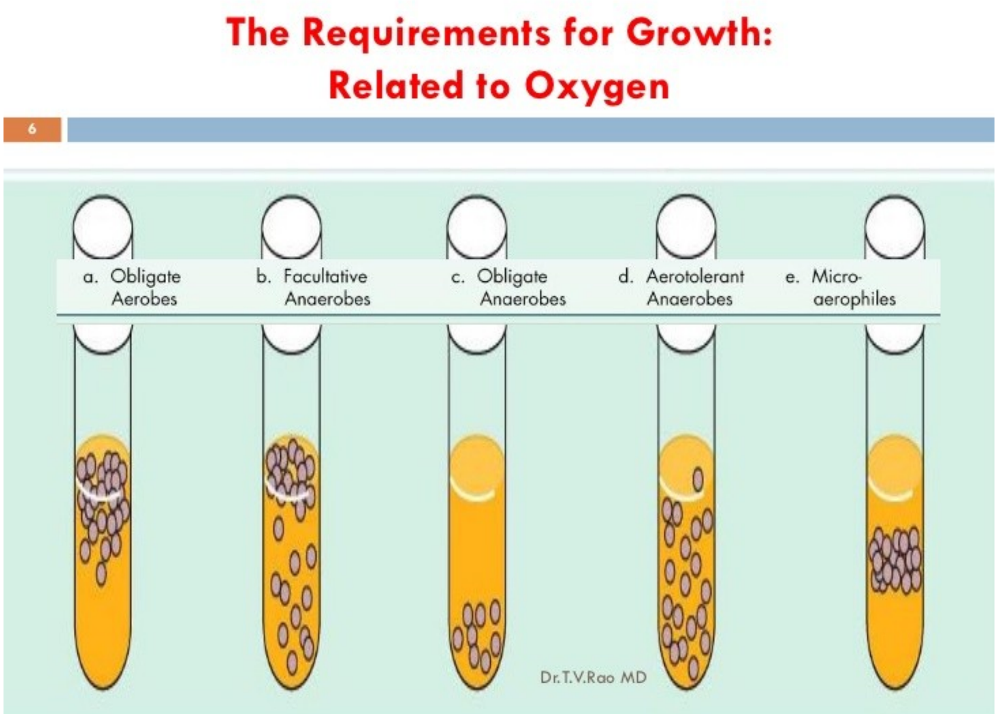
Lab Practical Study Guide # 2

Lab 18 - Anaerobic cultures

Purpose: One of the environmental factors to which bacteria and other microorganisms are quite sensitive is the presence of O2. Bacteria will only grow in the part of the agar deep culture that contains the proper O2 concentration



Obligate aerobes will only grow in this oxygen-rich top layer.
Obligate anaerobes will only grow in the lower areas of the tube.
Microaerophiles will grow in a thin layer below the richly oxygenated layer.
Facultative or aerotolerant anaerobes can grow throughout the medium but will primarily grow in the middle of the tube, between the oxygen-rich and oxygen-free zones.

Wrights tube- The anaerobic condition in Wright's tube is created using pyrogallol and NaOH. In the presence of NaOH, pyrogallol is oxidized and removes O2 very effectively in the process.

Thioglycollate Broth- sodium thiogycollate, thioglucollic acid and L-cysteine reduce the oxygen to water. The agar helps retard oxygen diffusion and helps maintain the stratification of organisms growing in different layers of the broth.

Candle jar- the candle flame will consume most of the oxygen in the jar and will produce an elevated level of carbon dioxide.

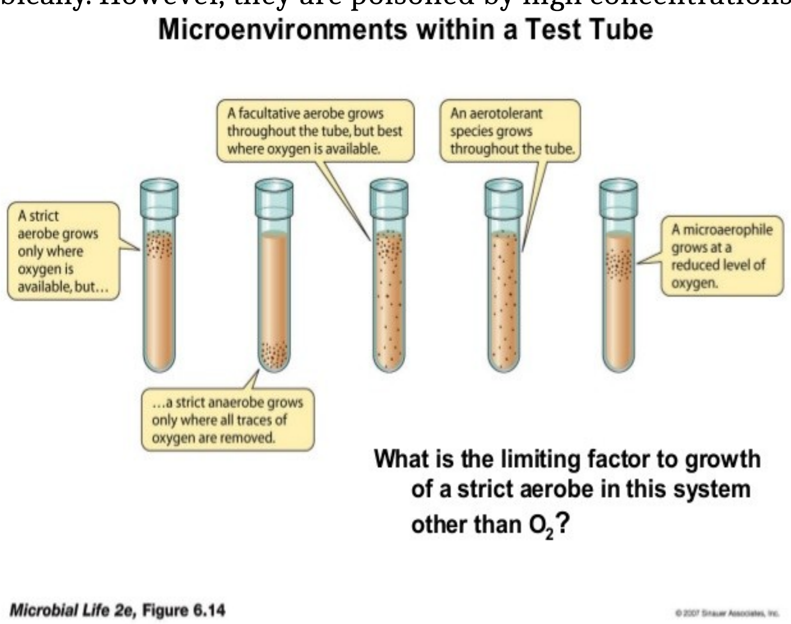
Gas Pack-most effective way of all. Hydrogen and CO2 are generated from package after addition of water. The hydrogen combines with O2 to produce condensation and thereby removing the 02 from the sealed chamber
Remember, Which one are the most effective in order?

<u>**Lab Report # 18**</u>

1.Explain how an anaerobic atmosphere can be create in a jar
By burning a candle inside a jar, any oxygen available will be used up as fuel to continue to burn of the candle until all O2 has been used up.

2. Explain what happens in a Wright's tube.
Using pyrogallol and NaOH creates an anaerobic condition. In the presence of NaOH, pyrogallol is oxidized and removes O_2 effectively.

