Wharton County Junior College

Microbiology Lab Manual

WCJC BIOL 2420

Fall 2018

Aseptic Technique Lab

A. Growth Medium

- contains nutrients that support microbial growth
- uses agar as a solidifying agent
- can be made selective due to nutrients, pH, antibiotics, etc.

1. Nutrient Agar
   - contains nutrients that support growth of a wide variety of bacteria
   - pH of 6.8
2. SDA – Sabouraud Dextrose Agar
   - higher dextrose content: 4%
   - lower pH: 5.6
   - favors fungal growth over bacterial
3. MacConkey Agar (used later in semester)
   - selective for Gram-negatives (Gram-positives inhibited by bile salts and dye)
   - differential: lactose fermenters turn agar pink; non-fermenters do not

B. Exercise

1. Obtain 1 NA plate and 1 SDA plate as well as 2 sterile swabs. You will be using the swabs to sample the environment. Samples may come from skin, nails, floor, shoes, money, cell phone, etc., but NO samples from bodily orifices/secretions or from the toilet!!!!
2. Label the bottom of each plate with your name, the date, the type of medium (NA or SDA), and the source of your specimen (doorknob, shoe, etc...). Each person in your group should select a different source so multiple sources are sampled.
3. Moisten a swab in the tube of sterile water provided at your table and proceed to roll the swab on the surface of your source to obtain a sample.
4. Transfer the sample to your NA plate by gently rolling the swab in a zig-zag pattern across the surface of the plate. Be careful not to gouge the agar. Dispose of the swab in the trash.
5. Repeat the process again with a fresh swab on the same source but this time transfer the sample to your SDA plate.

C. Growth Results

1. Distinguish between bacterial and fungal colonies (describe each).
2. Distinguish between types of growth on NA vs SDA medium (bacterial vs. mold vs. yeast).
3. Based on growth results, are microbes <u>ubiquitous</u>?

D. Aseptic Technique

1. Why is aseptic technique necessary/important?
2. Identify ways to practice aseptic technique.

Basic Microscopy

A. Types of Microscopes

1. Light Microscopes
   - use visible light as source of illumination
   a. Simple Microscope
      - contains a single magnifying lens
      - magnifies up to ~ 300x
   b. Compound Microscope
      - contains more than one magnifying lens
      - <u>total magnification</u> = ocular lens power x objective lens power
      - magnifies up to 1,000x
2. Electron Microscopes
   - use an electron beam as source of illumination
   - use magnets to focus the beam of e-s
   - <u>cannot</u> be used to view living specimens
   a. Transmission Electron Microscope
      - magnifies up to 1 million x
      - useful for studying interior of cell
   b. Scanning Electron Microscope
      - gives extremely detailed 3-D view of objects

B. Structure & Function of the Parts of the Microscope

1. Ocular (eyepiece) – magnifies 10x
2. Objectives: scanning – magnifies 4x
   low power – magnifies 10x
   high power – magnifies 40x
   oil immersion – magnifies 100x
3. Revolving nosepiece – supports objective lenses and rotates them into position
4. Stage – holds slide
5. Mechanical stage control – slide holder and knobs that position slide
6. Stage aperture – opening in stage that allows light to illuminate specimen
7. Diaphragm – controls amount of light entering stage aperture
8. Condenser – concentrates light source into a cone of light that passes through diaphragm
9. Coarse-adjustment knob – brings specimen into focus quickly
10. Fine-adjustment knob – slowly brings specimen into sharper focus
11. Arm – supports coarse- and fine-adjustment knobs; holds head and stage
12. Head – holds oculars and houses mirrors
13. Base – houses light source, supports and stabilizes entire microscope

C. Use & Care of the Microscope – Proper Microscopy Technique

1. Carry the microscope with one hand grasping the arm and the other hand underneath the base.
2. Use lens paper to clean the lenses.
3. Place a slide on the stage, secure with stage clips, and move the stage all the way up.
4. Turn on the light source and center the specimen in the beam of light.
5. View the specimen on scanning power. Use the coarse adjustment and then fine adjustment to focus.
6. Be sure to always center the specimen in your field of view before rotating the nosepiece to change objectives.
7. Under low power, again use both the coarse and fine adjustment knobs to focus.
8. Use ONLY fine adjustment to focus when using high power or oil. (**Never** use the coarse adjustment when viewing a specimen under high power or oil because it may damage the slide and lens.)
9. After examining the specimen, turn off the light source and allow the bulb to cool before moving.
10. Put the scanning objective back in position, coil up the cord properly, and cover with the dust cover before storing.

D. Oil Immersion Procedure for 1,000x magnification

1. Focus on specimen under high power.
2. Rotate between high power and oil immersion objective.
3. Place a drop of immersion oil on the slide. (Do not to touch tip of the oil dispenser to the slide.)
4. Rotate the oil immersion objective into place. (The lens will dip into the oil.)
5. Slowly use fine adjustment to bring into clear view.
6. **Never** return to a lower objective with oil on the slide!
7. When finished viewing, remove and dispose of slide.
8. **Clean objective lens by putting lens cleaner on lens paper!!**

E. Exercise

1. View each of the following slides using proper microscopy technique:
    a. prepared slide of human blood smear
    b. prepared slides of microorganisms as time allows
2. Sketch the specimens at different magnifications: 40x, 100x, and 400x. Observe the difference in appearance as you progress from lower to higher magnification. Use oil immersion only if directed to do so by the instructor.

