

VIDEO: MICROBIAL DIVERSITY

1. What characteristic of microbes drives the evolution resulting in the ability to survive in most any conditions?
2. DEFINE:
 - a. Microbial diversity:
 - b. Metabolic diversity:
3. Why is a termite's gut a good source of microbial diversity? What kingdoms are represented?
4. Since eubacteria and archaeobacteria are too difficult to differentiate visually, how is it done?
5. Electrophoresis separates DNA according to _____.
6. One project underway at TIGR is to map the entire genome of *Thermatoga*.
 - a. What is the purpose/goal/question of this research?
 - b. Where is this organism found in nature?
 - c. The data pointed to what conclusion?
 - d. How did the scientist researcher interpret the data?
7. What is the relationship between variations in the DNA sequence and where the organism is placed on the phylogenetic tree?
8. What are the three lines of evolutionary descent shown on the phylogenetic tree? How are they different? Give an example of each.
9. Where are viruses placed on the phylogenetic tree? Explain.
10. As the researchers tried to place analyze DNA from microbes to place them in the phylogenetic tree, what inherent problem slowed their progress?
11. The development of what procedure quickened the ability of researchers to analyze microbial DNA?
12. How do microbes contribute to the success of life on earth?

GOING TO EXTREMES

1. Define extremophiles.

2. What are extremozymes? How are these enzymes different than the ones humans make?

3. What enzyme is used in the PCR process? Why?

4. Describe 2 examples of how extremozymes are used in industry.

HOW A MICROBE GOT ITS NAME

1. Define hyperthermophiles.

2. What was the original name of this microbe? Why?

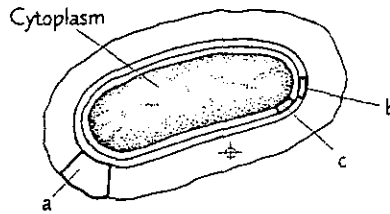
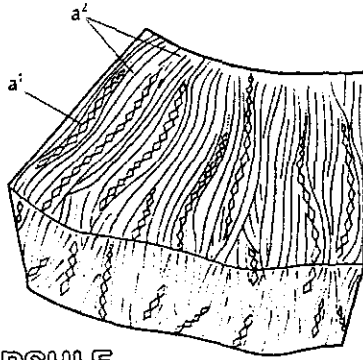
3. Why was it eventually called *Thermotoga maritima*?

4. Identify the genus and the species of this microbe.

Genus:

Species:

BACTERIAL CELL ENVELOPE

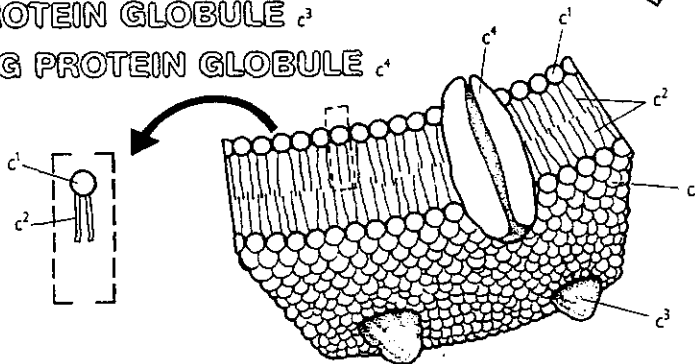
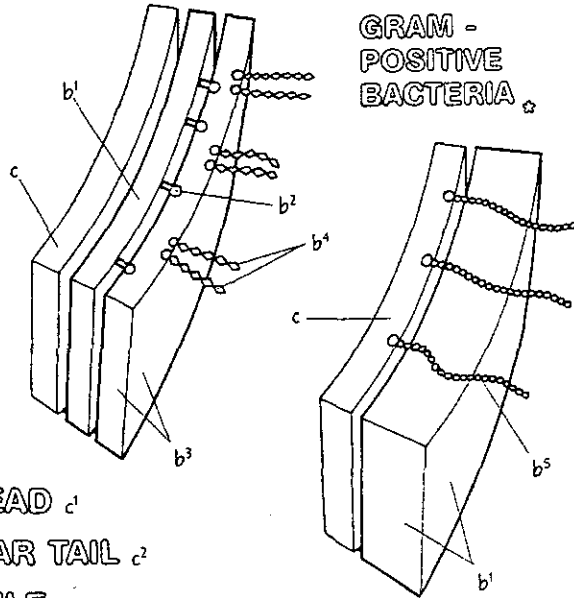


Bacterial cell envelope layers (magnified)

- CAPSULE a
- POLYSACCHARIDE a¹
- PROTEIN a²
- CELL WALL b
- PEPTIDOGLYCAN b¹
- LIPOPROTEIN b²
- OUTER MEMBRANE b³
- LIPOPOLYSACCHARIDE b⁴
- TEICHOIC ACID b⁵
- CELL MEMBRANE c
- PHOSPHOLIPID: POLAR HEAD c¹
- PHOSPHOLIPID: NON-POLAR TAIL c²
- SURFACE PROTEIN GLOBULE c³
- PENETRATING PROTEIN GLOBULE c⁴

GRAM -
NEGATIVE
BACTERIA ☆

GRAM -
POSITIVE
BACTERIA ☆



BCB: Bacterial Cell Envelope

1. What are the three layers of the bacterial cell envelope?
2. What are the three functions of the cell envelope?
3. Describe the structure and function of the capsule.
4. What is plaque?
5. Compare and contrast the cell wall in gram + and Gram - bacteria. Compare structure as well as reaction to the Gram stain.
6. Describe the structure and function of the cell membrane. What function is different than a eukaryotic cell membrane?

BCB: Bacterial Ultrastructure

Cell Structure	Type of Bacteria it is found in	Composition	Function
Flagella			
Capsule			
Cell wall			
Cell or plasma membrane			
Pili			
Mesosome			
Chromosome			
Nucleoid			
Plasmids			
Cytoplasm			
Ribosomes			
Inclusions			
Granules			