3. Differentiate between the following:
a. **An obligate anaerobe**— cannot survive in the presence of oxygen
b. **An obligate aerobe**— can only grow if O2 is present.
c. **A facultative anaerobe**— can use O2 in its metabolism and so grows better if O2 is present, but can survive without it.
d. **An aerotolerant anaerobe**— can survive in the presence of O2, but does not use O2 in its metabolism.
e. **A microaerophile**— needs oxygen because they cannot ferment or respire anaerobically. However, they are poisoned by high concentrations of oxygen



4. What are the ingredients in Brewer's anaerobic agar that remove O2 form the medium? Briefly explain how an Oxyrase plate works?
It contains chemical reducing agents such as thioglycollic acid and glutathione. An oxyrase plate has an enzyme that removes oxygen from the culture.

Lab 63 - Conjugation

Conjugation is the process by which a plasmid is transferred from an F+ cell into an F- cell. The F factor in the F+ cell contains genes, which express pili for attachment, and special membrane proteins for the transfer complex. DNA is transferred from a donor to a recipient. The ability of a bacterium to be a donor is a consequence of the presence in the cell of an extra piece of DNA called the F factor or fertility factor. The ability to act as a recipient is a consequence of the lack of the F factor.

Fertility Factor (conjugation with a plasmid results always results in a F+ recipient)
F+ donor of plasmid that carries genes (doesn't gain anything new)
F- recipient (gains new genetic material from donor)

The Process
Bacteria that have a F plasmid are referred to as F+ or male.
Those that do not have an F plasmid are F- or female.
The F plasmid consists of 25 genes that mostly code for production of sex pilli.
A conjugation event occurs when the male cell extends his sex pili and one attaches to the female. This attached pilus is a temporary cytoplasmic bridge through which a replicating F plasmid is transferred from the male to the female.
When transfer is complete, the result is two male cells.

Antibiotic resistance
Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth; in other words, the bacteria are "resistant" and continue to multiply in the presence of therapeutic levels of an antibiotic.

How do bacteria become resistant?
Some bacteria are naturally resistant to certain types of antibiotics. However, bacteria may also become resistant in two ways: 1) by a genetic mutation or 2) by acquiring resistance from another bacterium.

Bacteria can acquire antibiotic resistance genes from other bacteria in several ways. By undergoing a simple mating process called "conjugation," bacteria can transfer genetic material, including genes encoding resistance to antibiotics (found on plasmids and transposons) from one bacterium to another.

Any bacteria that acquire resistance genes have the ability to resist one or more antibiotics. Because bacteria can collect multiple resistance traits over time, they can become resistant to many different families of antibiotics.

Why do bacteria become resistant to antibiotics?
Antibiotic resistance is a natural phenomenon. When an antibiotic is used, bacteria that can resist that antibiotic have a greater chance of survival than those that are "susceptible." Susceptible bacteria are killed or inhibited by an antibiotic, resulting in a selective pressure for the survival of resistant strains of bacteria.

Selective pressure is the influence exerted by some factor (such as an antibiotic) on natural selection to promote one group of organisms over another. In the case of antibiotic resistance, antibiotics cause a selective pressure by killing susceptible bacteria, allowing antibiotic-resistant bacteria to survive and multiply.

Some resistance occurs without human action, as bacteria can produce and use antibiotics against other bacteria, leading to a low-level of natural selection for resistance to antibiotics. However, the current higher-levels of antibiotic-resistant bacteria are attributed to the overuse and abuse of antibiotics. In some countries and over the Internet, antibiotics can be purchased without a doctor's prescription. Patients sometimes take antibiotics unnecessarily, to treat viral illnesses like the common cold.

Plate 1 Tetracycline Plate
E. Coli BB4 is the donor – has tetracycline resistance, Ampicillin sensitive
E. Coli SCSI is the recipient – Ampicillin resistance, Tetracycline sensitive

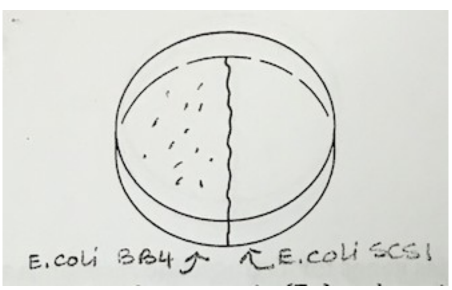


Plate 2 - Ampicillin Plate
E. Coli SCSI is the conjugation recipient strain (F-)
Ampicillin resistances, Tetracycline Sensitive

E. Coli BB4 is the donor strain (F+)
Tetracycline resistance and Ampicillin sensitive

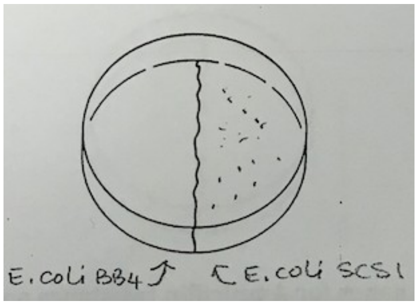
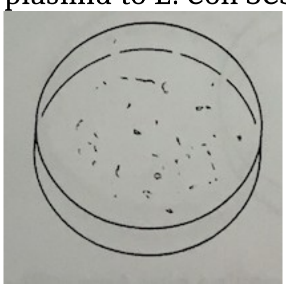


Plate 3 – Tetracycline and Ampicillin Plate
Upon plating, conjugation occurs, as E. Coli BB4 passes on its tetracycline resistance plasmid to E. Coli SCSI, creating genetically new bacteria.



Remember
Who is the donor? What does the recipient have already? If you combine the two, what will be the result? Will the organism grow within the plate, given what you know about both already?

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a plasmid, or DNA fragment. Certain bacteria can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome. After death or cell lyses, some bacteria release their DNA into the environment.

<u>Lab Report # 63</u>

1.Why is aseptic technique so important in this experiment?
a. To avoid contamination of the culture(s).
b. To avoid the spread of drug-resistant Escherichia coli to yourself, others, and the environment.
3. Would you expect that the new doubly antibiotic-resistant strain could grow well if it was released into the environment? Explain.
It would not grow well in most natural environments where antibiotic-producing microorganisms live and dominate. However, in the intestinal tract of humans

receiving kanamycin or rifampin therapy, this microorganism could dominate and become the new normal.

