

Microbiology assignment



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Microbiology Laboratory 7 MacConkey Agar PURPOSE: MacConkey agar selects for gram negative bacteria and also differentiates lactose fermenters (pink-red) from non-lactose fermenters(colorless). PRINCIPLE: Bile salts inhibit gram positive bacteria, which allows for the isolation of gram negative bacteria. Neutral red and crystal violet further inhibit the gram positive bacteria. Lactose is the only carbohydrate source. Neutral red indicator is brown in pH 6.8 to 8.0 and pink-red at pH less than 6.8 PROCEDURE: 1. Streak agar for isolation 2. Incubate at 35-37 °C for 18-24 hours and observe for growth and color. . INTERPRETATION: If lactose is fermented, the medium is acidified, and bile salts are precipitated. The precipitated dye is absorbed, resulting in a pink-to-red complex. MacConkey Agar ??? pink colonies (Enterobacter cloacae ??? rapid lactose fermenter) MacConkey agar ??? pink colonies (Escherichia coli ??? rapid lactose fermenter) MacConkey agar ??? colorless colonies (Salmonella typhimurium ??? nonlactose fermenter) MacConkey Agar Classification according to use: Differential medium for gram negative bacilli INDICATOR: Neutral red A. MAC showing LFO with pink or colored colonies Organism used : E. coli B. MAC showing LFO with mucoid colonies Organism used : Klebsiella pneumoniae C. MAC showing NLFO with colorless translucent colonies Organism used: Proteus vulgaris D. MAC Organism used: Enterobacter Salmonella-Shigella Agar PURPOSE: Salmonella-Shigella medium provides for inhibition of normal flora coliforms and differentiation of stool pathogens PRINCIPLE: Bile salts inhibit gram positive bacteria, and brilliant green agar and bile salts inhibit the gram negative coliforms. Lactose is the sole carbohydrate source. Neutral red indicator is red in acidic conditions.

Lactose fermenters appear pink-red, whereas non-lactose fermenters appear clear. To detect H₂S production, sodium thiosulfate serves as sulfur source.

When H₂S is formed, it combines with ferric ammonium citrate to form ferric sulfide (FeS), which is represented by black centered colonies. PROCEDURE:

1. Streak agar for isolation. 2. Incubate at 35-37 °C for 18-24 hours and observe for growth and color. INTERPRETATION: Normal flora coliforms =

pink to red colonies. Shigella = colorless colonies without black centers.

Salmonella = colorless colonies with black centers A. Salmonella-Shigella Agar (SSA) B.

SSA ??? Salmonella typhii C. SSA ??? E. coli Xylose Lysine Deoxycholate

(XLD) Agar PURPOSE: The XLD is used for the isolation and differentiation of

stool pathogens and inhibition of normal flora coliforms. PRINCIPLE: Sodium deoxycholate inhibits gram positive bacteria, partially inhibits the growth of

E. coli, and inhibits the swarming of Proteus. Phenol red indicator becomes yellow in acidic environments. Fermentation of xylose results in yellow

colonies. Most members of Enterobacteriaceae are xylose positive, except

Shigella. Most strains of Shigella cannot ferment lactose and thus produce red colonies.

Lysine positive bacteria first produce yellow colonies as xylose is fermented, followed by red colonies, indicating lysine decarboxylation. H₂S positive

colonies have black centers due to reaction of H₂S with ferric ammonium citrate. PROCEDURE: 1. Streak agar for isolation. 2. Incubate at 35-37 °C

for 18-24 hours and observe for growth and color. INTERPRETATION:

Salmonella = red colonies with black centers Citrobacter and Proteus =

yellow colonies with black centers Differential capabilities of XLD agar for

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lactose-fermenting, gram negative bacilli (e. g *E. coli*, arrow A), nonlactose fermenters (e. g *Shigella* spp. , arrow B) and H₂S producers (e. g *Salmonella* spp. , arrow C) Hektoen Enteric Agar PURPOSE: Hektoen enteric medium selects for stool pathogens by inhibiting the normal flora of the lower GI tract. PRINCIPLE: A high concentration of bile salts inhibits gram positive bacteria and gram negative coliforms. Lactose, sucrose, and salicin are carbohydrate sources. Bromthymol blue indicator has the following pH ranges: > 7.6 ??? blue 6.0-7.6 ??? green < 6.0 ??? yellow Sodium thiosulfate is the sulfur source of H₂S detection.