FLAGELLUM a

CAPSULE b

CELL WALL c

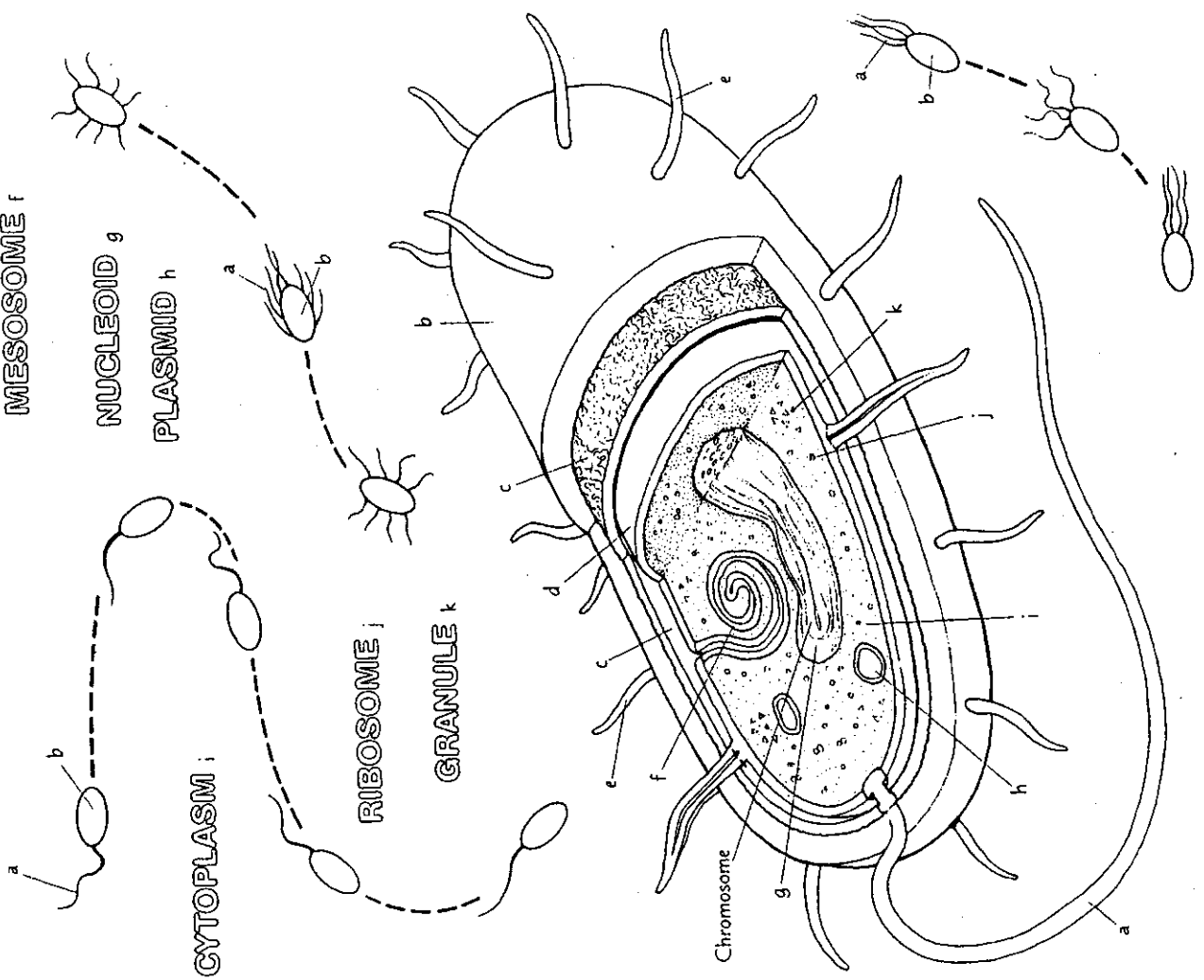
MESOSOME f

NUCLEOID g

PLASMID h

RIBOSOME i

GRANULE k



BCB: Bacterial Spores

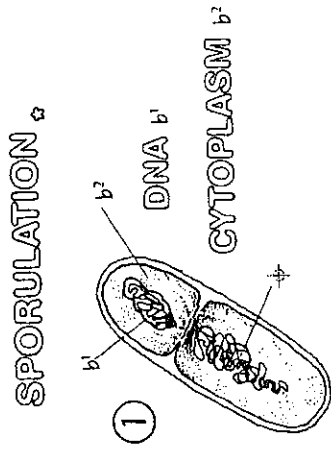
1. What type of bacteria form endospores? What two genus in particular?
2. Why can endospores survive for very long time periods?
3. How many endospores form from each bacterial vegetative cell? Why?

4. Summarize the structure of a spore in chart form. Include a description of composition and function.

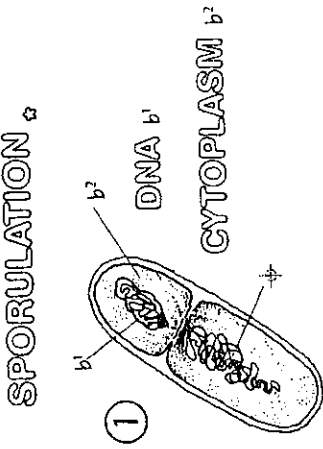
Spore layer	Composition	Function

5. Construct a simple, circular diagram showing the life cycle of the bacterial cell from one sporulation cycle to another. Briefly describe what is happening in each stage.

6. List four diseases that are caused by spore formers.



SPORULATION



DNA ^{b¹}
CYTOPLASM ^{b²}

1

2

3

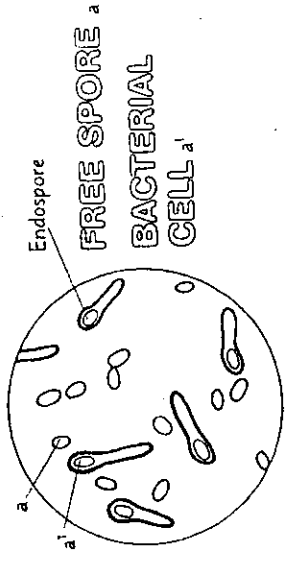
4

5

6

Disintegrating bacterium

SPORE STRUCTURE



Light microscopic view

SPORE CORE ^b

CORE MEMBRANE / WALL ^c

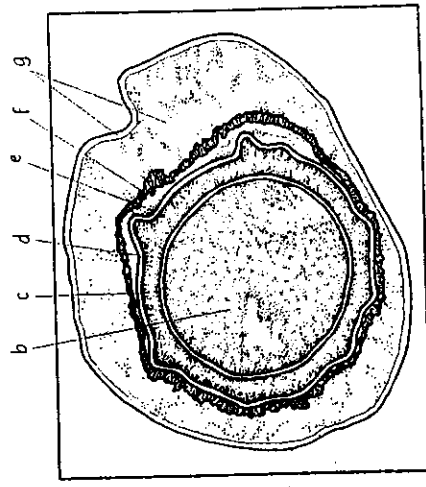
CORTIX ^d

INNER MEMBRANE ^e

OUTER MEMBRANE ^f

EXOSPORIUM

BASAL LAYER ^g



Electron microscopic view

Bacterial Growth and Cultivation

II. Inoculation is the introduction of _____ (the microbe sample) into _____

A. Sources of inoculums

1. _____: bodily fluids, discharges, tissue samples
2. _____: soil water, sewage, food, air, fomites

B. Types of Media classified by Form

- a) Liquid media (aka broths, milks, or infusions)
 - (1) _____ based plus various dissolved nutrients
 - (2) growth occurs _____ the substrate
 - (3) _____ indicates growth
 - (4) EX: _____

(a) Components: water, beef extract, _____ in _____ - partially digested protein rich

b) Solid Media

- (1) Contains _____
 - (a) Complex _____ derived from seaweed
 - (b) _____ at room temp. _____ at 100C, _____ at 42C
 - (c) Flexible, moldable
 - (d) Not digestible for microbes
- (2) EX: _____
 - (a) Components: water, beef extract, peptone and 1.5% agar

2. a) _____ Composition _____ (Chemically Defined Medium): exact chemical composition is known

b) _____: contains at least one ingredient that is _____ chemically definable such as _____ from plants or animals; blood infusion, peptone

I. Microbiologists use five basic techniques to manipulate, grow, examine and characterize microorganisms in lab.

1. Requires _____ to prevent contamination by unwanted microbes
2. Sample is placed in/on _____: a nutrient source

1. _____ controls the temperature and gas content to promote optimum growth.
2. Produces a _____: the visible growth of the microbe in the medium.

C. Separate microbes as evidenced by

- a) _____: large number of single cells clinging together to form discrete mounds on solid media.
- b) _____: cloudy appearance in liquid media. _____ further separates cells to make a _____ that contains only one species of microbe.