5. How do plasmids function?
A plasmid is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Often, the genes carried in plasmids provide bacteria with genetic advantages, such as antibiotic resistance.

6. Do bacteria reproduce sexually? Explain.
No, but in special cases involving conjugation with High frequency combination strains), a complete copy of the donor chromosome may be transferred to the recipient resulting in a diploid cell.

7. How would you define genetic engineering?
Genetic engineering is the process of manually adding new DNA to an organism using recombinant DNA technology. The goal is to add one or more new traits that are not already found in that organism. Examples of genetically engineered organisms include plants with resistance to some insects, and plants that can tolerate herbicides.

Lab 61 – Mutation

Mutation is the result of a stable, heritable change in the nucleotide sequence of DNA. By manipulating the chemical or physical environment of a bacterium, one can increase the frequency of mutations.

Method
If a mutant bacterium, by virtue of the change that it has undergone, is suited to the environment in which it is formed, its growth can be greater than the parent bacterium. The mutant will quickly become the dominant bacterium within the culture.

There are two ways for mutations to occur.
1. Spontaneous mutations arise occasionally in all bacteria and develop in the absence of any added agent.
2. Induced mutations are the result of the bacterium's exposure to a mutagen.

Spontaneous mutations that become resistant to antibiotics (such as streptomycin) can be readily detected in the laboratory, which can be easily found, as spontaneous mutations will grow in the presence of high antibiotic concentrations that inhibit the growth of normal bacteria.

Selective pressure
Exposing microbes to antibiotics will result in either adaptation or death, this is known as 'selective pressure'.

If a strain of a bacterial species acquires resistance to an antibiotic, it will survive the treatment. As the bacterial cell with acquired resistance multiplies, this resistance is passed on to its offspring. A whole new resistant strain of the bacterial species begins to become the dominant species within the culture.
Ultimately, putting the microbes into an antibiotic plate, didn't drive resistance, but selected for it.

Spontaneous mutations happen all the time, but can be induced further via induced mutations.

Lab Report # 61

1.How would you define a bacterial mutation?
A mutation is the result of a stable, heritable change in the nucleotide sequence of DNA. If a mutation in a bacterium gives it an environmental advantage compared to its parent bacterium, then the mutant will quickly become the dominant bacterium in the culture.

2.How would you describe a streptomycin-dependent mutant bacterium?
This type of bacterium is one that can grow in the presence of concentrations of streptomycin, which inhibit the growth of its parent cell(s).

3.How can bacterial mutations occur?
When DNA is copied or when damaged DNA is repaired, errors can be made that will be passed on to progeny cells.

4.What is a mutagen? Give several examples.
A mutagen is anything that changes the genetic material of an organism. The most common examples are X-Rays, UV light, and oxidizing agents.

5. What methods could be used to increase the frequency of the appearance of mutant?
a. Exposing the culture to mutagenic agents.
b. Using media or environmental conditions selective for the mutant cells and against normal (parent type) cells.

6. Could a gradient-agar plate be used for any antibiotic? Explain your answer.
This can be done only if the antibiotic is stable in the agar (heated) medium at 45 to 50 C, and if it will be evenly dispersed in the medium prior to pouring. If these conditions are satisfied, it should be possible to use this method

Lab 42 - Disinfectants

Disinfection is the killing, inhibition, or removal of microorganisms that may cause disease. Disinfection leads to the substantial reduction of the total microbial population and the destruction of potential pathogens. Moreover, it is the destruction of pathogens, but not endospores, on an object or in a material. The number of pathogens is reduced or growth is inhibited to a level that does not produce disease.

Many factors influence the effectiveness of chemical disinfectants and antiseptics. The microbicidal (to kill) or microbiostatic (to inhibit) efficiency of a chemical is often determined with respect to its ability to deter microbial growth.

Microbicidal efficiency of a chemical is often demonstrated through what is called a **phenol coefficient.**

Factors determining effectiveness

1. Number and Location of Microorganisms
The larger the number of microbes, the more time a germicide needs to destroy all of them.

2. Innate Resistance of Microorganisms
Microorganisms vary greatly in their resistance to chemical germicides and sterilization processes. To destroy the most resistant types of microorganisms, the user needs to employ exposure times and a concentration of germicide needed to achieve complete destruction.

3. Population Composition- microorganisms differ in their sensitivity to various agents. Ex. Do they have Spores?

4. Concentration and Potency of Disinfectants
The more concentrated the disinfectant, the greater its efficacy and the shorter the time necessary to achieve microbial kill.

5. Physical and Chemical Factors
a) Temperature
The activity of most disinfectants increases as the temperature increases. Increase in temperature causes the disinfectant to degrade and weakens its germicidal activity and thus might produce a potential health hazard.

b) PH
Increase in pH improves the antimicrobial activity of some disinfectants. The pH influences the antimicrobial activity by altering the disinfectant molecule or the cell surface

c) Relative Humidity
Relative humidity is the single most important factor influencing the activity of gaseous disinfectants.

d) Water Hardness
Reduces the rate of kill of certain disinfectants because cations (e.g., magnesium, calcium) in the hard water interact with the disinfectant to form insoluble precipitates.

6. Organic and Inorganic Matter
Organic matter in the form of serum, blood, pus, or fecal material can interfere with the antimicrobial activity of disinfectants in at least two ways.

1. Interference occurs by a chemical reaction between the germicide and the organic matter resulting in a complex that is less germicidal or non-germicidal, leaving less of the active germicide available for attacking microorganisms.
2. Alternatively, organic material can protect microorganisms from attack by acting as a physical barrier.

Inorganic contaminants of microorganisms to all sterilization processes can be a problem as well.

7. Duration of Exposure
Items must be exposed to the germicide for the appropriate minimum contact time.

8. Biofilms
Microorganisms may be protected from disinfectants by production of thick masses of cells called biofilms.
Biofilms are microbial communities that are tightly attached to surfaces and cannot be easily removed. Once these masses form, microbes within them can be resistant to disinfectants by multiple mechanisms, including physical characteristics of older biofilms as well as microbial production of neutralizing enzymes.

Phenol coefficient is the standard by which every disinfectant is measured.