Media Inoculation

A. Culturing Microbes

1. Inoculation

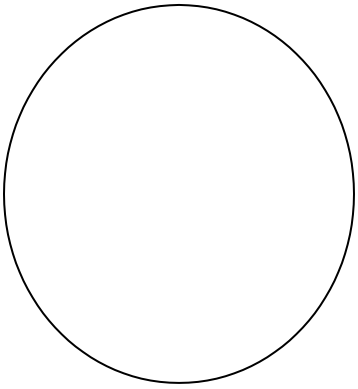
- a sample is transferred to the growth medium - provides ample nutrients and space; may be solid or liquid
- use sterile swabs, inoculating loops, or inoculating needles

2. Incubation

- allow to grow at optimum T: usually 20-40^{0}C (68-104 ^{0} F)
- may be at room temperature or inside incubator
- cell number increases rapidly

3. Isolation

- some inoculation techniques separate bacterial cells to form individual colonies – contain a single type of bacteria
- subculturing will eventually achieve a pure culture – a single species present in the medium
- we will use the streak plate technique to achieve isolation



Quadrant Streak

4. Inspection

- look at microbe's characteristics both macro- and microscopically
- macro: colony morphology, color, consistency, (smell),...
- micro: shape, arrangement, stain results,…

5. Information Gathering

- additional tests for function and characteristics
- biochemical tests, immunological tests, genetic typing,...

6. Identification

- use analysis and results of inspection and information gathering to identify species

B. Media Inoculation Technique

Always label tubes and plates to be inoculated.

Always practice aseptic technique when inoculating:

1. Wash hands and disinfect countertops.
2. Sterilize loop or needle by holding in open flame. Also flame the neck as well as the needle tip or loop when inoculating tubes. Be sure to allow tool to cool before touching culture.
3. Keep lids on plates and tubes when not in use.
4. Hold tubes at an upward angle.
5. Flame the opening of tubes.

Broth

- used to grow large numbers quickly

1. Use sterile loop to transfer sample from broth culture into sterile broth.

Plate

- used for isolating and determining colony morphology

1. Perform the streak technique as demonstrated by instructor.
2. Be sure to sterilize the loop between each successive section.

Slant

- same use as plate, but conserves material and space

1. Use sterile loop to obtain sample from broth culture.
2. Position loop at base of slant surface; zig-zag upward over surface.

Deep

- may be solid or semi-solid; used to determine O_2 requirements, motility

1. Use sterile needle to obtain sample from broth culture.
2. Perform stab by inserting needle into center of deep and extending downward almost to the bottom.
3. Withdraw needle up through same pathway.

Simple Morphological Stains

Simple Morphological Stains

- use only 1 dye; for determining size, shape, and arrangement
- include positive (direct) stains and negative stains

A. Positive Stain (Direct Stain)

- positively-charged (basic) dye binds to negatively-charged cell surface
- positive dyes: methylene blue, crystal violet, safranin

Procedure

1. Work in pairs, one partner staining a bacillus and one staining a coccus.
2. Smear Preparation:
   a. Place a loopful of distilled water onto the slide and then emulsify a loopful of bacteria into the water. Use the loop to spread the mixture out in a wide rectangular area.
   b. Allow the slide to air dry.
   c. Heat-fix the slide by gently passing it over the Bunsen burner flame a few times. This will cause the bacteria to adhere to the slide and not be rinsed off as easily during the staining process.
3. Apply a few drops of stain to the smear and allow to sit for 1 minute. Be sure to cover the entire smear with stain.
4. Gently rinse the stain with distilled water. Spray the water at the top of the slide and allow it to run over the smear rather than spraying directly onto the smear.
5. Blot the slide with bibulous paper.
6. Examine with the microscope and sketch observations. Sketch the bacillus at 400x and the coccus at 1,000x.

B. Negative Stain

- negatively-charged (acidic) dye is repelled by negatively-charged cell surface
- no heat fixation required, so no distortion of shape
- negative dyes: nigrosin, India ink, Congo red

Procedure

1. Work in pairs, one partner staining a bacillus and one staining a coccus.
2. Place a small drop of stain near the end of a clean slide.
3. Use a sterile inoculating loop to add a loopful of distilled water to the stain.
4. Use the loop to transfer a small amount of bacteria into the water/stain mixture.
5. Emulsify by using the loop to stir the mixture.
6. Use the end edge of another glass slide to spread the mixture along the slide.
7. Allow the smear to air dry.
8. Observe with the microscope and sketch observations. Sketch the bacillus at 400x and the coccus at 1,000x.

Bacterial Motility

A. Bacterial Motility

- true motility is achieved by the rotation of flagella
- motion due to collision with molecules of surrounding medium is *Brownian* movement and not true motility
- flagella are too thin to be seen with the compound light microscope

1. Flagella Stain

- special staining process leaves a precipitate around flagella so that they appear larger

a. Observe and sketch prepared slides of flagella stains:
   1) *Proteus vulgaris*
   2) *Spirillum volutans*

b. Label the flagellar arrangement of each specimen as amphitrichous or peritrichous.

2. Semi-solid Media

a. Inoculate motility medium by performing a stab.
b. Incubate until the following week.
c. Observe growth pattern:
   1) growth only along stab line → non-motile
   2) growth spreading through medium → motile

3. Hanging Drop Procedure

a. Place a small dab of petroleum jelly at each corner of a cover slip.
b. If using broth culture, place a loopful of broth onto center of cover slip. If using a plate culture, place 2 loopfuls of distilled water onto center of cover slip and stir in a small sample of bacterial culture.
c. Place depression slide on top of cover slip so that concavity covers drop.
d. Invert slide so that cover slip is on top and the drop is hanging into the concavity before placing slide on microscope.
e. Allow instructor to view on low or scanning before proceeding to high power. Observe movement at 400x on several different slides throughout the class.