D. 1. Characteristics (color, size, texture, margins) of _____ are observed _____

2. Characteristics (cell size, shape motility) of _____ are observed _____
3. _____ may be used to verify presence or absence of _____

1. Based on gathered information and _____
2. May include results from specialized tests:
 - a) _____ tests
 - b) _____ tests
 - c) _____ tests

Selective/Differential Medium	Selective Agent	Used for
		Isolation/differentiation of intestinal pathogens: <i>E. coli</i> produces an acid thus creates red/pink colonies; <i>Salmonella</i> does not produce acid therefore creates white/cream colored colonies
		Isolation of fungi- inhibits bacteria
		Differentiate bacteria that break down fats (dk. Blue) from those that do not (no color)
		Differentiates between species of <i>Staphylococcus</i>

e) _____ (1) Cultivated in live animals, _____ or cultures of _____
 (a) EX: Leprosy, typhus

3. _____
- a) _____
 (1) Grows a broad spectrum of microbes
 (2) _____
 (3) EX: Nutrient agar, TSA (Trypticase Soy Agar)
- b) _____
 (1) used for _____ bacteria
 (a) require special _____ supplements like blood or amino acids
 (2) EX: Blood agar, "chocolate agar"
- c) _____
 (1) _____ the growth of certain microbes and _____ the growth of others
 (2) Used to _____ a specific type of microorganism from samples containing _____ different species.
- d) _____
 (1) Grows several types of microorganisms and display _____ amongst them.
 (a) Shows as _____ media color changes, formation of _____ and _____ produce colonies of different _____ in depending on _____
 (i) measures _____ or _____ of a solution
 (ii) O is most _____; 7 _____
 14 most _____

4. _____ or _____ content to encourage growth of culture.

- a) _____ bacteria is transferred to a tube containing melted and cooled _____.
- (1) _____ poured into _____ dish and allowed to _____.
- (2) _____ the agar as well as on the _____.
- (3) Colonies grow _____.

- b) _____
- (1) Melted nutrient agar is poured into petri dish and cooled.
- (2) A sample of _____ bacteria is placed on the _____ and spread with a _____.
- (3) Colonies form on the _____ only.

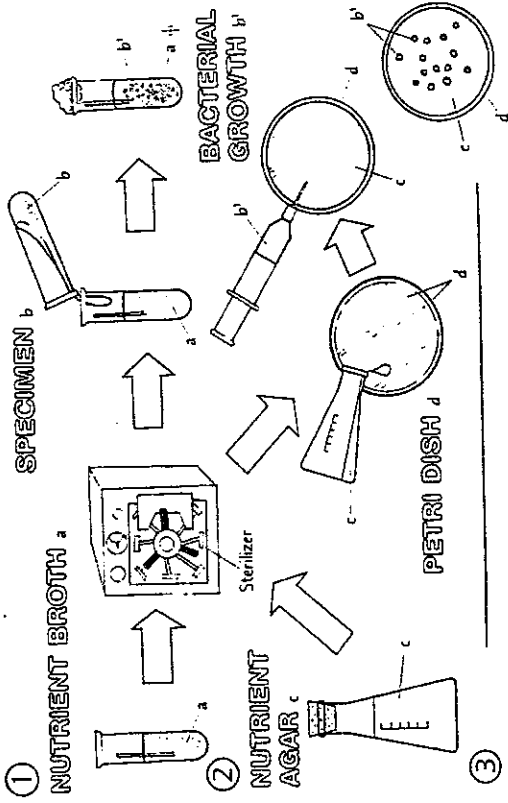
- c) _____
- (1) Melted nutrient agar is poured into petri dish and cooled.
- (2) An _____ is used to obtain a sample from a _____.
- (3) The bacteria is spread in a certain _____ on the surface of the agar.
- (4) Area of _____ set of streaks produce single _____ colonies.

iii. Incubation requires controlling _____ and _____ content to encourage growth of culture.

- A. Optimum temperature depends on species.
- 1. _____ bacteria prefer cold temps between 5C° and 20C°.
- 2. _____ bacteria grow best between 20C° and 40C°.
- 3. _____ bacteria grow best at higher temps between 40C° and 90C°.

- B. Oxygen requirement is also species specific.
- 1. _____ require a plentiful supply of O².
- a) grow close to supply of oxygen
- b) grow _____ the surface of media
- 2. _____ require lesser amount of O².
- a) can grow _____ from the surface
- 3. _____ can grow either in the presence of absence of oxygen.
- a) can grow _____ the media
- 4. _____ bacteria will not survive if any oxygen is present.
- a) grow near _____ of media far from the surface

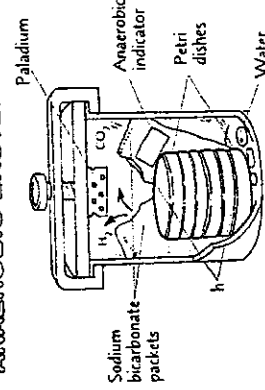
BACTERIAL CULTIVATION



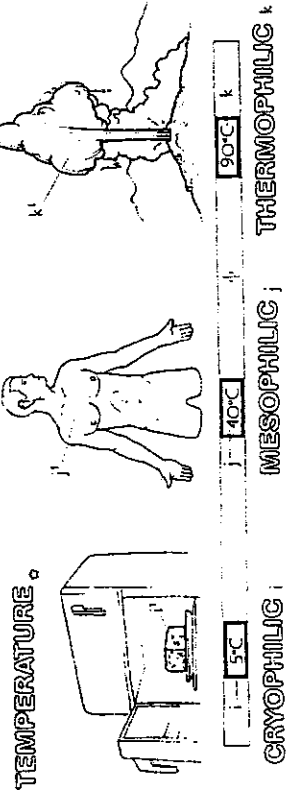
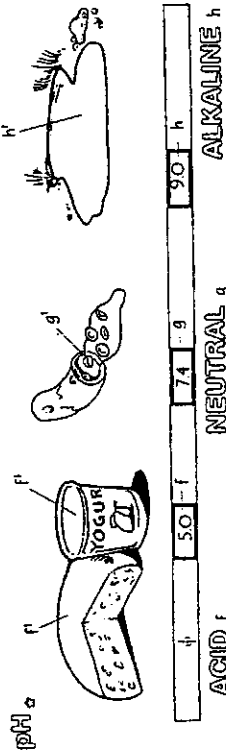
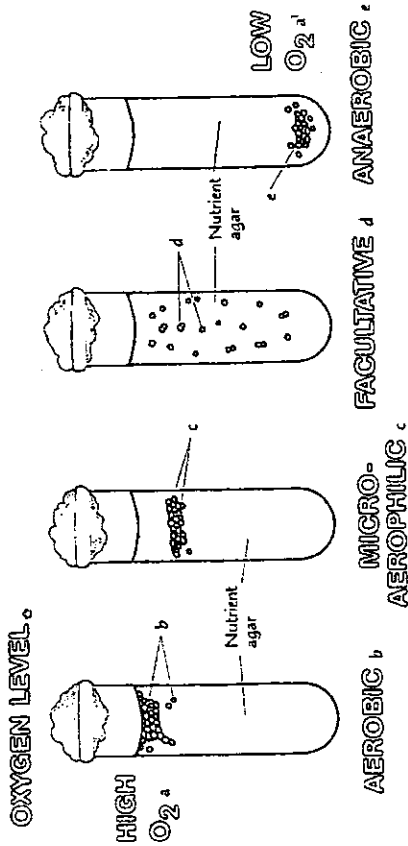
5 CHEMICALLY DEFINED MEDIUM

- GLUCOSE i
- AMMONIUM PHOSPHATE j
- SODIUM CHLORIDE k
- MAGNESIUM SULFATE l
- POTASSIUM PHOSPHATE m

4 ANAEROBIC CONTAINER ANAEROBIC BACTERIA h



CONDITIONS FOR BACTERIAL GROWTH



MAKING MEDIA FOR BACTERIA CELL CULTURE (MEDIA PREP)

DNA comes from cells. Scientists have learned how to grow many different cells using media prepared in the laboratory. Cells grown on media can be broken open and the DNA extracted and isolated from other cell molecules.

To grow cells in the laboratory, a scientist must provide an environment that the cells "like." Some cells grow well on liquid medium, called broth. Some cells prefer solid media, called agar. Some will grow well on either.

The decision to use one medium or another depends on the kind of bacteria to be grown and what they are to be used for. If single, isolated colonies are needed, cells must be grown on agar. The colonies result from a single cell being deposited on the agar surface and then being replicated hundreds of times. All the cells in a colony are clones of each other. A plate culture is ideal for separating bacteria into individual colonies and isolated in pure, non-contaminated cultures.