Greater than 1 (mystery is better than phenol)
Less than 1 (not as good as phenol)

For example, If the coefficient is 15, then it's 15 times stronger than phenol.

Problem:
A disinfectant diluted with 1/500 with water kills a bacterium after 10 minutes but not after 5 minutes. A 1/100 dilution of phenol kills the same bacterium after 10 minutes but not after 5 minutes. What is the phenol coefficient?

$(1/100) / (1/500) = 5$ times more effective than phenol.

Relationship to antibiotic resistance
At the correct strength, biocides kill bacteria and other microbes. However, if lower levels are used the bacteria can survive and become resistant to treatment.

Using disinfectants could cause bacteria to become resistant to antibiotics as well as the disinfectant itself.
Increase domestic usage of non-antibiotic antimicrobial agents may select for antibiotic resistant bacteria of clinical significance.
Bacteria like Staphylococcus aureus make proteins that pump many different toxic chemicals out of the cell to interfere with their antibacterial effects.
These pumps can remove antibiotics from the cell and have been shown to make bacteria resistant to those drugs.

Problem: we have added Triclosan to all sorts of stuff like toothpaste, shoes, and even mattresses). When microbes are killed, their DNA is released, which can offer other microbes something to play with like Triclosan resistance.

How does one increase the level of antibiotic resistance?

1. Don't Vaccinate
2. Antibiotics used in Food Supply
3. Taking antibiotics for no reason
4. Prescribe one at a time
5. Low dose for a long time
6. Not finishing your prescription correctly
7. Don't take it properly in the right dosage
8. Get them in water supply
9. Globalization
10. Larger populations in urban areas vs. rural areas.
11. Unsanitary conditions
12. Deny healthcare
13. Sending kids to daycare
14. Bioterrorism
15. Delivery system of antibiotics

Lab Report # 42

1. What are some limitations of a test such as you performed on the evaluation of a disinfectant?
a. Evaporation.
b. Dilution in natural environments
c. Presence of interfering substances.
All these factors plus others would tend to influence the effectiveness of a disinfectant.

2. List some criteria of a good disinfectant?
a. Can be easily and quickly diluted and applied.

b. Kills all microorganisms within a short time period (10 minutes or less).
c. Inexpensive.
d. Safe for use under normal use conditions.

3. What is the phenol coefficient technique?
It is a measure of the bactericidal activity of a chemical compound that kills all the microorganism(s) under study within 10 minutes but not within 5 minutes, with respect to phenol.

5. What is the difference between microbicidal and microbiostatic?
Bactericidal agents are used to kill microorganisms by inhibiting the synthesis of cell wall.
Bacteriostatic agents are used to limit the growth and reproduction of microorganisms by interfering with their protein production, DNA replication, or other aspects of bacterial cellular metabolism.

6. What physical factors can influence the activity of a disinfectant?
a. Temperature
b. Hydrogen ion concentration
c. Humidity

7. Why do microorganisms differ in their response to disinfectants?
The structure of microbial cell walls may differ resulting in variable effects upon different types of microorganisms.

Lab 43 - Antibiotic sensitivity testing

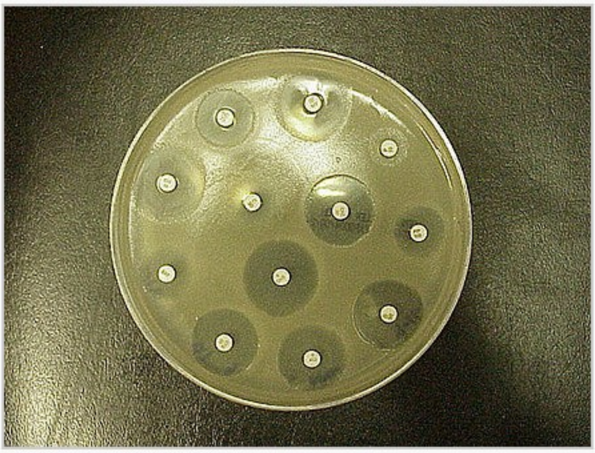
Certain bacteria can display resistance to one or more antibiotics. Determining bacterial antibiotic resistance, whether a bacterium can survive in the presence of an antibiotic is a critically important part of the management of infectious diseases in patients.

Kirby- Bauer method is used here to determine antibiotic susceptibility.
In this method, antibiotics are impregnated on paper discs using a dispenser.
Upon incubation, antibiotic diffuses from the disk into the surrounding agar. If susceptible to the antibiotic, the test organism will be unable to grow in the area immediately surrounding the disk, displaying a zone of inhibition

Determination of sensitivity-The zone of inhibition is measured to the nearest mm and compared to the sensitivity chart.

Look at the table as reference because the zone of inhibition alone is not telling us whether a microorganism is resistant or susceptible to the given antibiotic.

The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone, because a lower concentration of the antibiotic is enough to stop growth.



In Kirby–Bauer testing, discs containing antibiotics are placed on agar where bacteria are growing, and the antibiotics diffuse out into the agar. If an antibiotic stops the bacteria from growing, one can see circular areas around the wafers where bacteria have not grown.

Choice of antibiotic to treat with- Clinicians use KB test results to choose antibiotics effective against the specific bacteria causing a patient's infection.

Using specifically targeted antibiotics helps decrease the frequency of drug-resistant bacteria. The minimum inhibitory concentration is often used here
This is the lowest concentration of an antimicrobial drug that prevents visible growth of a microorganism after overnight incubation with media.

Remember that antibiotics from soil organisms, we do not make them in the lab.

When to do antibiotic sensitivity testing
Antibiotic sensitivity testing is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection.

The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection, and the ability of the bacteria to resist or inactivate the antibiotic.
Some antibiotics actually kill the bacteria (bactericidal), whereas others merely prevent the bacteria from multiplying (bacteriostatic) so that the host's immune system can overcome them.

In order for an antimicrobial drug to be useful, it must have selective toxicity.

The selective toxicity of antibiotics means that they must be highly effective against the microbe but have minimal or no toxicity to humans. In practice, this is expressed the ratio of the toxic dose (to the patient) to the therapeutic dose (to eliminate the infection). The larger the index, the safer is the drug (antibiotic) for human use.

