Lab: Selective and Differential Media

MATERIALS:
- 2 EMB plates
- 1 MacConkey plate
- 2 CNA plates
- 1 MSA plate
- 1 ssA plate
- 4 tubes SIM medium
- E.coli
- Salmonella sp.
- E.faecalis
- P.mirabilis
- C.albicans
- Candida glabrata
- S.aureus
- S.epidermidis
- S.epidermidis
- S.pyogenes
- S.pneumoniae
- Permanent marker
- Inoculating needle
- Bunsen burner
- Kovac’s Reagent

BACKGROUND:
Selective media are designed to suppress the growth of unwanted bacteria and encourage the growth of desired organisms. This is accomplished by the addition of antibiotics, high concentrations of salt or high acidity. Differential media make it easier to distinguish colonies of the desired organism from other colonies growing on the same plate. The colonies have different colors or cause changes in the surrounding medium. Sometimes selective and differential functions are combined in one medium.

ACTIVITY 1

EOSIN METHYLENE BLUE (EMB) MEDIA

PURPOSE:
EMB Agar is selective media used for the isolation and differentiation of enteric bacilli, especially coliforms, in clinical specimens, water, and dairy products. It is also used to differentiate Candida albicans for other yeast species.

PRINCIPLE:
Holt, Harris, Teague first developed Eosin Methylene Blue agar. The eosin dye inhibits growth of gram-positive bacteria and combines with the methylene blue indicator to produce a color change whenever lactose or sucrose are fermented. The modified formulation of Holt, Harris and Teague further balances eosin and methylene blue to optimize differentiation between organisms which ferment these carbohydrates and those that do not. This medium does not allow discrimination between which carbohydrate is fermented. Yersinia enterocolitica, which ferments sucrose, but not lactose, will produce the same purple-black colony as lactose-fermenting bacteria.

The Levine formula eliminates the sucrose and doubles the lactose concentration. As in the HHT-modified formulation, lactose fermenters appear as colonies with blue-black centers and non-lactose fermenters appear as clear to opaque colonies. Because the Levine formulation contains lactose as the only fermentable carbohydrate, reactions are more comparable with MacConkey Agar.

Candida albicans can also be differentiated using the Levine formula from other Candida and Cryptococcus species by its ability to produce germ tubes within 3 hours, and pseudohyphae and budding cells at 18-24 hours when incubated at 35°C in 5-10% CO2. The addition of tetracycline to the Levine formulation aids in the selection of C.albicans from clinical sources that are contaminated with bacteria.

FORMULAS:
Approximate, per liter deionized filtered water.

(1) EMB Agar, Levine:
- Pancreatic Hydrolysate of Gelatin .................10.0 g
- Lactose ......................................................10.0
- Dipotassium Phosphate .................................2.0
- Agar .........................................................15.0
- Eosin Y .....................................................0.4
- Methylene Blue .......................................65.0 mg
Final pH 7.1 ± 0.2 at 25°C

PROCEDURE:
Method of Use: Prior to inoculation, the medium should be brought to room temperature. Inoculate the specimen onto the media surface using standard microbiological procedures to obtain isolated colonies. Obtain 2 EMB plates, using a permanent marker apply lines to divide plate into four quadrants. Inoculate each of the seven organisms below, one per quadrant. Incubate aerobically at 35°C for 18-24 hours.

Interpretation:
- Escherichia coli Blue-black, dark centered colony with green, metallic sheen
- Salmonella species Colorless or transparent, light-purple colonies
- Klebsiella species Mucoid brownish colony with blue-black center
- Proteus species Smooth, translucent, colorless colonies
**Enterococcus faecalis** Small, pin-point, clear colonies

**Candida albicans** (in CO2) At 2-4 hours incubation, germ tubes can be observed when plate is examined under low magnification using a microscope. At 24 hours the characteristically feathery colonies will demonstrate pseudohyphae and budding cells under low magnification.