Agar media is a mixture of water and protein molecules. To prepare it, powdered agar is suspended in water and heated until the agar dissolves. The agar is sterilized and then poured into sterile Petri dishes. It cools into solid agar within 15-20 minutes. The poured plates may be used after about 24 hours.

Broth (also water and protein molecules) cultures result in suspensions of millions of floating cells. The cells are not isolated from each other and spread themselves throughout the liquid. Broth culture cells grow and reproduce quickly since they have better access to nutrients. Oxygen and food diffuses into these cells easily. Under ideal conditions, broth culture cells might replicate as often as every twenty minutes.

Materials: 250 mL media bottle w/cap or foil (for storing media)
 2 test tubes with caps for each team member
 400 mL glass beaker (for mixing agar)
 100 mL beaker (for mixing broth)
 Nutrient Agar, Nutrient Broth, Deionized water
 Stir rod, hot plate, hot hands or pot holder
 100 mL graduated cylinder
 electronic balance, weigh boats
 scoopula

Procedures:

- This recipe can be adjusted for any desired volume of agar.

Part A: For preparation of Nutrient Agar

- Prepare 125 mL of agar.
 - The recipe for making a 1 liter batch of Nutrient Agar is printed on most stock media containers.
 - Using $Mass_1/Volume_1 = Mass_2/Volume_2$, the proper amounts of media can be determined.
- Wash a 250 mL media bottle for storing the media. Label with your group # and class period.
 - Wash a 400 mL glass beaker. This is for mixing the media.
 - Calculate the amount of Nutrient Agar you need. See the example below for help with the calculation. Be sure to include your calculation in your lab procedures (show the set up as it is shown below but with your numbers). Record that mass (M_2).

For example if the recipe calls for 23 grams of Nutrient Agar base per liter and you only want 300mL:

$$Mass_1/Volume_1 = Mass_2/Volume_2$$

$$\begin{aligned} Mass_1 &= 23 \text{ grams} & Mass_2 &= \text{????? grams} \\ Volume_1 &= 1 \text{ liter} = 1000\text{mL} & Volume_2 &= 300 \text{ mL} \end{aligned}$$

$$\begin{aligned} \text{So,} & & & \\ 23 \text{ g} \times 300 \text{ mL} &= 6.9\text{g of nutrient agar media in distilled water to } 300 \text{ mL} \\ 1000 \text{ mL total} & & & \end{aligned}$$

- Measure out the amount of Nutrient Agar base required for the volume of agar desired and then pour the Nutrient Agar base into the clean 400 mL beaker.
- Very slowly add 90 mL of distilled water, stirring as it is added. The water should at first make a thick paste. As you add more water and stir the agar mixture the Nutrient Agar base will eventually become suspended.
- Add more water until a total volume of 125 mL of suspended agar is achieved.
- Use the stir rod to mix the solution. Move the beaker to the hot plate.
- Heat on high, gently stirring the entire time, until just before it boils. **DO NOT LET IT BOIL.** The agar suspension should become clear.

9. Carefully, using hot hands (or pot holders) remove the beaker from the hot plate. Set the beaker on the heat resistant lab station.
10. When the Nutrient Agar solution has cooled to the touch, pour the media into the 250 mL media bottle and cover with a piece of foil or the cap. Store as instructed by your supervisor.
11. CLEAN UP! Quickly clean all glassware before agar hardens.

Part B: For preparation of Nutrient Broth

- The recipe for making a 1 liter batch of Nutrient Broth is printed on most stock media containers.
 - Using $\text{Mass}_1/\text{Volume}_1 = \text{Mass}_2/\text{Volume}_2$, the proper amounts of media can be determined.
1. Obtain enough test tubes and caps so each member of your team has two. Label with initials, group # and class period.
 2. Wash a 100 mL glass beaker. This is for mixing the media.
 3. Calculate the volume of Nutrient Broth you need. You will need 10 mL for each member of your team. Add an extra 10mL to offset evaporation. See the example in Part A for help with the calculation. Be sure to include your calculation in your lab procedures (show the set up as it is shown below but with your numbers). Record that mass (M_2).
 4. Measure out the amount of Nutrient Broth base required for the volume of broth desired and then pour the Nutrient Broth base into the clean 100 mL beaker.
 5. Very slowly add the distilled water, stirring as it is added. As you add the water and stir, the Nutrient Broth base will eventually become suspended.
 6. Use the stir rod to mix the solution. Move the beaker to the hot plate.
 7. Heat on high, gently stirring the entire time, until just before it boils. **DO NOT LET IT BOIL.** The agar broth should become clear.
 8. Carefully, using hot hands (or pot holders) remove the beaker from the hot plate. Set the beaker on the heat resistant lab station.
 9. When the Nutrient Broth solution has cooled to the touch, pour 5 mL of the media into each test tube and loosely cap as instructed by your supervisor.

10. CLEAN UP!

Data:

Pre-Lab:

1. Compare and contrast the preparation and use of liquid media (broth) vs. solid media (agar), including
 - a. The ingredients from the side of the Nutrient Agar bottle and the Nutrient Broth.
 - b. How many grams of nutrient agar recommended to use to make 1L of solution.
 - c. How many grams of Nutrient Broth needed for 1L.
2. The difference in the use of each (Think about the type of result achieved).

2. Calculate the amount of agar powder required to mix 125mL of agar solution. **SHOW YOUR WORK!**

3. Calculate the amount of nutrient broth powder required to mix 45mL of nutrient broth. **SHOW YOUR WORK!**

PRE LAB
Questions

AGAR
Technique

BROTH
Technique

Post Lab:

1. Evaluate your ability to prepare 125mL of Nutrient Agar. Consider the final volume, color, mix, solidity and labeling of sample. Describe which were done well and what changes or modifications are needed next time.
2. Evaluate your ability to prepare 25mL of Nutrient Broth. Consider the final volume, color, mix, clarity and labeling of sample. Describe which were done well and what changes or modifications are needed next time.

Sterile Technique Pre-Activity Questions

ANSWER THE FOLLOWING IN YOUR SCIENTIFIC NOTEBOOK.

Get a stamp before you begin the lab.

1. Define *sterile technique*.
2. What are two goals of sterile technique?
3. How is the media sterilized after preparation- what equipment is used and how does it work to destroy unwanted microorganisms?
4. Paraphrase the 8 general principals of sterile technique.
5. How many plates is your lab group pouring today?
6. How many slant tubes will be poured?
7. What will be the first and last thing you do when you work at your lab station?
8. How will you insure unwanted organisms do not "fall" into your agar as you pour the plates?
9. Why do you pass the mouth of the agar stock bottle and mouth of the test tube through a hot flame?
 - a. How many times will you flame the mouth of the agar stock bottle?
 - b. How many times will you flame the mouth of the test tube?
10. What will you do with the cap of the test tube as you pour agar in?

PRE-LAB

TECHNIQUE

Sterile Technique and Pouring Plates and Slants

Background: Sterile, or aseptic, technique is a central concept in microbiology and good sterile technique is a very important part of working in a microbiology lab. The goal of sterile technique is two-fold. The first part is to prevent the contamination of a bacterial culture with bacteria from the environment. Clinical and research microbiology labs both depend on a bacterial culture containing only the type or types of bacteria of interest, and not other types of bacteria which entered the culture from the environment. Equally important is the prevention of possible contamination of the lab worker, or others, by the potentially pathogenic bacteria he/she is working with.