Culture Characteristics of Bacteria

A. Culture Characteristics

- unique growth pattern of a particular bacterial species exhibited on different forms of media such as broth, deep, plate
- used to help distinguish between types of bacteria and to identify species
- must be combined with other characteristics (staining, biochemical) to completely identify bacterial species

1. Inoculation

a. Work in groups of three to five people.
b. You will receive 5 broth cultures of bacteria: *Bacillus, Micrococcus, Pseudomonas, Staphylococcus*, and an Unknown.
c. Pre-label 1 sterile broth, 1 deep, 1 motility tube, and 1 plate for each of the bacterial broth cultures.
d. Use aseptic technique to properly inoculate each of the pre-labeled forms of media.
e. Incubate until next week.

2. Inspection & Identification

- inspect each form of media of each bacterial culture and record observations in the Culture Characterization Table

a. **Broth** (a species may have multiple broth characteristics)
   - turbid: cloudy
   - sediment: collection of cells at bottom of tube
   - pellicle: thin film across surface
   - ring: residue around perimeter of surface
   - flocculent: containing small masses or flakes

b. **Deep** – shows O_2 requirements
   - obligate aerobe: growth only on surface
   - facultative anaerobe: growth throughout with more at surface

c. Motility Tube
   - motile: growth spreading through semi-solid agar
   - non-motile: growth only along stab line; no spreading

d. **Plate** - shows colony morphology
   - pigmentation: color
   - appearance: shiny or dull
   - texture: smooth or rough
   - size: pinpoint, small, medium, large
   - shape: circular, irregular, filamentous

- use your findings to determine the species of your Unknown

Culture Characteristics Table

Bacteria	**Broth**	**Deep**	**Motility Tube**	**Colony Morphology (Plate)**
Bacillus				
Micrococcus				
Pseudomonas				
Staphylococcus				
Unknown				

What is the genus of your Unknown? _____

Fungi: Yeasts & Molds

A. Kingdom Fungi

- includes both macroscopic and microscopic members
- microscopic members include yeasts and molds

Yeasts	vs.	**Molds**
- single-celled		- multicellular
- oval shape		- consist of filamentous hyphae
- reproduce by budding		- reproduce by spore formation

B. Observations

- observe and sketch prepared slides of:

1. ***Penicillium*** mold
   - produces penicillin
   - sketch on high power and label conidia – identify as naked or covered, sexual or asexual

2. ***Rhizopus*** mold
   - black bread mold
   - sketch on low power
   - label sporangiospores enclosed w/in sporangia
   - label zygospores (on different area of slide)
   - identify structures as sexual or asexual

3. ***Candida albicans*** yeast
   - cause of yeast infections
   - note dimorphism
   - sketch on high power; label yeast form and hyphae

4. ***Saccharomyces*** yeast
   - used in production of bread and beer (baker's yeast)
   - sketch on high power and label buds – identify as sexual or asexual

Direct Measure of Microbial Growth

Microbial populations are usually very large, therefore most methods of measuring cell numbers involve diluting the original sample several times so that the diluted samples contain fewer cells for more manageable counting. The number of cells in a small amount of diluted sample is counted using methods such as the plate count method or direct microscopic count method; then calculations are used to determine the size of the total population in the original sample.

In the **plate count** method, a diluted sample is placed on a plate for growth. After incubation, the number of visible colonies is counted. Each colony represents a colony-forming unit (CFU), either a single cell or a group of cells that gave rise to the colony, so the number of colonies corresponds to the number of cells in the sample. An advantage of the plate count method is that it measures only viable cells. A disadvantage of this method is the time required for incubation.

In the **direct microscopic count** method, cells are viewed through the microscope and directly counted. A measured volume of diluted sample is placed on a counting chamber slide with defined areas that hold known volumes. Several cell counts in different areas of the counting chamber are done and averaged together to get a better estimate of the number of cells in the total sample. A disadvantage of the direct count method is that it measures both living and dead cells. An advantage of this method is that it can be done rapidly because no incubation time is required.

The original sample is diluted several times in both the plate count method and the direct count method. Therefore the dilution factor must be taken into account when calculating the size of the total population of the original sample.

Direct Microscopic Count of Yeast Cells

In today's lab, we will be using a counting chamber slide which is subdivided into defined areas called B squares, each holding a volume of $4x10^{-6}$ millliters. Once the number of cells has been counted in several different B squares, the average number of cells per B square can be calculated. The number of cells per milliliter can then be determined by dividing the average number of cells per B square by the volume of each B square. Also, since the original sample is diluted, the dilution factor will be taken into account in the final calculations to determine the cell number of the original sample.

1. Place a disposable counting chamber on the microscope stage. Using the scanning objective, find the ruled area and note the size and arrangements of the squares making up the grid.

2. Pipette 1mL of the yeast suspension provided by the instructor into a test tube containing 1 mL of water in your test tube rack. Replace the lid on the test tube. (You now have a 1:2 dilution of the yeast suspension.)