H₂S combines with ferric ammonium citrate to form ferric sulfide (FeS), which is represented by black-centered colonies. If one, two, or three of the carbohydrates are fermented, the colonies are orange in color.

Nonfermenters produce green colonies. PROCEDURE: 1. Streak agar for isolation. 2. Incubate at 35-37 ?? C for 18-24 hours and observe for growth and color. INTERPRETATION: Pathogens = green colonies or green colonies with black centers Normal flora (except *Yersinia enterocolitica*, which produce yellow colonies due to its fermentation of sucrose) = yellow colonies

Differential capabilities of HE agar for lactose-fermenting, gram negative bacilli (e. g *E. coli*, arrow A), nonlactose fermenters (e. g *Shigella* spp. , arrow B) and H₂S producers (e. g *Salmonella* spp. , arrow C) Eosin Methylene Blue (EMB) Agar PURPOSE: The EMB medium selects for gram negative bacteria and also differentiates lactose fermenters (purple color to “ green metallic sheen”) from non-lactose fermenters (colorless) PRINCIPLE: Eosin and methylene blue are dyes that inhibit the gram positive bacteria. Lactose is

the only carbohydrate source in most formulation. PROCEDURE: Streak agar for isolation. 1.

Incubate at 35-37 °C for 18-24 hours and observe for growth and color.

INTERPRETATION: If lactose is fermented, precipitated eosin and methylene are absorbed resulting in a purple color medium. A classical “green metallic sheen” is produced by *E. coli*, which is a rapid lactose fermenter. Non-lactose fermenters produce colorless colonies on EMB. Eosin methylene blue agar ??? greenish metallic sheen (*E. coli* ??? rapid lactose fermenter) Eosin Methylene Blue Agar Classification according to use: Differential medium for gram negative bacilli INDICATOR: Eosin and methylene blue A. A. EMB showing LFO with greenish metallic sheen

B. Organism used : *Escherichia coli* B. EMB showing LFO with mucoid colonies

Organism used : *Klebsiella pneumoniae* C. EMB showing NLFO with colorless translucent colonies Organism used: *Proteus vulgaris* UREASE TEST

INDICATOR: Phenol red MEDIUM: Urea broth PRINCIPLE: Microorganism that possesses the enzyme, urease, hydrolyze urea to ammonia and CO₂. Phenol red turns fuschia in the presence of alkaline end products. A. Uninoculated :

Salmon pink B. Positive result: Fuschia pink C. Negative result: Salmon pink

Urease Reaction PURPOSE: Urease is an enzyme that splits urea into alkaline end products.

The reaction is useful in the identification of rapid urease producers, such as *Proteus* and *Morganella*, as well as weak urease producers, such as *Klebsiella pneumoniae* and some species of *Enterobacter*. PRINCIPLE: Urease splits the urea molecule into ammonia (NH₃), carbon dioxide (CO₂), and water (H₂O).

Ammonia reacts in solution to form an alkaline compound, ammonium carbonate, which results in an increased pH of the medium and a color change in the indicator to pink-red. $\text{Urea} + 2\text{H}_2\text{O} \xrightarrow{\text{Urease}} \text{CO}_2 + \text{H}_2\text{O} + 2\text{NH}_3$
 $(\text{NH}_4)_2\text{CO}_3$ Ammonium carbonate
 Media and Reagents: Christensen's (urea) agar tubes or Stuart urea broth

PROCEDURE: 1. Using Stuart broth, dissolve one urea disk in 1 ml sterile, distilled water. 2. Using single, isolated, 18-24 hour colony and streak slant of agar tube or inoculate broth. 3. Replace cap loosely or parafilm broth. Incubate at 35°C for 18-24 hours. Urease Test (Christensen's Agar) A. Positive ??? rapid urease activity: red throughout medium B. Positive ??? slow urease activity: red in slant C. Negative ??? no urease activity: remains yellow Urease Test (Stuart Urea Broth) 1. Uninoculated control 2. Positive ??? red color in medium 3. Negative ??? no color change (buff to pale yellow)

INTERPRETATION:

Stuart Broth Positive: red color in medium Negative: no color change (buff to pale yellow) Strong positive reactions are seen only with *Proteus* spp. (*Proteus* and *Morganella*) and may be interpreted as early as after 4 hours of incubation. Weakly positive reactions (pink to orange) may be seen with *K. pneumoniae* and other slow urease producers. Christensen's agar Positive: (rapid urease activity) red throughout medium (seen only with *Proteus* and *Morganella*) Positive: (slow urease activity) red in slant (*K. pneumoniae*) Negative: (no urease activity) medium remains yellow QUALITY CONTROL:

Proteus vulgaris ??? positive: rapid urease producer *K. pneumoniae* ??? positive: slow urease producer *E. coli* ??? negative: no urease production

Urease (+) Slow SIM Medium (Sulfur Reduction, Indole Production, Motility) 1.

SULFUR REDUCTION PURPOSE: This test is used to identify those bacteria capable of reducing sulfur. This is particularly important in differentiating certain general enteric organisms. **PRINCIPLE:** Hydrogen sulfide, H₂S, can be formed by putrefaction or anaerobic respiration. The medium contains cysteine, an amino acid containing sulfur, and sodium thiosulfate plus peptonized iron or ferrous sulfate.

H₂S will react with the iron or ferrous sulfate, forming a black precipitate. If the black precipitate is present, the test is positive for H₂S production. No precipitate is a negative test. 2. **INDOLE PRODUCTION PURPOSE:** The indole test is used to identify bacteria capable of producing indole using the enzyme tryptophanase. It is one component of the IMVIC tests for differentiating Enterobacteriaceae. **PRINCIPLE:** The enzyme tryptophanase can convert the amino acid, tryptophan, to indole, ammonia and pyruvic acid. The by-product, indole, is the metabolite identified by this test.

When Kovac's reagent, which contains hydrochloric acid and dimethylaminobenzaldehyde and amyl alcohol, a red layer will form when indole is present. No color in this layer is a negative result. Indole production.

A. Positive ??? red ring at the interface of the reagent and broth; B.

Negative ??? no color development **INDOLE BROTH PURPOSE:** Indole broth is used for distinguishing Enterobacteriaceae based on the ability to produce indole from tryptophan. The test is particularly useful for the identification of lactose-fermenting members of Enterobacteriaceae.

Escherichia coli is indole positive, whereas Enterobacter and Klebsiella are indole negative. Indole is also useful in the inspection of Proteus. P. mirabilis is indole negative, P. vulgaris is positive. PRINCIPLE: Tryptophan present in peptone is oxidized by certain bacteria to indole, skatole, and indole-acetic acid. The intracellular enzymes that metabolize tryptophan are known as tryptophanase. Indole is detected in broth cultures of bacteria with an alcoholic p-dimethylaminobenzaldehyde reagent. Indole reacts with the acetaldehyde to form a red product. Two reagents may be used to detect indole, Kovac's and Ehrlich's.

Ehrlich's reagent is believed to be more sensitive than Kovac's reagent and is recommended for indole detection in anaerobes and nonfermentative bacteria. Kovac's reagent was initially used to classify members of the Enterobacteriaceae and should be used with these organisms.

Tryptophan Tryptophanase indole + Pyruvic acid + ammonia Indole + p-dimethylaminobenzaldehyde Red REAGENTS AND MEDIA: Tryptophan (1%) broth Kovac's reagent or Ehrlich's reagent Xylene or chloroform for extraction if using Ehrlich's reagent PROCEDURE: 1. Inoculate indole broth 2. Replace cap loosely and incubate at 35°C for 18-24 hours. . Add 5 drops of Kovac's reagent directly to the broth culture. Observe for red color in the upper alcohol layer. 4. If using Ehrlich's reagent, first add 1 ml xylene or chloroform to the broth culture. Shake gently and then add 5 drops of the reagent. INTERPRETATION: Negative reaction: no color development Positive reaction: red ring at the interface of the reagent and broth (or reagent and xylene or chloroform) Variable reaction: orange color, indicates production of

skatole, a methylated intermediate that may be a precursor to indole production. QUALITY CONTROL: *E. coli* ??? positive control (red ring)

Enterobacter cloacae ??? negative control (no color development) 3.