Sterile technique is the process of doing something without contamination by unwanted microorganisms or their spores. Sterile technique is used in virtually all biotechnology research applications. When cells, tissues, organs, or organisms are grown in the laboratory, they are maintained in sterile environments. Specimens are grown on sterile solid (agar) or in sterile liquid (broth) media. No matter the media, it must be sterilized under heat and pressure (for at least 15-20 min at 121°C and 15-20 pounds per square inch) before it is used. Sterile technique is used through media prep and cell culture. Media is prepared and sterilized in an autoclave.

Sterile technique includes all of the things done to one's person or equipment to decrease the possibility of transferring unwanted microorganisms to cultures. Achieving an effective aseptic technique may be one of the most difficult skills to learn in a microbiology lab, but it is also one of the most important. The difficulty lies in remembering to apply the fundamental principles of sterile technique. With practice, sterile technique will become second nature; until then, it requires constant attention.

General Principles:

1. Disinfect the work area with bleach solution before starting to reduce potential contaminants on the benchtop, and after work is finished to protect others from possible contamination.
2. Flame the inoculating loop before and after making a transfer of bacteria from one container to another. Never lay an inoculating loop on the benchtop. When in doubt, flame the loop again.
3. Flame the opening of glass containers before removing bacteria from them and again after bacteria have been removed. Likewise, flame the opening before transferring bacteria to a container and again after the transfer is completed.
4. Do not lay the cap to containers of bacteria on the benchtop while bacteria are removed from or transferred to the container. The cap should remain under your control throughout the transfer.
5. Work quickly and efficiently to minimize the time the culture is exposed to the environment.
6. Decrease air currents into the inoculation area through the use of laminar flow hoods. At the very least, choose a place to work that is free of drafts.
7. Remove baggy clothing and tie back long hair.
8. Keep your mouth CLOSED! DO NOT TALK! A bacteria laden mist comes from your mouth when you talk and could contaminate your work.

Purpose: To pour 8 plates and 1 agar slant under sterile conditions.

Materials:	Sterile Nutrient Agar	Matches	1 test tube with cap
Disinfectant	Parafilm	hot plate	
Bunsen Burner	stir rod	400mL beaker	
8 Petri Dishes (60 x 15 mm)			

Procedure:

Before Beginning:

- ✓ Label plates along the edge of the bottom of the plate with lab group #'s, date, period, and name of media.
- ✓ Label tubes with lab group #'s, date, period, and name of media.
- ✓ If media is solidified reheat on a hot plate in a beaker partially filled with water.

To Pour Plates:

1. Wipe the outside of the bottle of media with disinfectant.
2. Place petri dishes on your lab station.
3. Remove the cap and "flame" the bottle top (pass bottle top through the hot part of the flame).
4. Lift the lid of the first petri plate at a 45° (or less) angle and pour agar over $\frac{1}{2}$ of the petri plate. Be sure that the bottom of the petri plate is covered with agar. *Gently* swirl to insure even distribution.
5. Repeat pouring procedure with the other plates.
6. Leave undisturbed for at least 15 minutes.
7. When the agar has solidified, wrap each petri dish with parafilm.
8. Stack the plates on top of each other in two columns of four.
9. Store in a cool, dark place until needed (usually a refrigerator serves this purpose).

To Pour Slant tubes:

1. Wipe the outside of the bottle of media with disinfectant.
2. Remove the cap and "flame" the bottle top (pass bottle top through the hot part of the flame).
4. Remove the cap of the test tube but DO NOT put it on your lab station. Hold it between your fingers.
5. Flame the mouth of the test tube.
6. Slowly pour the agar into the test tube until it is half full.
7. Reflame the mouth of the test tube and loosely secure the cap.
8. Place the test tube on slant board on the front desk.
9. CLEAN UP!

Results:

- 1) On a scale of 1 to 10 (1 is low and 10 is high), how would you rate your groups ability to pour agar plates? _____ pour slant tubes? _____

Title: How Clean are Clean Surfaces?



Purpose: To determine the best cleaning solution.

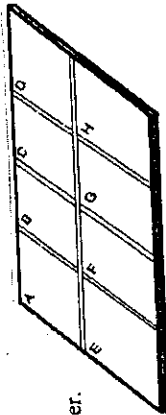
Materials:

- 8 sterile cotton swabs
- Test tube of sterile water
- Overhead pen
- 8 nutrient agar plates
- Tape
- 8 cotton balls

Procedure:

1. You will need 8 nutrient agar plates. Label them from "A" to "H".
2. Clear off but do not clean a desktop surface. Divide the surface into 8 different areas using overhead pen. Label each area from "A" to "H".
3. Use the following aseptic cleaning solution in each sector:

- A. Control (nothing done to sector)
- B. Cleaned with dry cloth
- C. Cleaned with wet cloth
- D. Cleaned with ordinary soap and water.
- E. Cleaned with Lysol.
- F. Cleaned with Awesome Spray.
- G. Cleaned with 10% bleach solution.
- H. Cleaned withTBD



4. Allow to air dry.
5. Moisten a swab in the sterile water, and then wiggle the swab around in the MIDDLE of an assigned sector. Using good aseptic technique, gently smear the swab around on the surface of the appropriately lettered agar plate. Use a new sterile swab for each sector. Dispose of the swabs appropriately.
6. Secure each plate with tape. Stack the plates, lids on the bottom, in groups of four. Rubber band each stack and bring to the front table. CLEAN UP!

Hypothesis:

Write a hypothesis, in "if/then" format stating which solution you predict will be most effective in killing microbes.

Data:

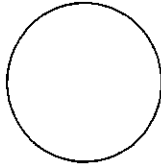
Take observations (drawings and descriptions) of each plate for 3 days (Yes, you will need a total of 24 circles in your data section). In particular note the presence of each of the following:

Types of things seen:

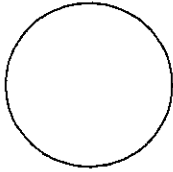
- Appearance:
 - colonies in terms of
 - number
 - size and color
 - texture
 - margins
- Succession:
 - Which colonies appeared first, second, etc.

Example:

Day 1 Observations: Inoculated on / / and observed on / /



A: Control



B: Dry Cloth

Analysis:

1. Which cleaning technique eliminated the most microbes?
2. Which technique was able to kill which sorts of microbes?
3. Door knobs, faucet handles, railing, coins, etc., are touched by many people. Might these surfaces be involved in the spread of disease? Do you think that it might be possible to make these daily items out of materials that are self-sterilizing? How would you test your hypotheses?

Reading Comprehension Questions

(Textbook Pg. 58-70)

Write your answers on a separate piece of paper using complete sentences when appropriate.

1. Biologists can rely on their senses to detect and evaluate major characteristics and to keep track of growth and developmental conditions while studying organisms. However, it is a different story for microbiologists. Name the 3 major difficulties mentioned in the reading that microbiologists encounter while studying microbes.
2. So much of learning science is becoming familiar with the language scientist's use to communicate with one another. Therefore, it is important to know the vocabulary necessary to understand scientific concepts. Define the following words:

- | | |
|------------------|-------------------------|
| a. inoculum | g. contaminants |
| b. medium | h. colony |
| c. inoculation | i. streak plate method |
| d. culture | j. incubated |
| e. pure culture | k. subculture |
| f. mixed culture | l. contaminated culture |

3. Why is a pure culture most frequently used for laboratory study?
4. How could one effectively achieve proper isolation of a bacterial cell?

5. What other condition can incubators (incubating ovens) control besides the temperature of the environment?
6. How does microbial growth materialize in the following media types:
a) liquid media b) solid media
7. When viewing bacterial colonies macroscopically, what type of characteristics can be observed?
8. When viewing bacterial colonies microscopically, what type of characteristics can be observed?
9. Bacteria are generally not identifiable by their appearances because their morphologies are frequently similar. What techniques are used to help scientist identify bacteria?
10. According to the textbook, what are 2 ways of effectively destroying microorganisms?