**Candida** species No germ tube production. Colonies will appear as smooth, circular, and cream colored.

### DATA TABLE:

<table>
<thead>
<tr>
<th></th>
<th>Salmonella Sp.</th>
<th>E. coli</th>
<th>E. faecalis</th>
<th>P. mirabilis</th>
<th>Candida albicans</th>
<th>K. pneumoniae</th>
<th>Candida, not albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td></td>
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<tr>
<td>Colony Description</td>
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</tr>
</tbody>
</table>

### ACTIVITY 2

#### MACCONKEY MEDIA

**PURPOSE:**
MacConkey media are selective, differential media used to isolate enteric microorganisms from mixtures of bacteria. MacConkey media is especially useful for the recognition of enterococci in the presence of coliforms and nonlactose-fermenting organisms. MacConkey broth is an enrichment media and meets the U.S. Pharmacopeia (USP) standards in performing microbial examination of nonsterile products.

**PRINCIPLE:**
In 1905 MacConkey first described the selective, differential media that he used to isolate enteric gram-negative bacilli. It consisted of a nutritious base media that also contained crystal violet and bile salts which inhibited the growth of gram-positive microorganisms. The original formula has been modified by an addition of sodium chloride and a modification of the concentration of bile salts, agar, and neutral red. These changes have enhanced the recovery of *Shigella* and *Salmonella* species, the differentiation of coliforms from enteric pathogens, and the inhibition of the swarming of *Proteus* species. Microorganisms capable of growing on MacConkey Agar and capable of metabolizing lactose, produce acid by-products that lower the pH of the media close to the colony. The lowering of the pH causes the neutral red indicator to turn red, and if sufficient acid is produced, a zone of precipitated bile develops around the colony. Microbes that do not metabolize lactose appear colorless and translucent.

**FORMULAS:**
Approximate, per liter of deionized filtered water.

1. **MacConkey Agar:**
   - Pancreatic Digest of Gelatin ......................... 17.0 g
   - Peptic Digest of Animal Tissue ..................... 1.5
   - Pancreatic Digest of Casein .......................... 1.5
   - Lactose ................................................. 10.0
   - Bile Salts Mixture ................................... 1.5
   - Sodium Chloride ...................................... 5.0
   - Agar .................................................... 13.5
   - Neutral Red .......................................... 30.0 mg
   - Crystal Violet ...................................... 1.0
   - Final pH 7.1 ± 0.2 at 25°C

**PROCEDURE:**

**Method of Use for MacConkey Agar:** Prior to inoculation, the medium should be brought to room temperature. Incubate the specimen onto the media surface using standard microbiological procedures to obtain isolated colonies. Obtain 1 MacConkey plate, using a permanent marker apply lines to divide plate into four quadrants. Inoculate each of the four organisms below, one per quadrant. Incubate aerobically at 35°C for 18-24 hours. Strong lactose and sorbitol fermenters will form deep red colonies on the respective media. Weak fermenters will form light pink colonies or colonies that have pink centers with a clear periphery. Nonfermenters will form colorless, translucent colonies.

**Interpretation:**

**MacConkey Agar**
- *Escherichia coli* Pink, smooth, circular, with zone of precipitation and entire edge.
- *Salmonella* species Colorless, translucent to opaque, smooth, circular, with entire edge.
- *Shigella* species Colorless, moderately transparent, smooth, circular, with entire edge.
- *Proteus* species Colorless, translucent, circular, smooth, some strains will show signs of spreading, but spreading is usually inhibited.
- *Enterobacter* species Colorless to pink with pink centers, mucoid, thick, smooth, with entire edge.
- *Pseudomonas* species Large, colorless to grayish-green with dark centers, translucent, with irregular edge.
DATA TABLE:

<table>
<thead>
<tr>
<th></th>
<th>E.coli</th>
<th>P.mirabilis</th>
<th>K.pneumoneae</th>
<th>S.epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Colony Description</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**ACTIVITY 3**

**COLUMBIA AGAR (CNA) MEDIA**

**PURPOSE:**
Columbia agar is nutrient media used for the cultivation of fastidious and nonfastidious microorganisms from clinical and nonclinical specimens. Sheep or horse blood is added to enhance the growth of bacterial species by providing the X factor (heme) necessary in the preliminary identification of hemolytic strains. The media becomes selective for streptococci and staphylococci with the addition of colistin and nalidixic acid (CNA). Various combinations of antibiotics are used as additives to further select for fastidious microorganisms. Columbia Agar (P8233) preparation meets the U.S. Pharmacopeia (USP) standards for use as an isolation media in performing microbial examination of nonsterile products.