MOTILITY (Test) PURPOSE: This procedure determines the motility of the bacteria through semisolid media. *Shigella* and *Klebsiella* are the only nonmotile Enterobacteriaceae. *Yersinia enterocolitica* is nonmotile at 37?? C but is motile at 22?? C. PRINCIPLE: The medium contains a small amount of agar, which allows motile bacteria to move out from the line of inoculation.

Nonmotile organisms grow only along the line of inoculation; 1% triphenyltetrazolium chloride may be added to medium to aid visualization of the reaction.

Bacteria incorporate this colorless dye and reduce it to a red pigment. Thus reddening of the medium can be used as an indication for the extent of bacterial growth. PROCEDURE: 1. With a sterile inoculating needle, select one colony and stab the needle to the bottom area of the agar. 2. incubate at 35?? C for 18-24 hours, and examine for growth around the line of

inoculation. INTERPRETATION: Motile ??? diffuse growth extending laterally from line of inoculation Nonmotile ??? growth only along line of inoculation

QUALITY CONTROL: *Proteus mirabilis* ??? motile *Klebsiella pneumoniae* ??? nonmotile Motility test. and 3 ??? Motile ??? diffuse growth extending

laterally from line of inoculation indicated by turbidity of the medium 2 ??? nonmotile ??? growth only along the line of inoculation MOTILITY (Medium)

PURPOSE: This medium can be stab-inoculated with an inoculating needle to indicate motility. PRINCIPLE: The lower agar concentration in the medium allows limited movement of motile bacteria from the area of stab. Motility

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will be detectable as diffuse growth radiating from the stab line. SIM medium control Nonmotile with no H₂S Motile with H₂S production A ??? positive indol red ring at the interface

B ??? negative indol; no color development MOTILITY INDOLE ORNITHINE MEDIUM (MIO) Indicator: Bromcresol purple Classification according to consistency: semisolid Results to be observed: 1. motility 2. indole production 3. ornithine decarboxylation A. Uninoculated: Cadaverine color B. Motility Test: Motile ??? showing a diffused growth or haziness in the medium C. Motility test: Nonmotile ??? showing growth along the stabbing line D. Ornithine decarboxylation test: positive ??? purple E. Ornithine decarboxylation test: negative ??? yellow F. Indole production test: positive ??? red ring G.

Indole production test: negative ??? absence of red ring NOTE: 1. Do not shake because the medium is semisolid. 2. Kovac's reagent for indole test should be added after reading the motility and ornithine decarboxylation reaction. Lysine Iron Agar (LIA) Purpose LIA can be used to determine the ability of the organism to deaminate lysine, decarboxylate lysine and produce H₂S gas. It is useful in the identification of Salmonella, Proteus, Providencia, and Morganella. Members of the Proteus group (Proteus, Providencia and Morganella) are the only members of the Enterobacteriaceae that are deaminase positive.

Principle LIA contains a small amount of protein, glucose, lysine, sulfur, H₂S indicator, agar, and the pH indicator bromcresol purple. As glucose fermentation occurs, the deep of the tube turns yellow. Lysine

decarboxylation produces alkaline cadaverine and leads to reversion of the deep from yellow to purple. Lysine deamination occurs in the presence of oxygen (on the slant) and results in production of a red color. H₂S production is noted by a black precipitate in the deep as H₂S gas reacts with ferric ammonium citrate. Media LIA slants Procedure 1. inoculate LIA by using straight wire to stab the deep (?) and to streak the slant. 2. incubate at 35C for 18 to 24 hours, if necessary, incubate for 48 hours. Interpretation ???

Lysine decarboxylase positive: purple/purple ??? Lysine decarboxylase negative: purple/yellow ??? Deaminase positive: red/yellow ??? H₂S positive: blackening Indicator: Bromcresol purple ??? Acid: Yellow ??? Alkaline state: Purple Results to be observed: 1. deamination on the slant portion only: ??? positive: red slant ??? negative: purple slant 2. Lysine decarboxylation on the butt portion only: ??? positive: purple butt ??? negative: yellow slant