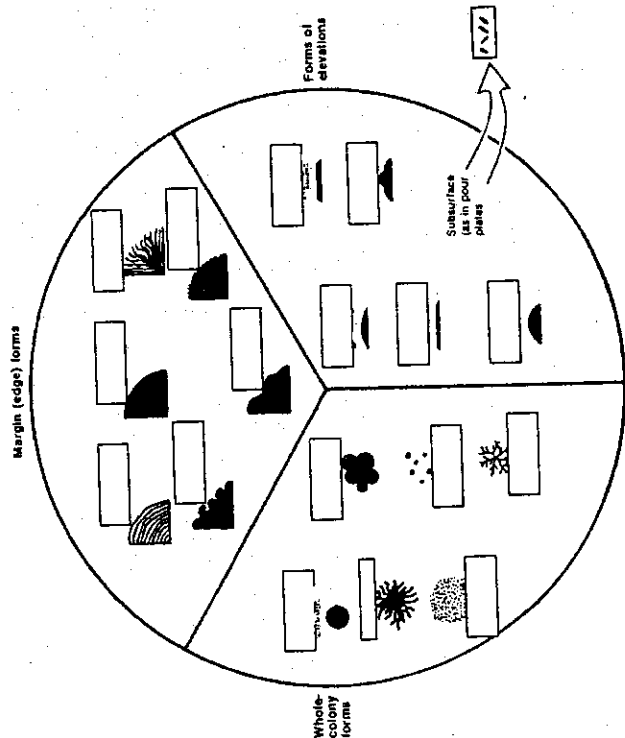
VIDEO: Microbial Techniques

- 1) What is the primary concern of all of the techniques when handling microbes?
- 2) DEFINE sterile medium
- 3) What is the difference between "clean" and "sterile" technique?
- 4) When is an autoclave appropriate to use to sterilize equipment?
- 5) What two factors does an autoclave use to sterilize?
- 6) Why are the Petri dishes inverted?
- 7) List several sources of microbes both biotic and abiotic.
- 8) Outline the steps and list the sterile techniques used when performing a slant to slant transfer.
- 9) Why is it necessary to cool the loop before touching the bacterial colonies?
- 10) Outline the steps and list the sterile techniques used in a broth to plate transfer.
- 11) What is the goal of streaking?
- 12) Draw two circles. Diagram two streaking patterns used in the streak plate method.
- 13) After inoculating, what is the next step?
- 14) For each procedure listed
 - a) State the purpose or use
 - b) Outline the steps and list the sterile techniques used
 - (1) serial dilution
 - (2) pipetting
 - (3) mixing solutions

INSPECTION

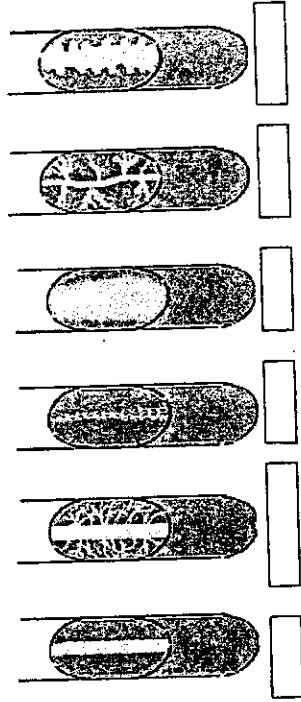
- I. Bacteria species on solid agar media can be distinguished by colony _____ which will permit the selection and transfer of _____, and allow transfer of a single colony to _____.
- different species from a _____, a sterile medium for cultivation of a _____.
- a. When a single bacterial cell is deposited on the surface of a nutritive medium, it begins to _____. After thousands (up to billions) of cells are formed, _____ mass appears. This mass of cells is called a _____. Each species of bacterial or fungal organism will exhibit characteristic colonies.
- b. Colony characteristics are described in _____ to permit _____.

1. Colony form:
2. Margin (edge):
3. Elevation:
4. Color:
5. Texture:



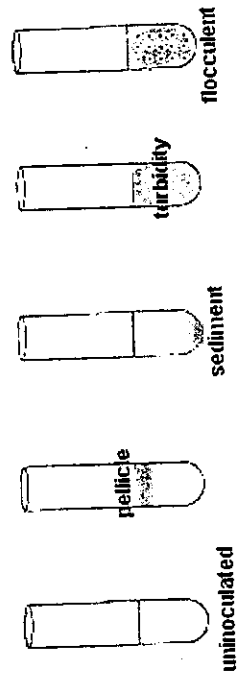
II. Bacteria streaked on slants demonstrate specific growth patterns.

- a. Filiform:
- b. Arborescent:
- c. Beaded:
- d. Effuse:
- e. Rhizoid:
- f. Echinulate:



III. Bacteria can also be grown in liquid, or broth, culture. Evidence of growth in a broth is shown by

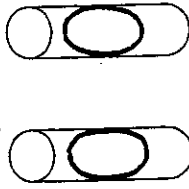
- a. turbidity:
- b. pellicle formation:
- c. sediment:
- d. flocculent:



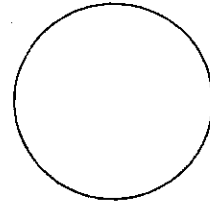
Manipulation of Cultures Pre-lab

1. What is the purpose/objective of this lab?
2. Define the following:
 - a. Pure culture:
 - b. Mixed culture:
 - c. Subculture:
3. Define sterilization by incineration. How does this apply to aseptic technique.

4. When transferring from plate to broth, how many times will you flame the inoculating loop? _____. How many times will you flame the lip of the broth tube? _____



5. Draw the two types of slant inoculation patterns in the culture tubes to the right.



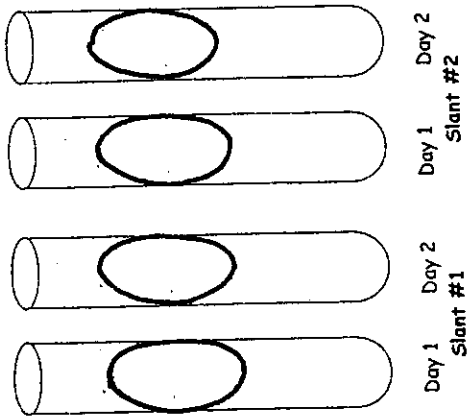
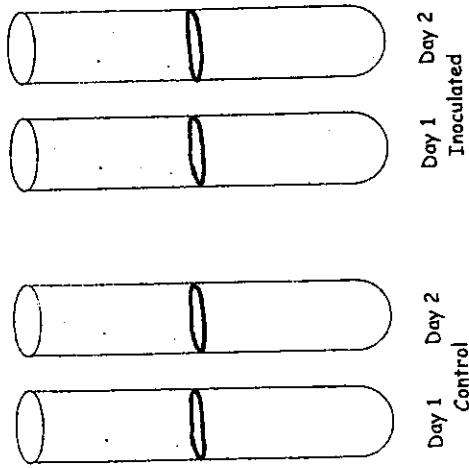
6. Use the side of a pencil to draw the streak pattern used in lab.

7. What is true about one-sixth of the steel used to build the Landry's home in Simi Valley?

Results: Observation of Cultures

A. Transfer from Plate to Broth

1. Examine each tube for evidence of growth. Draw the results. Describe the differences in appearance of the growth between the two broths.
2. Do the practice control tubes have any evidence of growth? If there is growth, good aseptic technique was not followed!