3. Use the vortex stirrer in the room to shake your test tube of yeast mixture for a few seconds in order to distribute the cells evenly. **Be sure the lid is on the test tube when you do this!**

4. Take the counting chamber from the microscope (without changing the focus on the 4x objective) and use a pipette to transfer some of the yeast mixture from your test tube into a well opening of the counting chamber. Note: the tip of the pipette needs to touch the well opening to prevent air bubbles. Capillary action will occur to fill the chamber and move the yeast suspension from the opening of the well into the grid area. If you do not observe capillary action occurring within 3 minutes, repeat the process with the other counting chamber on the same slide.

5. Once you see the capillary action occurring, use a paper towel to gently dab any excess yeast mixture from the surface of the slide. Then carefully place the counting chamber back onto the microscope stage and observe the cells under scanning power. You may need to reduce the amount of light by closing the diaphragm.

6. Work up to the high power objective to count the number of yeast cells in a **B square** (see image on next page). To avoid counting the same cell twice: if it falls on a line, include in your count those cells on the top and left-hand lines and exclude those on the bottom and the right-hand lines. (If the yeast cells are too dense to count, dilute your sample as in step 2 above and start again.) Each person will select 2 B squares to count and average.
   Record your numbers below and compare results with your partner.
   Number of cells in first B square selected _____
   Number of cells in second B square selected _____
   Total number of cells in the two B squares selected _____
   Average number of cells per B square _____

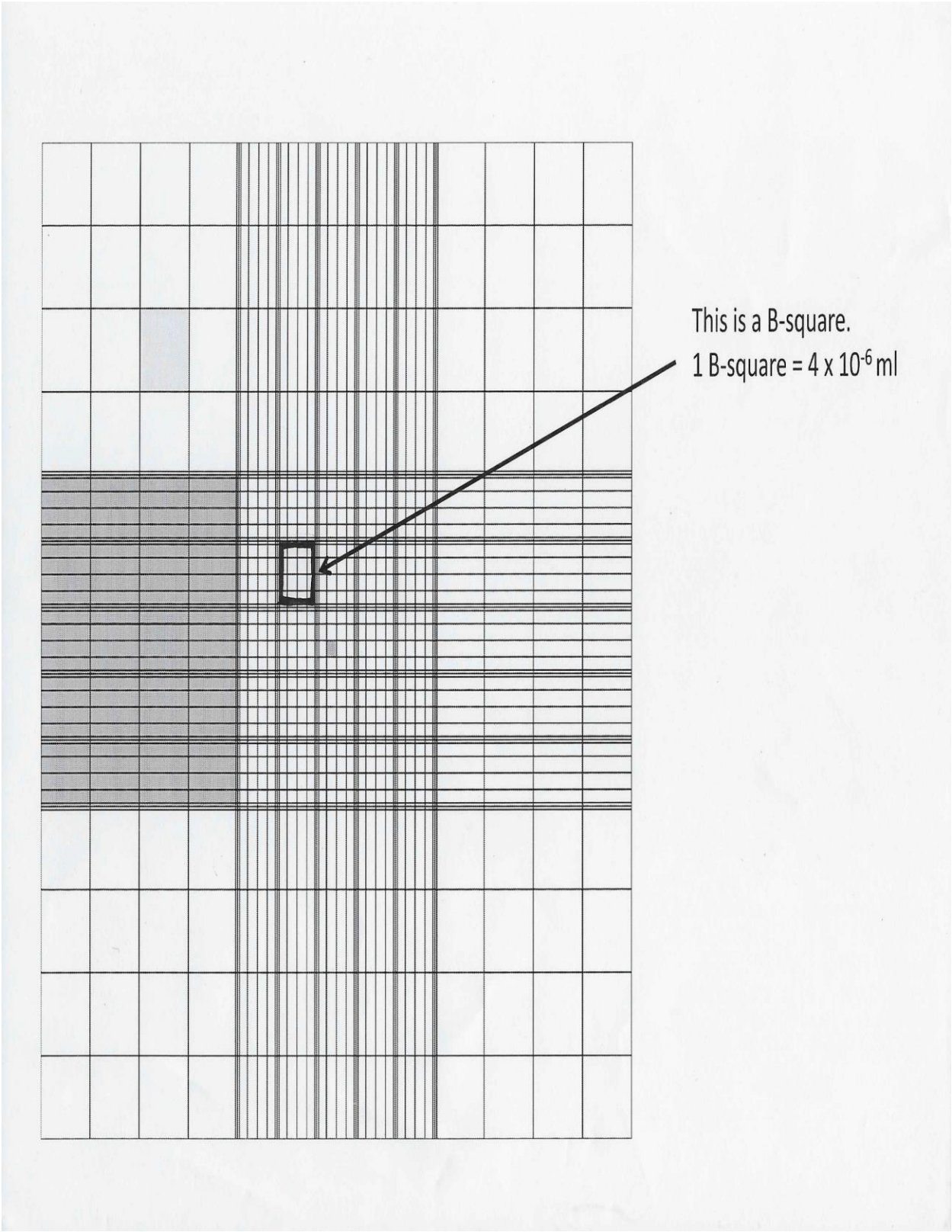
7. Now calculate the number of yeast cells per milliliter by dividing the average number of cells per B square by the volume of each B square. (The volume of each B square is $4x10^{-6}$ millliters.)

   Concentration of cells = $\frac{\text{Average number of cells from step 6}}{\text{Volume of each counting square}} = \frac{\text{Average number of cells from step 6}}{4x10^{-6}\text{ millliters}}$ = _____

8. If you diluted the sample you must also multiply your results by the dilution factor to determine the concentration of yeast cells in the *original* sample. (You know you have a dilution factor of at least 2 from step 2 above).