A. Uninoculated: Cadaverine color B. K/K ??? alkaline slant (purple): negative deamination ??? alkaline butt (purple): positive lysine decarboxylation C. K/A ??? alkaline slant (purple): negative deamination ??? acid butt (yellow): negative lysine decarboxylation D. R/Y or R/A ??? red slant: positive deamination ??? acid butt (yellow): negative lysine decarboxylation Lysine iron agar. A. Alkaline slant/alkaline butt (K/K). B. Alkaline slant/alkaline butt, H₂S positive (K/K H₂S +). C. Alkaline slant/acid butt (K/A). D. Red slant/acid butt (R/A). E. Uninoculated tube Decarboxylase Reactions Purpose:

Moeller decarboxylase medium is used for determining the production of decarboxylase by bacteria. Principle: The decarboxylases are enzymes that attack the carboxyl group of specific amino acids, forming amines and carbon dioxide. The amines formed are alkaline, and they alter the color of

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the pH indicator. The amino acid to be tested is added to the Moeller base medium in a 1% concentration. Each decarboxylase reactions is specific for a particular amino acid. Tests for lysine decarboxylase, ornithine decarboxylase, and arginine dihydrolase are generally performed on the enteric bacteria.

Lysine is carboxylated to cadaverine; ornithine is decarboxylated to putrescine, and arginine undergoes a dihydrolase reactions to form citrulline, which is then converted to ornithine in a decarboxylation. Media and Reagents Moeller decarboxylase broths containing ??? 1% lysine ??? 1% ornithine ??? 1% arginine Sterile mineral oil Procedure 1. inoculate test cultures into the tubes of decarboxylase media for each amino acid to be tested. Include a control tube for each organism 2. Overlay all tubes with 5-10 mm of sterile mineral oil Replace cap. 3. Incubate at 35oC for 24 hours.

Interpretation

Glucose fermentation indicates the organism is viable and the medium turns yellow. Decarboxylation is indicated by a blue-purple color in the medium Incubate all tubes negative for decarboxylation for another 24 hours and read again. Quality control All should be read at 24 hours. Arginine ??? enterobacter cloacae: positive (purple); alkaline ??? Klebsiella pneumoniae: negative (yellow), acidic Lysine: ??? Klebsiella pneumoniae: positive (purple), alkaline ??? Enterobacter cloacae: negative (yellow), acidic Ornithine: ??? Enterobacter cloacae: positive (purple), alkaline ??? Klebsiella pneumoniae: negative (yellow), acidic

Control: inoculate the control with the same organisms that are tested in the amino acid tubes. All reactions for the control should be negative (yellow), acidic. Decarboxylase reactions. Positive ??? purple Negative ??? yellow

ONPG Reaction Purpose The ONPG (o-nitrophenyl-beta-D-galactopyranoside) reaction determines the presence of late or slow fermenting strains. The test is useful in detecting late lactose fermenting strains of *Escherichia coli* and distinguishing some *Citrobacter* species and *arizonae* subspecies, which are ONPG positive, from similar *Salmonella* subspecies, which are ONPG negative.

It is also useful in speciation of *Shigella*, since *S. sonnei* is the only ONPG-positive *Shigella* species. **Principle** Lactose fermentation requires two enzymes: lactose permease, which actively transfers lactose into the bacterial cell, and beta-galactosidase, which degrades lactose into glucose and galactose. Non-lactose fermenters lack both enzymes, and those known as slow or late lactose fermenters possess the beta-galactosidase but lack the permease. Lactose fermenters possess the beta-galactosidase but lack the permease. Lactose fermenters possess both enzymes.

ONPG is useful in detecting late lactose fermenters because the ONPG molecule is structurally similar to lactose. ONPG can enter the bacterial cell without a permease. In the presence of beta-galactosidase, ONPG (colorless) is converted into galactose and O-nitrophenyl, which is yellow chromogen and the alkaline end product. **Media and Reagents** ??? ONPG tablets or disks ??? Sterile distilled water ??? Sterile 1.0 mL pipettes **Procedure** 1. if using ONPG tablets, dissolve one tablet in 1.0 ml of sterile distilled water, ONPG disks are dissolved in 0.5 ml sterile distilled water. 2. mix and allow