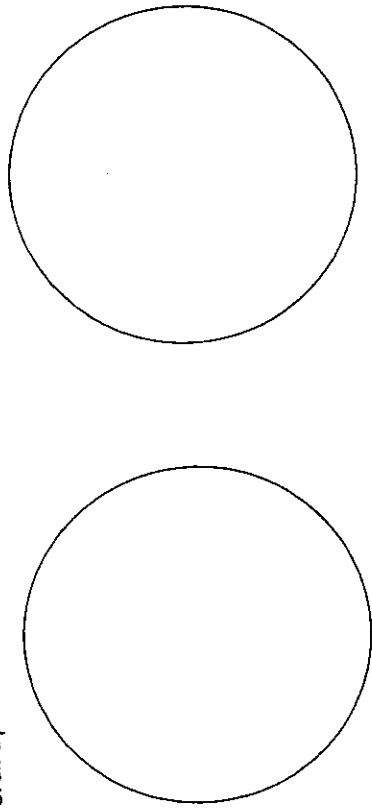


B. Transfer from Plate to Slant

1. Draw your observations in your data section. Trade slants with another group so you have sketches of both types of inoculations.
2. Describe the type of growth pattern evident in each tube.

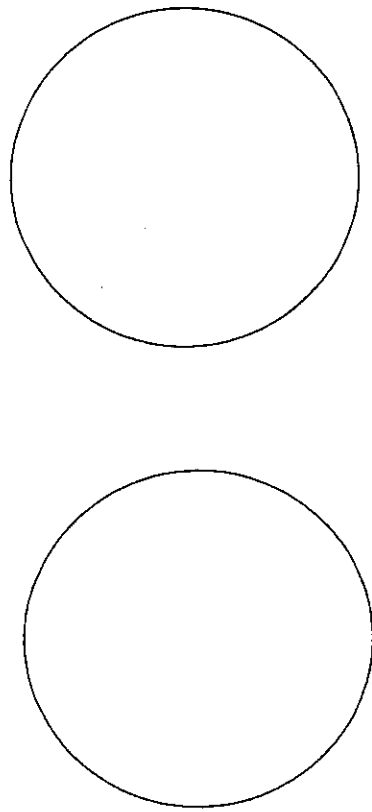
C. Transfer from Broth to Plate

1. Observe the plates for isolated bacteria. If successful, individual colonies (spots) of bacteria will be isolated.
2. Do the bacteria all look the same, or are there different kinds? (If they are different, good aseptic technique was not accomplished and the culture was contaminated somewhere along the way!)
3. Draw a picture of the streaked plate.



D. Transfer from Plate to Plate

1. Observe the plates for isolated bacteria. If successful, individual colonies (spots) of bacteria will be isolated.
2. Do the bacteria all look the same, or are there different kinds? (If they are different, good aseptic technique was not accomplished and the culture was contaminated somewhere along the way!)
3. Draw a picture of the streaked plate.



Enumeration (counting) of Bacteria

Cell _____ : the determination of the number of cells or concentration of cells in a sample

A. Methods used to count microorganisms:

1. _____ : actual counting of every organism present in a sub-sample of a population.

a. _____ : only _____ cells are counted.

- used in plate counts (pour plate, spread plate)
- assumes that every colony is founded by a _____, and that a cell must have been _____ in order to _____.

• method used is _____ (SPC) - average number of colonies per unit extrapolated to the _____ of the dish.

➢ only so many colonies that may be present before it becomes impossible to _____

➢ use _____ to dilute cultures before enumerating.

- By keeping track numerically of the degree of diluting employed, the concentration of bacteria in the pre-diluted culture may be estimated
- large dilutions are typically handled

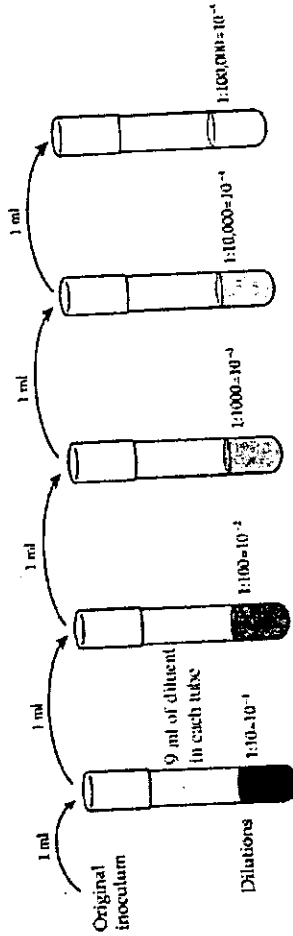
- inoculate from each _____ and perform _____ on most distinct
- multiply SPC by _____ to determine _____

b. _____ : all cells are counted, whether dead or alive.

- Generally taking total counts requires the employment of _____

2. _____ (MPN): a way of determining approximate viable count by _____ then growing the dilution cultures in _____ tubes.

- a. Observe _____ at different concentrations.
- The more _____ that show _____, especially at greater _____, the _____ organisms were _____ in the sample



Estimating the Number of Bacterial Colonies:
Standard Plate Count

Pre lab estimate: _____

Table 1: Estimating Bacteria Colonies

Square #	# Colonies Present
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
TOTAL	

Equation #1: Average Colonies Per Square

Total Colonies / 10 squares = _____ colonies/sq

Equation #2: Total Colonies Present

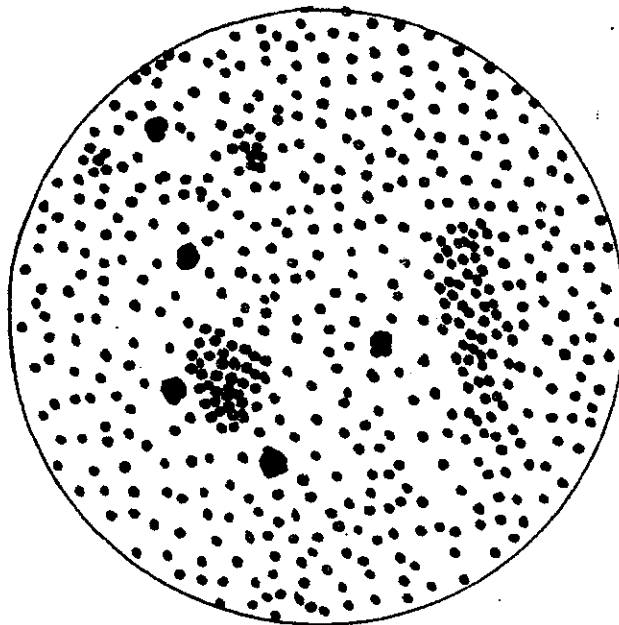
Average colonies per square X Total # Squares = _____

Equation #3: Colonies per cm²

Step 1: Area of Petri dish: ($A = \pi r^2$)

(Diameter of small dish = 6cm; lrg dish = 10cm)

Step 2: Total colonies present ÷ Area of Petri dish = _____ colonies/cm²



Procedure Part 2:

7. Using your grid, practice estimating the number of bacterial colonies on the plates below: (SHOW YOUR WORK!)

Square #	# Colonies Present
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
TOTAL	

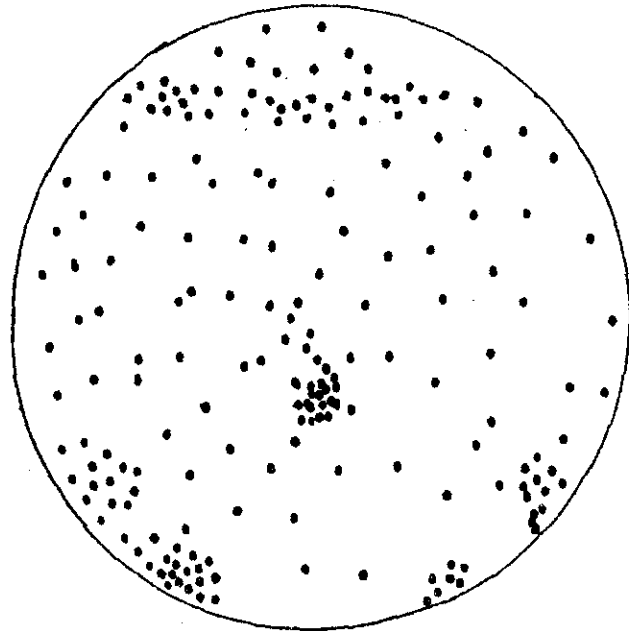


Plate A: _____

Square #	# Colonies Present
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
TOTAL	