Factors that affect the test
The size of this zone is dependent on a number of factors, including:

1.the sensitivity of the microbe to the antibiotic.
2.The rate of diffusion of the antibiotic through the agar
3.The depth of the agar.
4.Incubation period
5.Mistakes in the lab
6.Patient physiology
7. Obstructions- Children's ear canals often bend in unusual ways that cause ear infections because it gives the bacteria a place to hang out in.

Lab Report # 43

1. How can you determine whether the zone of inhibition is due to death or to inhibition of a bacterium?
Swab the zone of inhibition and place on a new plate. If no new colonies grow then the bacteria in the zone are dead.

2. What factors must be carefully controlled in the Kirby-Bauer method?
1. Size of inoculum
2. Distribution of the inoculum
3. Incubation period
4. Growth rate of bacterium
5. Depth of agar
6. Diffusion rate of the antibiotic
7. Concentration of antibiotic in the disk

3. In which growth phase is a bacterium most sensitive to an antibiotic?
During the log phase when the cells are dividing exponentially, any bacterium can be sensitive to an antibiotic.

1.Antibiotics inhibit cell wall formation
2. Inhibit DNA replication
3.Antibiotics inhibit protein synthesis

4. If the clinical laboratory reports bacterial susceptibility to an antibiotic but the patient is not responding to it, what could have gone wrong?
a. The infection may still be present in part of the body that the antibiotic is not reaching.
b. The test could have been done on a contaminant.
c. The wrong culture could have been tested.
d. There could have been a secondary infection that was not cultured, & this antibiotic may not be able to kill it.

5. What are the similarities and differences in response to plates with gram-positive and gram-negative bacteria? Between enterics and nonenterics?

Gram-positive bacteria are overall more susceptible to antibiotics.
Gram-negative bacteria are considered less susceptible, but gram-negative bacteria are still susceptible to some antibiotics.

Enterics could be more susceptible since they have less exposure to antibiotics since they are protected inside the body.
Non-enterics could be less susceptible because they are exposed to more antibiotics since they are not protected in the body.

6. What is the difference between an antibiotic and an anti-microbic?
An antibiotic is produced by a microorganism and is effective against microorganisms.
On the other hand, an anti-microbic is a naturally occurring or man-made chemical agent effective against microorganisms.

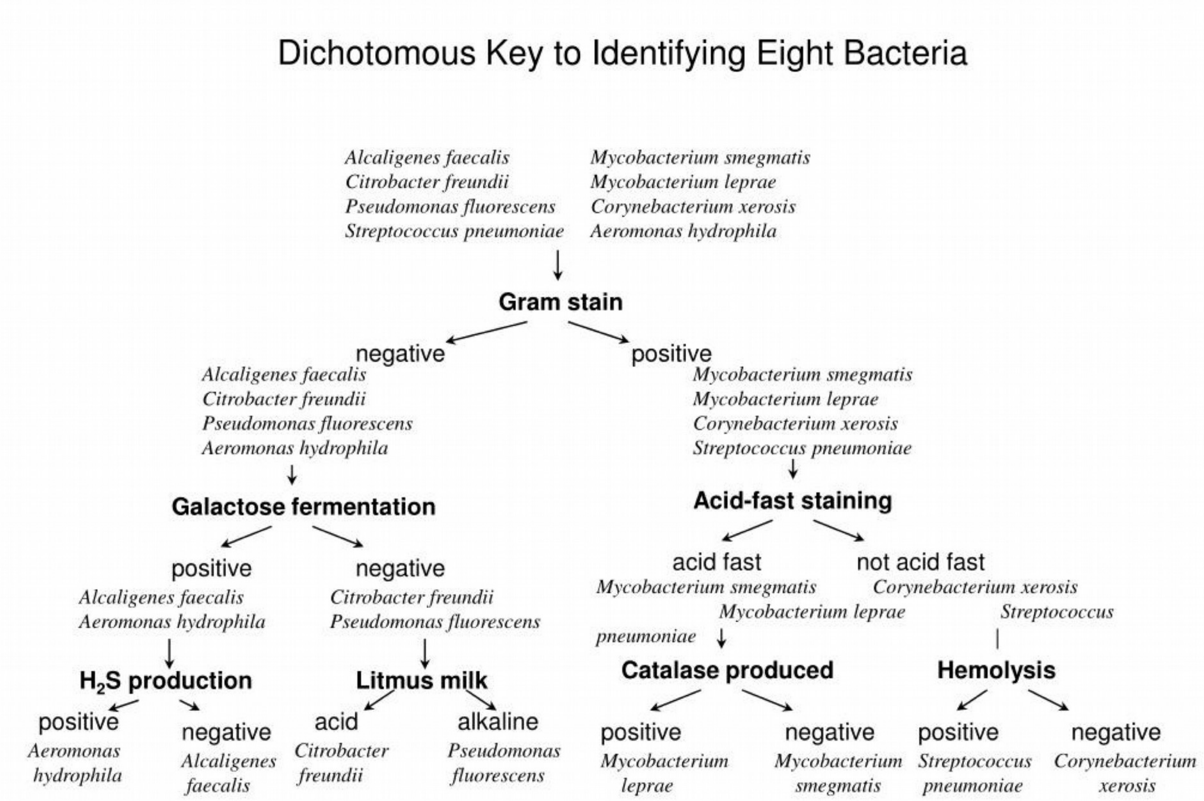
7. What are some reasons bacteria are becoming more resistant to antibiotics?
a. The more they are used, the greater the probability for selecting resistant mutants.
b. Most antibiotics are now used orally allowing more contact with common organisms in the alimentary and digestive tracts and resulting in more resistant strains.
c. The use of antibiotics in animal feeds to increase size and growth rates of commercial animals for food can result in the selection of resistant microbial strains.

Lab 37 - Bergey's manual

What information it contains

Bergey's manual is the main resource for determining the identity of prokaryotic organisms, emphasizing bacterial species, using every characterizing aspect.