   Concentration of yeast cells in original sample = _____ x _____ = _____
   (cells/mL) (dilution factor)

Counting Chamber Slide



Biochemical Tests

A. Biochemical Tests

- used to evaluate metabolic properties of specific bacteria
- used in conjunction with staining and culture characterization to definitively identify bacteria

1. Catalase Test

The catalase enzyme catalyzes the breakdown of H_2O_2 (hydrogen peroxide) into H_2O and O_2. If bacteria have the catalase enzyme, bubbling will occur from the breakdown of H_2O_2.

Negative result: no bubbling
Positive result: bubbling

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2 \text{ (bubbles)}$$

2. Denitrification Test

In denitrification, the substance potassium nitrate is converted to nitrogen gas. If bacteria have the nitrate reductase enzyme to break down the nitrate (NO_3) in nitrate broth, there will be nitrogen gas (N_2) bubbles produced. Note the small inverted tube in the bottom of the nitrate broth medium. This is called a durham tube and collects gases generated by the culture.

Negative result: no bubbles in durham tube
Positive result: bubbles in durham tube

$$NO_3 \xrightarrow{\text{nitrate reductase}} N_2 \text{ (bubbles)}$$

3. Hydrogen Sulfide (H_2S) Production

Sulfide Indole Motility (SIM) media contains the amino acid cysteine as well as iron. If bacteria have the cysteine desulfurase enzyme to catalyze the breakdown of cysteine to produce H_2S, the H_2S will combine with the iron to produce iron sulfate which blackens the medium.

Negative result: no color change
Positive result: blackening of the medium

$$\text{cysteine} \xrightarrow{\text{cysteine desulfurase}} H_2S$$
$$H_2S + Fe \rightarrow \text{blackening}$$

4. Indole Production

SIM media also contains the amino acid tryptophan. If bacteria have the tryptophanase enzyme that catalyzes the breakdown of tryptophan to indole, the indole will react with Kovac's reagent to form a red color.

Negative result: no color change
Positive result: red color

$$\text{tryptophan} \xrightarrow{\text{tryptophanase}} \text{indole}$$
$$\text{indole} + \text{Kovac's} \rightarrow \text{red}$$

5. **Methyl Red Test**
   Methyl red pH indicator is a yellow to orange color at slightly acidic levels (5-6), but will turn red at more acidic levels (below 4.4). If bacteria have multiple enzymes that break down sugars and produce enough acid to drop the pH to about 4, the pH indicator will turn red. If bacteria do not have multiple enzymes for the breakdown of sugar, fewer acids will be produced and the pH indicator will remain yellow.
   Negative result: yellow — glucose → pyruvic acid only
   Positive result: persistent red color — glucose → pyruvic, acetic, formic

6. **Oxidation-Fermentation Test**
   The Oxidation-Fermentation test determines oxygen requirements for the breakdown of sugar. O-F media contains sugar as well as a color indicator that is green at neutral pH and turns yellow when the pH drops below 6. If bacteria have the enzyme to catalyze the breakdown of sugar, acid will be produced and the medium will turn yellow. The addition of oil to a tube creates an anaerobic environment; the absence of oil renders the environment aerobic.
   Inert to the medium (O_2 is irrelevant): both tubes stay green
   Obligate aerobe: tube w/ oil remains green; tube w/out oil turns yellow
   Facultative anaerobe: both tubes turn yellow

B. Session 1 – Inoculation

Work in groups of 3-5. Label all biochemical media tubes with the genus with which it is to be inoculated as indicated below.

1. **Catalase Test**
   Inoculate 1 plate with *Staphylococcus*.

2. **Denitrification Test**
   Inoculate 1 nitrate broth tube with *Alcaligenes* and 1 with *Pseudomonas*.

3. **H_2S Production**
   Inoculate 1 SIM tube with *E. coli* and 1 SIM tube with *Proteus*.

4. **Indole Production**
   Inoculate the remaining SIM tube with *Enterobacter*.

5. **Methyl Red Test**
   Inoculate 1 MR-VP tube with *Enterobacter* and the other with *E. coli*.

6. **Oxidation-Fermentation Test**
   Inoculate 2 O-F tubes with *E. coli* and 2 with *Pseudomonas*.
   Place a dropper of mineral oil in 1 of the *E. coli* and also in 1 of the *Pseudomonas*.

C. Session 2 – Results

1. Catalase Test

Place 2 drops of hydrogen peroxide on a clean slide. Emulsify a loopful of *Staphylococcus* into the peroxide.

Expected results*: Staphylococcus* – catalase-positive

2. Denitrification Test

Examine the durham tubes in the nitrate broth for bubbles.

Expected results: *Pseudomonas* – denitrification-positive
Alcaligenes – denitrification-negative

3. H_2S (hydrogen sulfide) Production

Examine the SIM tubes inoculated with *Proteus* and *E. coli* for color change.

Expected results: *Proteus* – H_2S-positive
E. coli - H_2S-negative

4. Indole Production

Place 5 drops of Kovac's reagent into the SIM tubes that were inoculated with *E. coli* (also used previously in test 3) and *Enterobacter*.

Expected results: *E. coli* – indole-positive
Enterobacter – indole-negative

5. Methyl Red Test

Place 5 drops of methyl red pH indicator in each MR-VP tube. Gently swirl the tube and examine for color change.

Expected results: *E. coli* – methyl-red-positive
Enterobacter – methyl-red-negative

6. Oxidation-Fermentation Test

Examine the O-F tubes for color changes. Be sure to note whether or not oil is in each tube.