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tablet to dissolve (5 to 10 minutes) 3. inoculate with four or five colonies of an 18 to 24 hour culture. Use sterile needle to select colonies and mix well 4. parafilm all tubes and incubate at 35°C for 4 to 6 hours. Interpretation Positive reaction: yellow color within 20 minutes to 24 hours Negative reaction: colorless after 24 hours Quality control E. coli: positive-yellow Salmonella typhimurium: negative ??? no color change ONPG (O-nitrophenyl-beta-Dgalactopyranoside) test A- negative ??? no color change or colorless after 24 hours B- positive ??? yellow color w/in 20 min to 24 hrs

Methyl Red-Voges Proskauer (MR-VP) Tests Purpose MR-VP broth is a dextrose broth medium buffered with peptone. Glucose is fermented to pyruvic acid by one of two pathways, which results in either a positive MR or a positive VP test. The tests are particularly useful for the lactose-fermenting Enterobacteriaceae. Escherichia coli is MR positive and VP negative, whereas most members of the Klebsiella-Enterobacter-Serratia-Hafnia group are VP positive. Principle In the first pathway, mixed acid products (lactic, acetic, formic and succinic) result, leading to a decrease in the pH of the medium and a positive MR test.

The pH must drop to 4.4 or less for the MR indicator to take on its acidic red color. In the second pathway, acetylmethyl carbinol (acetoin) is an intermediate product to butylenes glycol. Acetoin is the neutral product detected in the VP reaction. The broth should be heavily inoculated, with a small volume of broth used for the VP test to obtain favorable results at 24 to 48 hours of incubation. In the presence of oxygen and 40% potassium hydroxide (KOH), acetoin is converted to diacetyl form, which results in a red color in the presence of alpha-naphthol.

Metabolism of glucose using MR and VP pathways
Media and Reagents MR-VP broth; glucose base MR pH indicator 5% alpha-naphthol in absolute methyl alcohol 40% KOH containing 0.3% creatine
Procedure
1. inoculate medium with a heavy suspension of an 18 to 24 hour culture.
2. Incubate for 48 hours or until sufficient growth occurs in broth.
3. After 48 hours, split broth by pipetting half into a clean test tube.
4. Perform the MR test on one tube:
a. Add five drops of MR indicator to the aliquot with a Pasteur pipette
b. Interpret the color result immediately
5.

Perform the VP test on the second aliquot
a. Add 0.6 ml (6 drops) of alpha-naphthol reagent to VP aliquot and shake well
b. Add 0.2 ml (2 drops) of 40% KOH reagent to aliquot
c. Gently shake tubes for 30 seconds to 1 minute to expose reaction to atmospheric oxygen. This oxidizes acetoin to obtain a color reaction.
d. Allow tubes to stand at least 10 to 15 minutes before attempting to interpret color results, although the reaction is often immediate. Note The order of adding reagents is very important. A reversal of the order may lead to false-negative results
Interpretation
positive MR test: distinct red color at surface of the medium
Negative MR test: yellow color at surface of the medium
Delayed reaction: orange color. Continue incubation and repeat test in 4 days. No attempt should be made to interpret an MR test before 48 hours' incubation, since false-positive results may occur
Positive VP test: pink-red color at surface of the medium
Negative VP test: yellow color at the surface of the medium
A copper-like color is interpreted as negative, since this is caused by the action of the reagents when mixed.

Quality Control ??? E. coli: MR positive- red, VP negative ??? no pink-red color
??? Enterobacter cloacae: MR negative- no color, VP positive pink-red color
Methyl red test. A ??? positive ??? distinct red color at surface of the medium
B ??? negative ??? yellow color at surface of the medium Voges Proskauer
test A ??? Positive ??? pink red color at surface of the medium B ??? Negative
??? yellow color at surface of the medium Triple Sugar Iron Agar (TSIA)

Purpose TSI agar can be considered an initial step in the identification of the
Enterobacteriaceae. Principle

The medium contains protein sources (beef extract, peptone, yeast extract, proteose peptone) that permits the growth of most bacterial strains. Lactose, sucrose, and glucose are present as well as phenol red indicator. Glucose is in a concentration one-tenth that of the other carbohydrates. Ferrous sulfate is present as an indicator of hydrogen sulfide production. The TSI is a two reaction chamber with an aerobic slant portion and an anaerobic deep portion. The slant of the tube is exposed to atmospheric oxygen and will become alkaline due to oxidative decarboxylation of peptides and amino acids.