**PRINCIPLE:**
Columbia agar base (CAB) is a general purpose media with the basic ingredients necessary for microorganisms to replicate and grow. The media contains peptones, which provide a mixture of nitrogenous compounds and amino acids, and yeast and beef extracts to provide additional nitrogenous compounds, carbohydrates and vitamins. Ellner et al1 discovered peptones from both animal and vegetable protein to be complementary, and the growth of the microorganisms to be better than on the then more frequently used base media (casein hydrolysate or meat infusion media). In addition, yeast and beef extracts were added and appeared to increase the growth of *Neisseria* species, while cornstarch, by neutralizing the inhibitory effects of glucose, decreased the formation of a green coloration (alpha hemolysis) by beta-hemolytic streptococci.

Columbia agar base (CAB) was made a selective media by adding colistin and nalidixic acid (CNA), which inhibit gram-negative microorganisms. CNA was found to be more effective in suppressing *Proteus, Klebsiella,* and *Pseudomonas* species than Phenylethyl

**FORMULAS:**
Approximate, per liter of deionized filtered water.

Columbia Agar + 5% Sheep Blood:

- Peptic Digest of Animal Tissue .................5.0 g
- Pancreatic Digest of Casein........................5.0
- Yeast Enriched Peptone................................10.0
- Pancreatic Digest of Heart Muscle ..............3.0
- Cornstarch ....................................................... 1.0
- Sodium Chloride.............................................. 5.0
- Agar ..............................................................14.0
- Sheep Blood....................................................50.0 ml

Final pH 7.3 ± 0.2 at 25°C

**PROCEDURE:**
*Method of Use:* Prior to inoculation, the medium should be brought to room temperature. Inoculate the specimen onto the media surface using standard microbiological procedures to obtain isolated colonies. Obtain 2 CNA plates, using a permanent marker apply lines to divide plate into three sections. Inoculate each of the six organisms below, one per section. Incubate aerobically at 35°C for 18-24 hours.

**Interpretation:**
*Organism: Colony Morphology:*

- *Streptococcus pyogenes* Small, beta-hemolytic, transparent to opaque, domed, smooth and entire edge
- *Enterococcus faecalis* Small, gamma-hemolytic, transparent to opaque, domed, smooth and entire edge
- *Streptococcus pneumoniae* Small, alpha-hemolytic, round and mucoid with entire edge
- *Staphylococcus aureus* Average, ± hemolysis, opaque, circular, smooth, raised, white to golden yellow pigment
- *Staphylococcus epidermidis* Average, ± hemolysis, opaque, circular, smooth, raised, usually white to colorless
- *Escherichia coli* ± growth. Large, grayish colonies

For other clinically significant organisms, a reference such as Murray et al.5 should be consulted.

**DATA TABLE:**

<table>
<thead>
<tr>
<th></th>
<th>S.pyogenes</th>
<th>E.faecalis</th>
<th>S.pneumoniae</th>
<th>S.aureus</th>
<th>S.epidermidis</th>
<th>E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Colony Description</td>
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<td></td>
</tr>
</tbody>
</table>
**ACTIVITY 4**

**MANNITOL SALT AGAR**

**PURPOSE:**
Mannitol Salt Agar is a highly selective medium designed for the recovery and isolation of pathogenic staphylococci. This medium meets the U.S. Pharmacopeia (USP) standards in performing microbial examination of nonsterile products.

**PRINCIPLE:**
In 1942 Koch described the tolerance of *Staphylococcus aureus* to high concentrations of sodium chloride. Chapman formulated a medium which incorporated 7.5% sodium chloride into an agar containing mannitol and a phenol red indicator for the recovery of pathogenic staphylococci. Most strains of coagulase-positive staphylococci grow on the medium, producing colonies with yellow zones as a result of the fermentation of mannitol. Coagulase-negative strains may be inhibited or produce small colonies with no color change in the surrounding medium. Other bacteria are generally inhibited, so that a heavy inoculum of a culture containing mixed flora will not result in an overgrowth.