Expected results: *Pseudomonas* – obligate aerobe
E. coli – facultative anaerobe

Biochemical Test Results Table

Biochemical Test	Medium	Reagent	Observations	Results	Interpretation
Catalase Test:					
Staphylococcus					
Denitrification:					
Pseudomonas					
Alcaligenes					
H_2S Production:					
Proteus					
E. coli					
Indole Production:					
E. coli					
Enterobacter					
Methyl Red Test:					
E. coli					
Enterobacter					
Oxidation-Fermentation:					
E. Coli					
Pseudomonas					

Protozoa

Protozoa

- single-celled, heterotrophic protists

A. Prepared Specimens

1. Amoeba

- agent of amebic dysentery
- observe and sketch prepared slide on low power
- label nucleus and pseudopodia

2. Trypanosoma

- blood parasite (Chaga's disease; African sleeping sickness)
- observe and sketch prepared slide on high power
- label erythrocytes and flagella
-

3. Paramecium

- free-living
- observe and sketch prepared slide high power
- label cilia

4. Plasmodium

- non-motile agent of malaria; vector is a mosquito
- observe and sketch prepared slide at high power
- label erythrocytes and ring stage

B. Live Specimens

- if time allows, observe live protozoan specimens available in a culture mix from your instructor or in water collected from a ditch or horse trough
- note the use of motility structures in any motile specimens observed

Helminths

Helminths
- parasitic worms

A. Roundworms

1. Ascaris
- intestinal roundworm parasite
- observe and sketch adult specimens in jars and mounts
- distinguish between male and female; label spicule of male

2. Enterobius
- **pinworm**; intestinal roundworm parasite
- observe and sketch prepared slide of adult female on low power
- note pointed female tail for depositing eggs

3. Trichinella
- roundworm parasite of final host; larvae encyst in muscle
- observe and sketch prepared slide of larvae on low power
- label larvae and muscle tissue

B. Flatworms

4. Fasciola
- **fluke**: observe adult specimen in jar, noting flat body

5. Taenia
- **tapeworm**; intestinal parasite of carnivores
- observe prepared slide of adult on low power
- note: slide has 4 sections – start with scolex and progress to more mature proglottids as you move toward opposite side
- sketch scolex and label hooks and suckers
- observe immature proglottids (not filled with eggs)
- observe gravid / mature proglottids (filled with eggs)

Identification of Unknown

Week 1

1. Record broth characteristics.
2. Inoculate TSA and MacConkey agar plates using quadrant streak technique.

Week 2

1. Record colony characteristics from TSA streak plate.
2. Use culture sample from TSA plate to perform a Gram stain and record cell shape, cell arrangement, and Gram result.
3. Double check your Gram result with the MacConkey agar plate results: Gram-positives will not grow on MacConkey agar; only Gram-negatives will exhibit growth on MacConkey agar. (Lactose-fermenters will turn the agar pinkish/red, but this color change does not factor into our analysis.)
4. Use the dichotomous key to determine which biochemical test should be run next and begin the test. (Remember that most of the tests require a particular medium be inoculated and results will not be available until the following week.)

Week 3

1. Record biochemical test results from previous week.
2. Use the dichotomous key to determine which biochemical test should be run next and begin the test.

Week 4

1. Record biochemical test results from previous week.
2. Use the dichotomous key to identify unknown by genus name.
3. Turn in Laboratory Report and Dichotomous Key (traced out).
   - be sure your name and student ID are on each paper