Dichotomous Key (always start with gram stain and morphology)
Determine organism as specifically as possible based on characteristics.
You can only go as far as you can. Stick to the information you are given.



Method to identify an unknown organism
The most fundamental technique for classifying bacteria is the gram stain, developed in 1884 by Danish scientist Christian Gram.

Microbiologists have accumulated and organized the known characteristics of different bacteria in a reference book called **Bergey's Manual**

The identification schemes of Bergey's Manual are based on **morphology** (e.g., coccus, bacillus), **staining** (gram-positive or negative), **cell wall composition** (e.g., presence or absence of peptidoglycan), **oxygen requirements** (e.g., aerobic, facultatively anaerobic) and **biochemical tests** (e.g., which sugars are aerobically metabolized or fermented).

In addition to the **gram stain**, other stains include the **acid-fast stain**, used to distinguish *Mycobacterium* species, **endospore stain**, used to detect the presence of endospores; **negative stain**, used to demonstrate the presence of capsules; and flagella stain, used to demonstrate the presence of flagella.

Which organisms are found in Bergey's manual?
Unknown bacteria.

<u>Lab Report # 37</u>

1. What is found in each section of the first edition of Bergey's Manual of Systematic Bacteriology?
Microorganisms with specific features in common such as:
1. Size,
2. Morphology
3. Gram stain reaction
4. Oxygen dependency
5. Motility
6. Presence or absence of endospores
7. Mode of ATP production

2. In articles on the bacterial genera, what types of tables are used to summarize data?
a. A table to differentiate between related genera.
b. A table to differentiate species within the genus.
c. And tables that provide extra information about particular species.

3. How would you use Bergey's Manual to identify an unknown?
a. Determine which volume to use based upon morphology and Gram stain reaction.
b. Look at descriptive titles for each section within the volume and select the appropriate one in the table of contents.
c. Use the tables and keys within the appropriate section and select the appropriate genus.
d. Use keys and tables within the genus to select the species.

4. When using the Table of Contents in Bergey's Manual, why must you be very careful?
If your Gram-staining technique is not absolutely correct, you could end up trying to identify your unknown in the wrong section of the manual.

5. Of what value is the Bibliography in Bergey's Manual?
This section alphabetically lists all the papers cited within each volume of the manual. This allows the student to further explore the bases behind the data within the manual.

6. How many current volumes are there of Bergey's Manual?
There are four volumes in the complete Bergey's Manual with 33 sections.

7. What are the three types of tables found in Bergey's Manual?
1. A table of characteristics distinguishing this genus and related genera.
2. A table showing differentiation between species within the genus.
3. A description of each species in tabular form.

Lab 53 - Normal Flora

Why normal flora is important?
1.The normal flora can synthesize and excrete vitamins in excess of their own needs, which can be absorbed as nutrients by their host. For example, in humans, enteric bacteria secrete Vitamin K and Vitamin B12, and lactic acid bacteria produce certain B-vitamins.

2.The normal flora prevents colonization by pathogens by competing for attachment sites or for essential nutrients. This is thought to be their most important beneficial effect, which has been demonstrated in the oral cavity, the intestine, the skin, and vaginal epithelium.

3.The normal flora may antagonize other bacteria through the production of substances, which inhibit or kill nonindigenous species.

4.The normal flora stimulate the development of certain tissues, like certain lymphatic tissues (Peyer's patches) in the GI tract.

5.The normal flora stimulates the production of natural antibodies. Antibodies produced against antigenic components of the normal flora are sometimes referred to as "natural" antibodies.

Why it is important to know the normal flora of specific body sites?

1. Diagnosis purpose: if you know where the normal flora are, then you won't chase down E-coli, for example, as a pathogen or source of disease in your gut.

2. Lab: understanding what the normal flora are, allows you to selectively inhibit the growth of normal flora to isolate any known pathogen.

Species of some obvious normal flora
*Gut- E. Coli (Not Salmonella Typhy)
*Skin- Staphylococcus Epidermidis
*Vagina –Lactobacillus

Urine - Blood Agar Plate
Rectal Culture- EMB (selective and differential test- Look at your handout)
Throat- Blood Agar Plate

Skin- TSA Plate.

Lab Report # 53

1. **When doing a throat culture, what specific area of the throat is swabbed, and why?**
The area around the tonsils is swabbed, because this area is more likely to harbor potential pathogens.

2. When doing a skin culture, why is the swab first moistened with saline?
To more readily absorb microorganisms from the skin onto the swab.

3. What are four reasons for knowing which microorganisms are associated with different parts of the body?
(1) To gain an understanding of the different microorganisms at specific body locations, which provides greater insight into the possible infections that might result from injury to these body sights
(2) To develop a knowledge of the native microorganisms in any one part of the body, which helps the clinician put in perspective the possible source and significance of microorganisms isolated in clinical infections
(3) To gain a knowledge of indigenous flora that aids in understanding the consequences of overgrowth of those microorganisms normally absent at a specific body site
(4) To develop an increasing awareness of the role these indigenous flora play in stimulating the host in immune response, which provides protection against microorganisms that might otherwise cause disease.

4. Why were the blood agar plates incubated at 35°C, whereas the TSA plates were at room temperature?
To more accurately reflect the temperatures of the body sites where the microorganisms are normally found.

5. Why is EMB used for rectal cultures?
It is selective for gram-negative enteric rods and inhibits the growth of most other rectal bacteria.

6. What are fastidious streptococci?
They are streptococci that require additional nutrients not normally found in standard culture media.

Lab 46 - Most Probable Number Test

Use

MPN is used to test if there are coliforms in water. Is the water potable?
Used to test huge quantity of water (city water supply) so it's not an exact number but rather a statistical probability based on a small amount of water that represents the characteristics of the entire water supply.

Method

This test involves a multiple series of Durham fermentation tubes, and is divided into three parts: presumptive, confirmed, and completed tests.

1.Presumptive Test
Dilutions from the water sample are added to lactose or lauryl tryptose broth fermentation tubes. **After a 24-48-incubation cycle, one looks for bacteria capable of fermenting lactose with gas production.**
---the lauryl tryptose broth is selective for gram-negative bacteria.