**FORMULA:**
Approximate, per liter deionized filtered water.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Extract</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>5.0</td>
</tr>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>75.0</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>10.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Phenol Red</td>
<td>25.0 mg</td>
</tr>
</tbody>
</table>

Final pH 7.4 ± 0.2 at 25°C

**PROCEDURE:**
**Method of Use:** Prior to inoculation, the medium should be brought to room temperature. Inoculate the specimen onto the media surface using standard microbiological procedures to obtain isolated colonies. Obtain 1 MSA plate, using a permanent marker apply lines to divide plate into three sections. Inoculate each of the three organisms below, one per quadrant. Incubate aerobically at 35°C for 18-24 hours. Most strains of *S. aureus* capable of fermenting mannitol will do so within 24 hours. However, delayed fermentation of mannitol may occur with a few strains of *S. aureus*, so negative plates should be incubated for an additional 24 hours before being discarded.

**Interpretation:**
Positive: Growth of smooth, raised colonies; yellow color change in the medium. (Possible growth of *Staphylococcus aureus*; further identification of cation required.)
Negative: Growth of smooth, raised colonies; no color change in the medium; or inhibition of growth.

**DATA TABLE:**

<table>
<thead>
<tr>
<th></th>
<th><em>S. aureus</em></th>
<th><em>S. epidermidis</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colony Description</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ACTIVITY 5**

**SULFIDE-INDOLE-MOTILITY (SIM) MEDIA**

**PURPOSE:**
SIM media is a semisolid agar used for the identification of members of the family *Enterobacteriaceae* by detecting indole formation, sulfide production, and motility.

**PRINCIPLE:**
Green et al. first described the use of SIM medium, suggesting that a reduced amount of medium would improve the incubation times for motility detection. SIM medium is designed to detect three biochemical characteristics of bacteria through the following mechanisms:

**Motility:** When organisms are stabbed into the semisolid agar with a straight wire, the bacteria will migrate by means of their flagella away from the stab line. This produces turbidity throughout the medium. Nonmotile organisms grow only on the stab line, leaving the surrounding medium clear.

Triphenyltetrazolium chloride (TTC) is a soluble compound incorporated in the medium. When taken up by the bacterial cells, the substance is reduced releasing the acid formazan, a highly pigmented red, insoluble compound. SIM with TTC demonstrates motility by means of a diffuse pink color throughout the medium. Nonmotile organisms will produce a straight pink line.
Hydrogen Sulfide (H\textsubscript{2}S): Sulfur is incorporated into the medium in the form of sodium thiosulfate, with the indicator ferric ammonium citrate. If H\textsubscript{2}S is produced, it reacts with the sodium thisulfate, to produce ferrous sulfide which is precipitated in the medium, producing a blackish color.

Indole: Tryptophan is incorporated in the medium in the form of peptones. If the organism produces tryptophanase, tryptophan will be broken down into by-products; in particular, indole. This compound reacts with the aldehyde in Kovacs reagent to form a red or purplish-red color. A negative reaction will show no pink color change after the addition of the reagent. (**H\textsubscript{2}S and motility must be observed before Kovacs reagent is added!**)

**FORMULAS:**
Approximate, per liter deionized filtered water.

**SIM Medium**
- Pancreatic Digest of Casein……………..20.0g
- Peptic Digest of Animal Tissue……………..6.1g
- Ferrous Ammonium Sulfate……………..0.2g
- Sodium Thiosulfate………………………………....0.2g
- Agar…………………………………………………3.5g
  Final pH 7.3+/- 0.2 at 25˚C

**PROCEDURE:**
**Method of Use:** Prior to the inoculation, the medium should be brought to room temperature. Obtain 4 tubes of SIM medium. Inoculate each tube with one of the organisms listed below, obtain colonies from a pure 18-24 hour culture. Using a straight needle, stab the center of the medium to about one half its length. Incubate the tubes with caps loose at 35˚C for 18-24 hours. After incubation observe for production of H\textsubscript{2}S and motility. After reading the above reactions, add a few drops of Kovacs reagent and observe for indole production.