Laboratory Report

Identification of a Nonclinical Bacterial Unknown

Name: _____ Unknown Code: _____

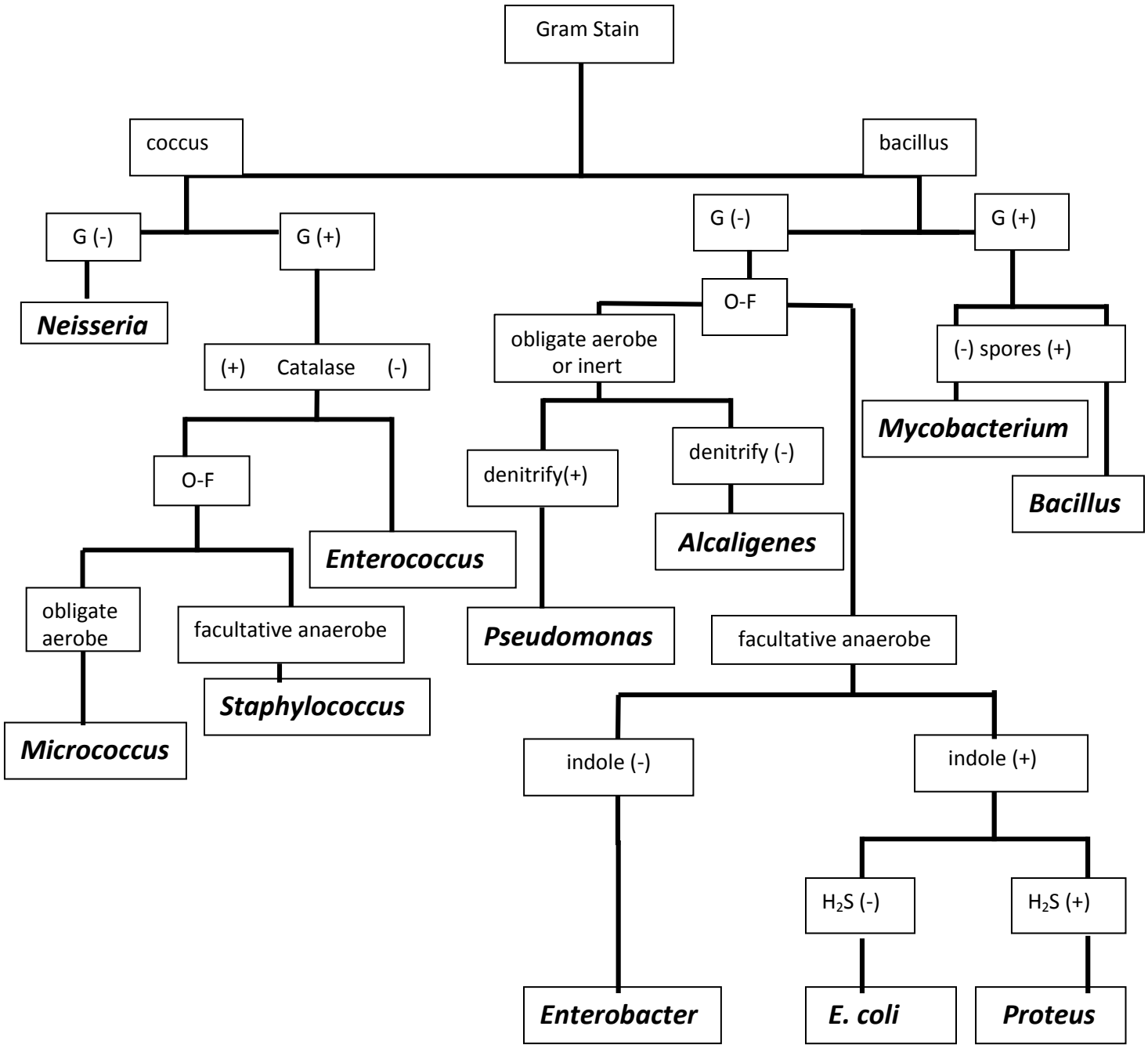
Culture Characteristics	Observations
Broth Characteristics	
Colony Characteristics from TSA plate	
	Observation and Interpretation
MacConkey Agar	

Staining Characteristics	Observations	Results	Interpretation
Cell Shape		xxxxxxxxxx	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
Cell Arrangement		xxxxxxxxxx	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
Gram Stain			

Biochemical Characteristics	Reagent Added	Observations	Results	Interpretation
Catalase Test				
Denitrification Test				
H_2S Production				
Indole Production				
Methyl Red Test				
Oxidation-Fermentation Test				

Conclusion
I conclude that my unknown is _____

Dichotomous Key for Identification of Unknown



Differential Stains: Capsule, Endospore, and Gram Stains

Differential Stains

- use 2 different dyes to differentiate between cell types or cell structures

A. Capsule Stain - differentiates between capsule, cell, and background

1. Observe a prepared slide of a capsule stain.
2. Sketch on high power, noting the capsule.

B. Endospore Stain - differentiates between spores and vegetative cells

1. Observe prepared slides of spore stains.
2. Sketch on high power, noting whether the endospore has a central or terminal location within the vegetative cell. Also note any free spores.

C. Gram Stain - differentiates chemical composition of cell wall:

Gram-positive	vs.	**Gram-negative**
- thick peptidoglycan		- thin peptidoglycan
- no outer membrane		- outer membrane (highly negative)
- stain purple		- stain pink

Components of Gram Stain

Primary stain: crystal violet – stains all cells purple
Mordant: Gram's iodine – fixes crystal violet into cell wall of G+ cells
Decolorizer: ethyl alcohol – removes crystal violet from G- cells
Counterstain: safranin – stains G- cells pink

Gram Stain Procedure

1. Prepare a smear of bacteria: Place 2 loopfuls of distilled water side by side on the slide. Add *E. coli* to one loopful of water and add *Staphylococcus* to the other. As you emulsify, overlap the 2 bacteria in between the original loopfuls of water so that there is a mixture of the bacteria in the center. Allow to air dry, then heat fix.
2. Apply crystal violet to the smear. Allow to sit for 1 minute and then rinse with distilled water.
3. Apply Gram's iodine and allow to sit for one minute. Rinse with distilled water.
4. Rinse gently with ethyl alcohol. Do not over-rinse - stop rinsing after a few seconds. Rinse with distilled water.
5. Apply safranin and allow to sit for 1 minute. Rinse with distilled water.
6. Blot dry with bibulous paper.
7. Observe on high power. Continue on to oil immersion as necessary.
8. Sketch and label the Gram-positive control, the Gram-negative control, and a mixed sample.
   Purple indicates Gram-positive while pink indicates Gram-negative.
   *Remember that the Gram-positive/Gram-negative designation is important for describing cell wall structure and thus appropriate treatment measures!