Note: An estimate of the number of coliforms can also be done in the presumptive test
In this procedure, 15 lactose broth tubes are inoculated with the water sample.
5 tubes 10ml of water
5 tubes 1ml of water
5 tubes 0.1 ml of water

A count of the number of tubes showing gas production in then made, and the figure is compared to a table developed by the American Public Health Association.

2.Confirmed Test
One transfers material from the highest dilution of those lactose broth tubes that showed growth and gas production into brilliant green lactose bile broth.
---this broth is both differential and selective for coliforms.
After incubation, one is looking for any gas formation in the Durham tube, which is a confirmed test for total coliforms.

3.Complete test
A sample from the positive green lactose bile broth is streaked onto Levine's EMB and incubated for 18-24 hours. Media contains lactose for coliform bacteria.
EMB has methylene blue, which is inhibitory for gram-positive bacteria.

On EMB agar, coliforms produced small colonies. Samples are then inoculated into brilliant green lactose bile broth. **If gas is produced in the lactose broth, and the isolated bacterium are gram-negative, then the completed test is positive.**

Difference between a presumed and definitive MPN Test

The presumed test is used to estimate the number of coliforms while the definitive MPN test doesn't give you a statistical estimate of the total number of bacteria in the water source.

Lab Report # 46

1. Why are coliforms selected as the indicator of water potability?
They indicate the presence of lactose-fermenting gram-negative rods. If they are present, the water may contain fecal microorganisms (from the intestinal tract of humans or animals) and is not potable (fit to drink).

2. Does a positive presumptive test indicate that water is potable?
No, it detects the presence of lactose fermenters that produce gas, therefore it is presumed that coliforms are present.

3. Why is the MPN test qualitative rather than quantitative?
It estimates the probable existence of microorganisms in water without actually counting them.

4. What is the function of the following in the MPN test?
a. Lactose broth—presumptive indication of coliforms.
b. Levine's EMB or LES Endo agar—used for the completed test indicating the presence of coliforms.
c. Nutrient agar slant—used for Gram staining the culture to determine the presence of gram-negative rods.
d. Gram stain—shows whether the organisms present in the presumptive and confirmed tests are really gram-negative rods.

5. What does a metallic green sheen indicate on an EMB plate? Pink to dark red colonies with a metallic surface sheen on LES Endo agar?
In both cases, these results indicate the presence of coliforms, or lactose fermenters in the completed test.

6. What bacterial diseases can be transmitted by polluted water?
a. Dysentery
b. Typhoid fever
c. Cholera
d. Urinary tract infections

Lab 49 - Soil Microorganisms

Use of selective media-
To support the three different groups of microorganisms, you will use three types of media.

Media
Fungi- Sabouraud Dextrose Agar
Acitinomycetes- Glycerol yeast agar
Bacteria- Tryptic Soy Broth

Each media provides optimal environment to the specific organism and sometimes selectively prohibits the growth of other organisms.

Calculation of number in the soil sample example
Take your count from the given plate, and multiply it by it dilution reciprocal
25 count (10^-3) = 25000
201 count (10^-6) = 201000000
141 count (10^-7) = 1410000000

Example Problem
Actinomycetes = 164000
Bacteria= 6100000
Fungi=60000
total=907800000

#A 1640000/ X% = 907800000/100% =1.8%
Calculation of percentage of each type of organism in the soil sample

Formula
#F/ % = # Total/ 100

<u>Lab Report # 49</u>

1. Why were three different media used in this experiment?
Because not all the microorganisms present will grow on any one medium.
One medium is particularly appropriate for fungi (dextrose agar)
One is appropriate for actinomycetes (glycerol yeast extract agar)
One is appropriate for common "typical" bacteria (tryptic soy agar).

2. Would the same results be obtained in this experiment if the soil sample was collected during a different time of the year? Explain your answer.
Yes. The abundance of plants at certain times of the year would favor some microorganisms, while decaying vegetable matter at other times would also be selective.

3. What general group of soil bacteria cannot be determined using the media and procedures in this exercise?
Most obligate anaerobes in the soil will not be isolated using the above procedures.

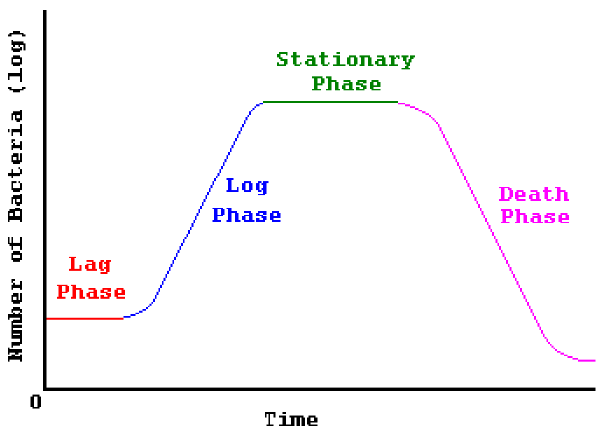
4. What generalizations can you make from this exercise with respect to your garden soil?
a. One can estimate whether it is alkaline or acidic (fungi predominate in acidic soil).
b. One can estimate the soil fertility based upon numbers of microorganisms present.

5. What are some physical features of soil that influence microbial populations?
a. Porosity (airspaces) that allow adequate moisture to enter and permeate the soil.
b. The pH of the soil (too high or too low restrict microbial species).
c. The quantity and availability of nutrients in the soil.

