrogen sulfide gas.

# Indole Production

Some organisms will convert tryptophan into indole. In order to see this, the organisms need to be grown in a medium that is high in tryptophan such as BHI broth. To determine the presence of indole after incubating the organism overnight, several drops of Kovac’s reagent is added to the tube and observed for a red colored ring to form on top of the medium.

1. Obtain two tubes of BHI broth and inoculate with a couple of loopfuls of the *E. coli* (Eca) and *Salmonella* (St) culture.

slant. Remove the loop and gently inoculate the slant. The instructor

1. incubate the tubes at 37oC overnight

**Detection of Bacterial Enzymes** We will be using two media to detect the presence of the enzymes DNase and urease. Both of these enzymes are used in the identification of a variety of clinically significant bacteria.

# DNase Production

The detection of the enzyme DNase, which degrades polymerized DNA), can be done in a variety of ways. We will be using a special medium that contains DNA and methyl green. Methyl green only binds to highly polymerized DNA and the color will fade if the DNA is degraded.

1. obtain one plate of the DNase medium and divide it in half with your wax pencil.
2. using aseptic technique, inoculate the plate in the appropriate half with a small amount of the bacterial culture

*S. aureus* (Sa) and *S. epidermidis* (Se). The instructor will give specific instructions on how to perform the inoculation. *Be sure not to spread the organism beyond the bounds of the half.*

1. incubate the plates at 37oC overnight

# Urease Assay

Urease is an enzyme that breaks down urea into ammonia and carbon dioxide. As the ammonia content of the medium increases, so will the pH of the medium. A pH indicator will detect this change and indicate that the urea is being degraded.

1. Obtain two tubes of urea broth and inoculate with a couple of loopfuls of the *E. coli* (Ec) and *P. vulgaris* (Pv) culture.
2. incubate the tubes at 37oC overnight

# NOTES

**Day Two:**

You will need to examine your plates and tubes and record your results in the tables below.

# Nutritional Requirements of Bacteria

* 1. examine your plates for growth. Growth is indicated by colonies on the medium.
	2. record your results in table 1. **Isolation of Bacteria from Mixed Cultures**
1. observe your plates for growth and record what the colonies look like.
2. perform a Gram stain on representative colonies from each plate and record your results in table 2.

# The use of differential media TSI agar

1) observe your tubes and record your results in table 3.

# Indole production

1. observe your tubes for growth and add about 5 drops of the Kovac’s reagent
2. gently shake the tube and observe for the presence of a red color at the top

of the tube. A red color indicates a the presence of indole.

1. record your results in table 3. **Detection of Bacterial Enzymes DNase agar**
2. observe the plate for growth and determine the color of the medium adjacent to the colonies. A green color indicates no DNase activity and a clearing of the medium indicates the presence of DNase.
3. record your results in Table 4.

# Urease broth

1. observe your tubes for growth and color change. If the medium looks the same as when it was inoculated, salmon color, it is negative for urease activity. If the medium has a bright pink color, it is positive for urease.
2. record your results in Table 4.

# Table 1 - Nutritional Requirements of Bacteria

|  |  |
| --- | --- |
| **Medium** | **Organism** |
|  | **Pa** | **Spy** | **Se** | **Ec** |
| **Plain agar** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** |
| **M9 + glucose** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** |
| **Nutrient agar** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** |
| **Blood agar** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** | **Growth [ ]****No Growth [ ]** |

**Table 2 - Isolation of Bacteria from Mixed Cultures**

|  |  |  |
| --- | --- | --- |
| **Medium** | **MSA** | **EMB** |
| **Gram Stain Results** |  |  |

**Table 3 - The use of differential media**

|  |  |
| --- | --- |
| **TSI agar** | **Organism** |
|  | **Ec** | **St** |
| **Butt** | **Yellow [ ] Red [ ]****H2S [ ]** | **Yellow [ ] Red [ ]****H2S [ ]** |
| **Slant** | **Yellow [ ] Red [ ]****H2S [ ]** | **Yellow [ ] Red [ ]****H2S [ ]** |
| **Indole Test** | **Ec** | **St** |
| **Indole Production** | **Yes [ ]****No [ ]** | **Yes [ ]****No [ ]** |

**Table 4 - Detection of Bacterial Enzymes**

|  |  |
| --- | --- |
| **Test** | **Organism** |
| **Dnase** | **Sa** | **Se** |
| **Presence of Dnase** | **Present [ ] Absent [ ]** | **Present [ ] Absent [ ]** |
| **Urease** | **Ec** | **Pv** |
| **Presence of Urease** | **Present [ ] Absent [ ]** | **Present [ ] Absent [ ]** |

**Questions**

1. Did all the organisms tested grow equally well on all the media used?
2. Was there a difference between the organisms growing on the EMB and MSA plates?
3. Was there a noticeable difference between the Ec and St on the TSI agar slants and in the indole test?
4. Could easily differentiate between Sa and Se based on their reactions on the DNase test agar?
5. Was there a difference between Ec and Pv when grown in the urease medium?