**Interpretation:**
- Motility: turbidity or fuzzy growth throughout medium denotes motility. Growth only on stab site is indicative of a nonmotile organism.
- H\textsubscript{2}S Production: Blackening of the medium is a positive result. Absence of blackening denotes a negative test.
- Indole: A pink to red color after the addition of Kovac’s Reagent is a positive result. A yellow color denotes a negative test.

**DATA TABLE:**

<table>
<thead>
<tr>
<th>Hydrogen sulfide production</th>
<th>Control</th>
<th>E.coli</th>
<th>Salmonella sp.</th>
<th>K.pneumoneae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**ACTIVITY 6**

**BBL. Group A Selective Strep Agar with 5% Sheep Blood**

**INTRODUCTION**
Group A Selective Strep Agar with 5% Sheep Blood (ssA) is a selective medium for use in the isolation and presumptive identification of group A streptococci from throat cultures and other specimens. Trypticase Soy Agar with 5% Sheep Blood (TSA II) is used for the growth of fastidious organisms and for the visualization of hemolytic reactions.

**PERFORMANCE TEST PROCEDURE**
1. Inoculate representative samples with the cultures diluted to contain 103.104 CFU/0.01 mL.
   a. To each plate, add 0.01 mL of the dilution and streak for isolation. Make a stab in the primary streak area before streaking the rest of the plate.
   b. Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
   c. Include Trypticase Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18.24 h for beta hemolysis in the stabbed area and for amount of growth, inhibition, colony size and hemolytic reactions.
**PRODUCT INFORMATION**

**INTENDED USE**

Group A Selective Strep Agar with 5% Sheep Blood (ssA) is recommended as a primary selective plating medium for the primary isolation of group A streptococci (S. pyogenes) from throat cultures and other specimens in which the presence of S. pyogenes is suspected. Group B streptococci will also grow on this medium; most other streptococci, neisseriae, staphylococci and gram-negative bacteria are inhibited. The medium is designed for use in conjunction with Taxo A (bacitracin, 0.04 unit) discs for presumptive identification of S. pyogenes.

**Trypticase Soy Agar with 5% Sheep Blood (TSA II)** is used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

**SUMMARY AND EXPLANATION**

Infection with Lancefield group A streptococci (S. pyogenes) may produce serious sequelae such as rheumatic fever and acute glomerulonephritis. Therefore, early detection and identification are important.

The nutritional composition of Trypticase Soy Agar has made it a popular medium, both unsupplemented and as a base for media containing blood. Trypticase Soy Agar with 5% Sheep Blood (TSA II) is extensively used for the recovery and cultivation of fastidious microbial species and for the determination of hemolytic reactions which are important differentiating characteristics for bacteria, especially Streptococcus species.

Because of the overgrowth of normal flora present in throat culture specimens plated on routine blood agar plates, selective ingredients have been added to sheep blood agar to enhance the detection of group A streptococci.

Evaluation of various antimicrobial agents in our laboratories resulted in a combination with improved selectivity over other selective media tested. This medium (ssA) allows presumptive identification of group A streptococci, based on bacitracin susceptibility and beta hemolysis, within 24 h after inoculation with the specimen when the medium is incubated in a CO₂-enriched atmosphere.1

The divided bi-plate, containing the nonselective blood agar (TSA II) in the sector marked "I" and the selective blood agar (ssA) in the sector marked "II," permits the recovery of group A streptococci and evaluation of the total specimen microbiota with one dish.

**PRINCIPLES**

The combination of casein and soy peptones in the Trypticase Soy Agar base renders the medium highly nutritious by supplying organic nitrogen. The sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood provides proper hemolytic reactions of streptococci. In addition, growth of Haemophilus hemolyticus, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

**Trypticase Soy Agar with 5% Sheep Blood (TSA II)** provides excellent growth and beta hemolysis by Streptococcus pyogenes (Lancefield group A) and also provides excellent growth and appropriate hemolytic reactions with other fastidious organisms.

Group A Selective Strep Agar with 5% Sheep Blood (ssA) incorporates a unique combination of selective ingredients in Trypticase Soy Blood Agar (TSA II) to suppress normal throat flora for improved recovery of S. pyogenes. Defibrinated sheep blood supplies enrichment for the growth of such fastidious organisms and allows detection of the typical beta hemolysis of S. pyogenes.