The slant tends to become and remain alkaline (red). Amino acid degradation is minimal in the deep (anaerobic) portion, and thus a small quantity of acid produced can be detected because few amines are being formed from amino acids. Bacteria that ferment glucose, but not lactose or sucrose, only produce small quantities of acid and cannot counteract the degradation of amino acids at the slant, which results in an alkaline pH due to oxidative decarboxylation. Such organisms characteristically produce an alkaline slant over an acid deep (K/A)

Organisms that ferment both glucose and lactose and/or sucrose produce large quantities of acid, which overcome the alkaline reaction of the slant, yielding an acid slant over an acid deep (A/A). An organism incapable of fermenting glucose produces no change in the indicator and is characterized by an alkaline slant over an alkaline deep (K/K). A sulfur source, sodium thiosulfate, provides sulfur atoms to detect the production of H₂S, H₂S reacts with iron salts (ferrous sulfate or ferric ammonium citrate) to produce the black precipitate of ferrous sulfide.

The production of gas during fermentation is indicated by the presence of cracks in the medium or the "pulling away" of the medium from the walls of the test tube. Materials Selected members of Enterobacteriaceae

Nonfermentative gram-negative bacilli TSI slants Procedure 1. use single, isolated 18 to 24 hour colony 2. select colony with sterile needle and stab within 1/4 inch of the bottom of the agar 3. streak colony up slant 4. leave cap on loosely and incubate at 35 to 37°C for 18 to 24 hours. 5. Read and interpret results Quality Control Salmonella typhimurium: alkaline/alkaline (purple/purple) ??? H₂S positive ??? Shigella flexneri: alkaline/acidic (purple/yellow) ??? H₂S negative ??? Proteus vulgaris: red/yellow Notes 1.

H₂S producing strains of Proteus may not blacken this media 2. Morganella morganii does not consistently produce a red color after 24 hours incubation

Indicators: Phenol red H₂S indicators: ammonium iron citrate, sodium thiosulfate ??? acid state ??? yellow ??? alkaline state ??? red

Sugars present: glucose or dextrose, lactose and sucrose Results to be observed 1.

fermentation of sugar 2. reduction of gas 3. production of hydrogen sulfide A.

Uninoculated: Orange butt & slant B. A/A G ??? acid slant: yellow ??? acid

butt: yellow ??? gas production: positive o bubbles or displacement of the medium ??? hydrogen sulfide production: negative o no blackening of the butt ??? sugars fermented: glucose/dextrose, lactose and sucrose (LFO) C. K/A G w/ H₂S ??? Alkaline slant: red ??? Acid butt: yellow ??? Gas production: positive o bubbles or displacement of the medium ??? Hydrogen sulfide production: negative o no blackening of the butt ??? Sugar/s fermented: glucose/dextrose only (NLFO) D.

K/K or K/N ??? alkaline slant: red ??? alkaline or neutral butt: red or orange ??? gas production : negative o no bubbles & no displacement of medium ??? hydrogen sulfide production: negative o no blackening of the butt ??? sugars fermented: none (NLFO) E. K/A G+ ??? alkaline slant: red ??? acid butt: yellow ??? gas production: positive o bubbles or displacement of medium ??? hydrogen sulfide production: positive o blackening of the butt ??? sugars fermented: glucose/dextrose only (NLFO) F. A/A G+ ??? acid slant: yellow ??? acid butt: yellow ??? gas production: positive o bubbles or displacement of the medium hydrogen sulfide production: positive o blackening of the butt ??? sugar/s fermented: glucose/dextrose, lactose and sucrose (LFO) G. K/A ??? alkaline slant: red ??? acid butt: yellow ??? gas production: negative o no bubbles/no displacement of the medium ??? hydrogen sulfide production: negative o no blackening of the butt ??? sugar/s fermented: glucose only (NLFO) Quality Control ??? Proteus mirabilis: motile ??? growth extending laterally from line of inoculation ??? Klebsiella pneumoniae: non-motile ??? growth only along line of inoculation Summary of TSI Reactions Reactions | Carbohydrate Fermented | Typical Organisms | | A/@ H₂S | Glucose with acid and gas | Escherichia, Klebsiella | | | | | |