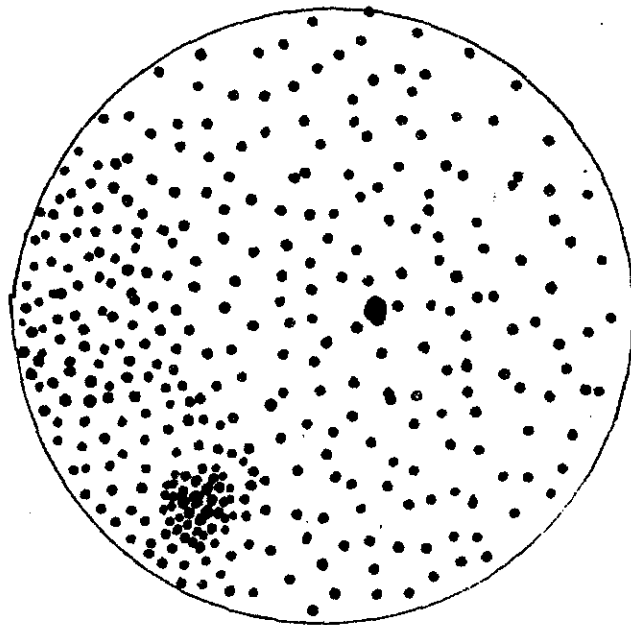
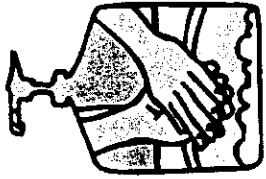


Plate B: _____

EFFECTIVENESS OF HAND WASHING



PURPOSE/OBJECTIVE:

- 1) Evaluate the effectiveness of hand washing.
- 2) Explain the importance of aseptic (sterile) technique in the hospital and laboratory environment.

BACKGROUND:

The skin is sterile during fetal development. After birth, a baby's skin is colonized by many bacteria for the rest of its life. As an individual ages and changes environments, the microbial population changes to match the environmental conditions. The microorganisms that are more or less permanent are called **normal flora**. Microbes that are present only for days or weeks are referred to as **transient flora**.

Discovery of the importance of hand and skin surface disinfection in disease prevention is credited to Ignatz Semmelweis at Vienna Hospital in 1846. He noted that the lack of aseptic methods was directly related to the incidence of diseases. Medical students would go directly from the autopsy room to the patient's bedside and assist in child delivery without washing their hands. Semmelweis established a policy for the medical students of hand washing with a chloride of lime solution that resulted in a drop in the death rate due to bacterial disease infections from 12% to 1.2% in one year.

A layer of oil and the structure of the skin prevent the removal of all bacteria by hand washing. Soap helps remove the oil, and scrubbing will maximize the removal of bacteria. Hospital procedures require personnel to wash their hands before attending a patient, and a complete surgical scrub- removing the transient and many of the resident microflora- is done before surgery. Transient flora is usually removed after 10 to 15 minutes of scrubbing with soap. The surgeon's skin is never sterilized. Only burning or scraping it off would achieve that.

In this lab, we will examine the effectiveness of washing skin with soap and water. Only organisms capable of growing in the presence of oxygen and on nutrient agar will be observed. Because organisms with different nutritional and environmental requirements will not grow, this procedure will involve only a minimum number of the skin microflora.

HYPOTHESIS: (in "If...then..." format)

MATERIALS:

- 4 Petri Plates containing nutrient agar
- soap as assigned
- Incubator
- sharpie
- Parafilm
- paper towel
- pipette

PROCEDURE: Part I:

1. Divide two nutrient agar plates into four quadrants. Label both dishes with your lab group number. Label one dish "Control" and the other dish "Soap".
2. Inoculate the control plate first. Touch Q. I with the pads of your middle and ring fingers of your right hand. Roll your fingers over the agar without puncturing it.
3. Wash well **without** soap. Shake off the excess water and while still damp, touch Q. II.
4. Do not dry your fingers with a towel. Wash again and while damp touch Q. III.
5. Wash a final time and touch Q. IV.
6. Have your lab partner repeat steps #1-5 using the soap assigned to your group and inoculating the "----- soap" plate.
7. Close lid, secure with parafilm, invert and place in the incubator.

Part II:

1. Label the remaining two nutrient agar plates with your lab group number. Label one dish, "Clean" and the other, "Dirty". Using the sterile pipette, aseptically add 5 drops of the respective water sample to the agar surface. Swirl to distribute.

DATA:

1. Sketch the appearance of your plates after incubation. Remember your drawings should be to scale (same size as the actual plate) and in color if appropriate.
2. Written description of bacterial growth (include color, texture, general morphological characteristics).

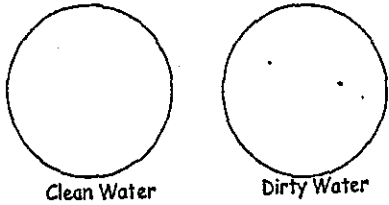
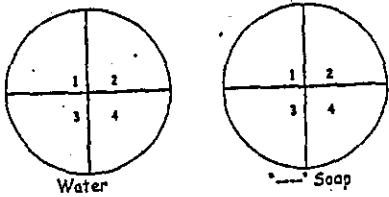
DATA ANALYSIS:

1. Make a data table (see sample) and record the SPC for each quadrant.
2. Create a graph that compares the # of colonies in each quadrant.

CONCLUSION:

Summarize your results, explain if your data supported your hypothesis or not, and discuss any experimental errors that may have occurred to skew your data.

Inoculated on / ; Observed on /

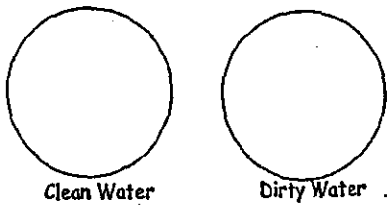
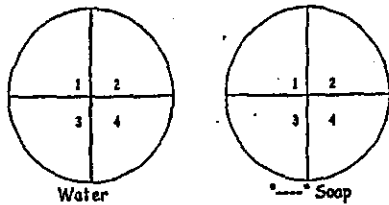


Quad		SPC	SPC	Description
		11/	11/	
1	Water			
	soap			
2	Water			
	Soap			
3	water			
	Soap			
4	Water			
	Soap			

Table 2

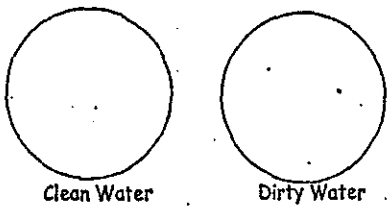
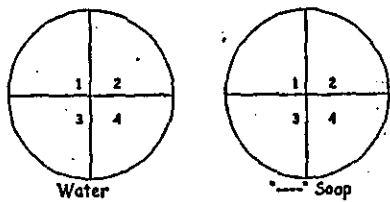
	SPC	SPC	Description
	11/	11/	
Clean Water			
Dirty Water			

Inoculated on / ; Observed on /



Results

Inoculated on / ; Observed on /



Data:

Table 1

Quad		SPC	SPC	Description
		11/	11/	
1	Water			
	soap			
2	Water			
	Soap			
3	water			
	Soap			
4	Water			
	Soap			

Table 2

	SPC	SPC	Description
	11/	11/	
Clean Water			
Dirty Water			

Inoculated on / ; Observed on /