**FORMULA:**

**Group A Selective Strep Agar with 5% Sheep Blood (ssA)**

Approximate Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>14.5 g</td>
</tr>
<tr>
<td>Papaic Digest of Soybean Meal</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>14.0 g</td>
</tr>
<tr>
<td>Growth Factors</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Selective Agents</td>
<td>40.2 mg</td>
</tr>
<tr>
<td>Sheep Blood, defibrinated</td>
<td>5%</td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.

**PROCEDURE:**

**Method of use:** Prior to inoculation, the medium should be brought to room temperature. Inoculate the specimen onto the media surface using standard microbiological procedures to obtain isolated colonies. Obtain 1 ssA plate, using a permanent marker apply lines to divide plate into three sections. Inoculate each of the three organisms below, one per section. Incubate aerobically at 35oC for 18-24 hours.

**Interpretation:**

After 18.24 h of incubation in an atmosphere enriched with carbon dioxide, group A streptococci (S. pyogenes) on ssA will appear as translucent or opaque, white to gray, small (1.2 mm) colonies surrounded by a zone of beta hemolysis. A decrease in size as compared to the nonselective control, Trypticase Soy Agar with 5% Sheep Blood, is typical. Pinpoint or very small colonies of alpha-, nonhemolytic or other beta-hemolytic streptococci may grow in small numbers, but they should not interfere with the recovery of group A streptococci or interpretation of the results. Neisseria species, viridans streptococci, staphylococci, gram-negative rods and most beta-hemolytic streptococci other than groups A and B are inhibited on the ssA medium. Bacitracin susceptibility may be used to differentiate group A streptococci from group B. Fair to heavy growth of
beta-hemolytic colonies demonstrating a zone of inhibition around the Taxo A disc may be presumptively reported as *S. pyogenes*. A PYR (pyroglutamic acid) test may also be performed. It is more specific and as sensitive as the bacitracin test for this purpose.7 Gram stains should be made and examined.

**DATA TABLE:**

<table>
<thead>
<tr>
<th></th>
<th><em>S.pyogenes</em></th>
<th><em>S.pneumoniae</em></th>
<th><em>E.coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Colony Description</strong></td>
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</tr>
</tbody>
</table>

**DISCUSSION:**

1. Complete the following table indicating whether the media is selective or differential by placing check mark in the appropriate column.

<table>
<thead>
<tr>
<th>Media</th>
<th>Selective</th>
<th>Differential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin Methylene Blue (EMB) media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macconkey media (MAC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Columbia media (CNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannitol Salt Agar (MSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfide-Indole-Motility (SIM) media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A Selective Strep Agar (ssA)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Complete the following table by recording the inhibitive/selective agent, if any, of the listed medias and what organism(s) are inhibited.

<table>
<thead>
<tr>
<th>Media</th>
<th>Inhibitive/Selective Agent</th>
<th>Type of Organism(s) Inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin Methylene Blue (EMB) media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macconkey media (MAC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Columbia media (CNA)</td>
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</tr>
<tr>
<td>Mannitol Salt Agar (MSA)</td>
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<tr>
<td>Sulfide-Indole-Motility (SIM) media</td>
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<tr>
<td>Group A Selective Strep Agar (ssA)</td>
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</tbody>
</table>

3. Complete the following table by recording the method or type of differentiation, if any, for each of the medias listed.

<table>
<thead>
<tr>
<th>Media</th>
<th>Method or type of differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin Methylene Blue (EMB) media</td>
<td></td>
</tr>
<tr>
<td>Macconkey media (MAC)</td>
<td></td>
</tr>
<tr>
<td>Columbia media (CNA)</td>
<td></td>
</tr>
<tr>
<td>Mannitol Salt Agar (MSA)</td>
<td></td>
</tr>
<tr>
<td>Sulfide-Indole-Motility (SIM) media</td>
<td></td>
</tr>
<tr>
<td>Group A Selective Strep Agar (ssA)</td>
<td></td>
</tr>
</tbody>
</table>