Microbial Control

A. Chemical Control

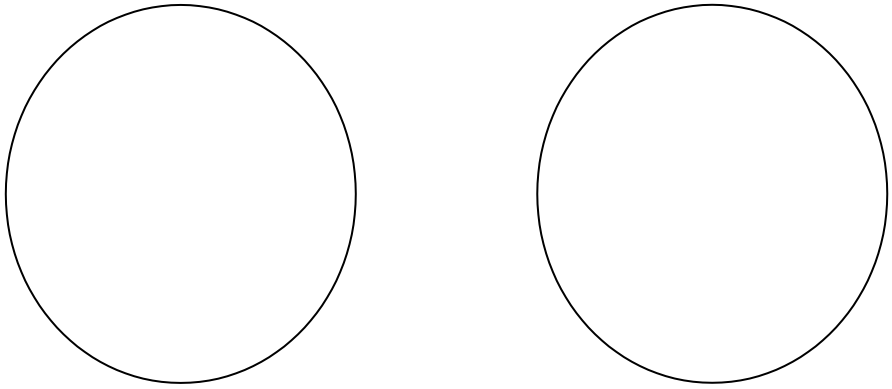
1. You will work in groups to test different active ingredients of antiseptics and disinfectants.
2. Obtain plates of growth medium and divide into “before” and “after” sides.
3. Label the plates as to which active ingredient is being tested.
3. Swab hands/surface before cleansing and place sample on the “before” side of the plate.
4. Cleanse your hands/surface using the antiseptic/disinfectant you have been assigned.
5. Swab hands/surface after cleansing and place sample on the “after” side of the plate.
6. Let incubate and then observe the following week. Record a description of growth for each side of the plate, noting both number and diversity of colonies.

Antiseptics - used on living tissue	**Description of Growth BEFORE**	**Description of Growth AFTER**
1.		
2.		
3.		
4.		

Disinfectants - used on inanimate objects	**Description of Growth BEFORE**	**Description of Growth AFTER**
1.		
2.		
3.		
4.		

B. Antibiotic Sensitivity Testing

- the Kirby-Bauer Method is done to test for antibiotic sensitivity
- Mueller-Hinton agar is used because of its density
- a bacti-spreader tool is used for spreading out a bacterial sample to produce solid growth called a lawn
- an antibiotic disc is then placed on the plate; the antibiotic will diffuse out from the disc through the growth medium
- a zone of inhibition indicates that the bacteria is sensitive to the antibiotic
- if bacteria are resistant, no zone of inhibition will be significantly apparent
- narrow spectrum antibiotics are effective against a small range of cell types
- broad spectrum antibiotics are effective against a wide range of cell types



Antibiotic	*Bacillus* (G+ rod)	*Staphylococcus* (G+ coccus)	*E. coli* (G- rod)
Ampicillin (Am)			
Bacitracin (B)			
Penicillin (P)			
Tetracycline (T)			
Vancomycin (Va)			

Food Microbiology

A. Yogurt

- prepared by the action of fermentative bacteria (i.e., *Streptococcus thermophilus*, *Lactobacillus acidophilus*) on milk
- the lactic acid produces a tart taste and causes the milk protein (casein) to precipitate, thickening the product

B. Procedure

1. Pipet 1 mL of distilled water into test tube.
2. Add a small sample (1 loop) of yogurt to the tube and mix.
3. Replace cap and gently shake for 5 to 10 seconds.
4. Transfer 2 loopfuls of tube mixture to slide and prepare a smear (including air drying and heat fixing).
5. Proceed with Gram stain procedure.
6. Observe on high power. Check with instructor before proceeding to oil.

C. Results

1. Sketch the 2 types of bacteria observed in the yogurt.
2. Label cell shape and cell arrangement.
3. What is the Gram reaction of these bacteria? Describe their cell wall.

Live Bacterial Cultures for BIOL 2420 - Microbiology

Alcaligenes faecalis
Intestinal flora. Alpha hemolysis on blood agar growth medium. Motile. Coccal rods. Gram negative reaction. Aerobic incubation temperature 37°C. Tryptic soy agar growth medium.

Bacillus cereus
Isolated from food. Presence in large amounts causes food poisoning. Central terminal spores. Motile. Rods; often in chains. Gram positive reaction. Aerobic. Incubation temperature 30°C. Tryptic soy agar growth medium.

Bacillus subtilis
Found in soil. Central spores, chains. Produces antibiotics bacitracin, subtilin, and bacillin. Motile. Rods. Gram positive reaction. Aerobic. Incubation temperature 30°C. Tryptic soy agar growth medium.

Escherichia coli
Often isolated from urinary tract infections. Normal intestinal flora. Lactose positive, indole positive, citrate negative. Motile. Rods. Gram negative reaction. Aerobic. Incubation temperature 37°C. Tryptic soy agar growth medium.

Enterobacter aerogenes
Found in soil, water, and sewage, as well as serves as food for protozoans. Normal intestinal flora. Motile. Rods. Gram negative reaction. Aerobic. Incubation temperature 37°C. Tryptic soy agar growth medium.

Micrococcus luteus (Kocuria rhizophila)
Found in water, air, soil, and on skin. Chromogenesis — yellow pigment produced. Nonmotile. Cocci; in tetrads. Gram positive reaction. Aerobic incubation temperature 30°C; will grow at 0–37°C. Tryptic soy agar growth medium.

Proteus vulgaris
Isolated from human urinary tract and wound infections. Putrefactive odor. Motile. Rods. Gram negative reaction. Aerobic. Incubation temperature 37°C. Tryptic soy agar growth medium. Note: moderate potential pathogen; only used after learning proper infectious control technique.

Pseudomonas aeruginosa
Isolated from human urinary tract infections, polluted water, and sewage. Noticeable odor of trimethylamine (grapes). May turn agar light green due to production of pyocyanine (blue pigment). Motile. Rods. Gram negative reaction. Aerobic. Incubation temperature 37°C. Tryptic soy agar growth medium. *Note:* moderate potential pathogen; only used after learning proper infectious control technique.

Staphylococcus epidermidis
Normal flora of human skin. Coagulase negative, catalase positive. Nonmotile. Cocci; grape-like clusters. Gram positive reaction. Aerobic. Incubation temperature 37°C. Tryptic soy agar growth medium.