Lactose and/or sucrose with acid | Enterobacter | | | and gas | | | K/@ H₂S |
 Glucose with acid and gas | Salmonella, Proteus | | | | | | Lactose or sucrose
 not fermented | | | | Citrobacter | | K/A H₂S - | Glucose with acid; no gas |
 Shigella, Providencia | | | | | | Lactose or sucrose not fermented | Serratia,
 Anaerogenic | | | Escherichia coli | | K/K H₂S - | Glucose not fermented |
 Pseudomonas | | | | | | Lactose or sucrose not fermented | | | | Alkaligenes |
 | A/@H₂S + | Glucose fermented with gas; | Citrobacter freundii | | | lactose
 or sucrose fermented | | | Note: those species of Proteus that ferment
 sucrose may produce an acidic | | slant. | | *A-acid; @- acid and gas; K-
 alkaline (no change) | Triple sugar iron agar.

A ??? acid slant/acid butt with gas no H₂S (A/@) B ??? alkaline slant/acid
 butt, no gas, H₂S positive (K/A H₂S +) C ??? Alkaline slant/no change butt,
 no gas, no H₂S (K/NC) D ??? Uninoculated tube Simmons Citrate Reaction
 Purpose The citrate reaction is used to determine if a member of the
 enterobacteriaceae is capable of utilizing citrate as the sole source of
 carbon. No other protein or carbohydrate that might provide another carbon
 source must be present in the medium. The reaction is useful in
 identification of the lactose fermenting Enterobacteriaceae. Escherichia coli
 is a citrate negative, whereas Enterobacter and Klebsiella are positive.

Principle

Simmons citrate agar contains sodium citrate, which serves as the only
 carbon source. If the organism can utilize citrate, the sodium citrate is
 converted to ammonia, which is then converted to ammonium hydroxide.
 The alkalinity of indicator takes on its alkaline color, which is blue Medium
 Simmons citrate agar Procedure 1. Use single, well-isolated 18 to 24 hour

colony 2. select colony with sterile needle and streak citrate slant lightly 3. leave cap on loosely and incubate at 35°C for 18 to 24 hours Interpretation A positive test is indicated by growth with an intense blue color on the slant or solely the presence of growth. Compare to a uninoculated tube for correct interpretation.

A negative test is indicated by the absence of growth and no color change in the medium (remains green). False positive results may occur with an inoculum that is too heavy. Quality Control *Klebsiella pneumoniae*: blue with growth ??? positive *E. coli*: no growth without color change Indicator

Bromthymol Blue Test to be observed: Citrate utilization test Principle

Utilization of citrate as the sole source of carbon A. Uninoculated: green B.

Positive Reaction: Prussian blue (left tube) C. Negative Reaction: Green

Deaminase Reactions Purpose Deaminase activity can be determined using the amino acids phenylalanine or tryptophan. Only *Proteus*, *Providencia* and *Morganella* species possess the deaminase enzyme. Principle

Deamination of the amino acid results in a colored compound with the addition of 10% ferric chloride (FeCl_3): Phenylalanine Phenylpyruvic acid + 10% FeCl_3 (green) Tryptophan Indole-pyruvic acid + 10% FeCl_3 (brown)

Media and Reagents Phenylalanine or tryptophan agar or tablets 10% FeCl_3

Procedure 1. inoculate the agar slant (or tablet dissolved into 1 ml sterile distilled water) with a few pure colonies of the test organism. Replace cap 2. Incubate 24 hours at 35°C 3. Add four to five drops of 10% FeCl_3 to the agar surface or tube. Rotate the tube and mix gently to provide contact for reagent and media Interpretation appearance of an intense green color indicates a positive deamination for Phenylalanine ??? Appearance of a

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brown color indicates a positive deamination for tryptophan. Quality

Control ??? Proteus vulgaris: positive (green or brown color with FeCl₃) ???

Escherichia coli: negative (no color development with FeCl₃) Phenylalanine

deamination A ??? negative ??? slant remains colorless B ??? positive ???

appearance of an intense green color - fin -

comcom thanks, leigh, for getting half of

the work done! Hi to 2nd yr vbelles, roch, aina and andree! ----- A

B C B C A B C Glucose Pyruvic acid Mixed acid fermentation Acetoin pH < 4.

4 (red) + methyl red KOH + air Diacetyl Napthol + creatine Pink-red complex

Positive Phenylalanine deaminase Tryptophan deaminase