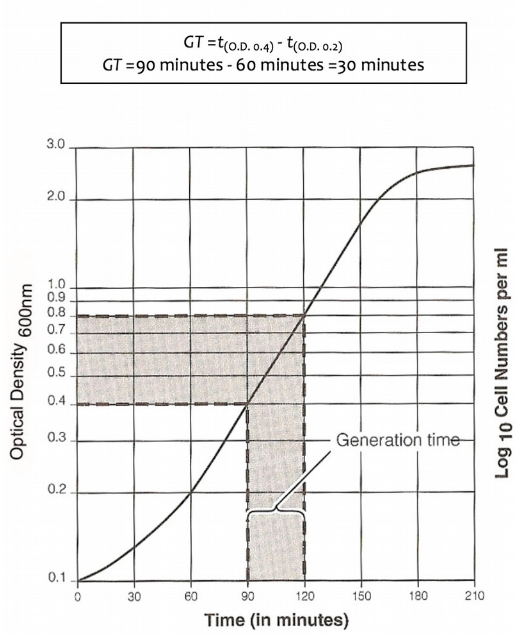
7. Why are different dilutions used for bacteria, fungi, and actinomycetes?
Because in most soils there will be a greater number of "typical bacteria," followed by the actinomycetes, and, finally, the least, comprising fungal isolates.

Lab 45 - Growth Curves

Method of determination
Be able to plot absorbance versus time (cell numbers versus time and able to calculate and identify different phases.



Be able to look at the graph and be able to come up with generation time.
Look for a place in exponential phase where absorbance doubles.
----Generation time is optimal in exponential (log) phase



Remember, Spectrophotometer is indirect and needs to be compared to growth plate to establish relationship.

<u>Lab Report # 45</u>

1. Define generation time.

The generation time is the time taken by the bacteria to double in number during a specified time period. The generation time tends to vary with different organisms. E. Coli divides in every 20 minutes, hence its generation time is 20 minutes, and for Staphylococcus aureus it is 30 minutes.

2. When following bacterial growth, why is absorbance plotted instead of percent transmission?

Because when we read absorbance, it's the amount of light absorbed by the bacteria itself. Absorbance is directly related to the amount of bacteria. More absorbance = more bacteria.

3. Can generation time be calculated from any phase of the growth curve? Explain your answer.

No, it can only be calculated from the log phase, which is where constant growth occurs. Moreover, in the lag phase, bacteria are just beginning to explore their surroundings and in stationary phase growth stops.

4. What is occurring in a bacterial culture during the lag phase? During the growth phase?

Lag phase

When a microorganism is introduced into the fresh medium, it takes some time to adjust with the new environment. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate and therefore no increase in cell mass.

Exponential or Logarithmic (log) phase

During this phase, the microorganisms are in a rapidly growing and dividing state. Their metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate. The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases logarithmically (exponentially) and finally the single cell divide into two, which replicate into four, eight, sixteen, thirty two and so on (That is 2^0, 2^1, 2^2, 2^3.........2^n, n is the number of generations) This will result in a balanced growth.

6. What is meant by the turbidity of a culture?
Turbidity or Optical density is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganisms present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism.

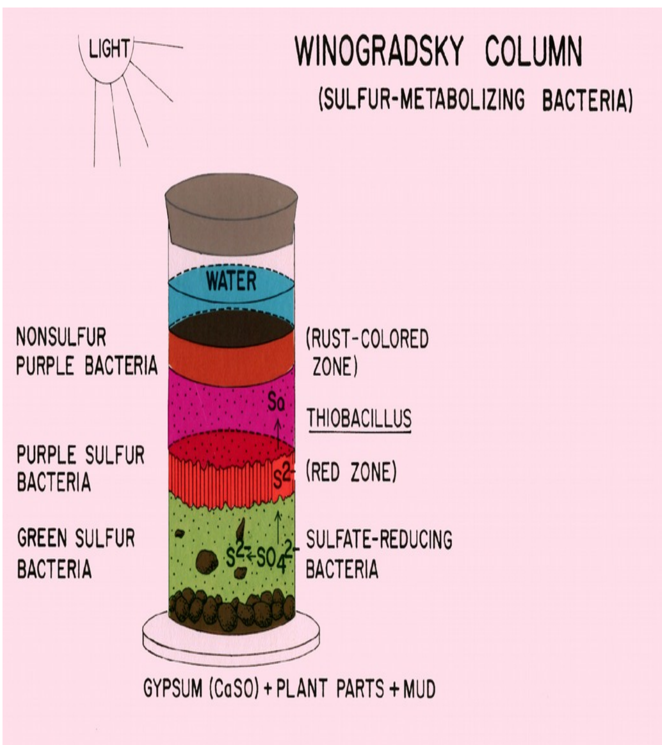
Winogradsky Columns

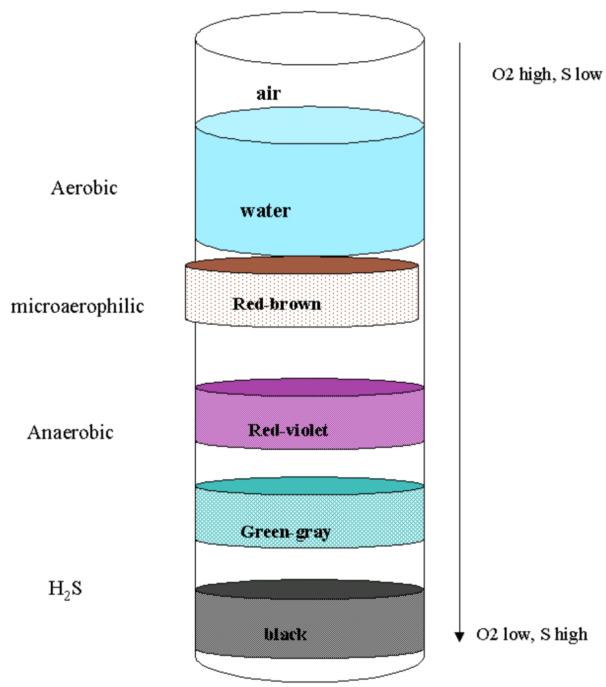
Winogradsky Column is a simple device for constructing a stratified ecosystem and provides a visual example of various modes of metabolism and zonation in the microbial world. It is a classic demonstration of the metabolic diversity of prokaryotes.

Purpose
The purpose is to realize that what is waste of one organism is the food of another (connected food loop). A given ecosystem might have several biogeochemical cycles going on within it. Some important nutrients that are recycled through an ecosystem are oxygen, carbon and sulfur. Different soil microbes play key roles in recycling these and other nutrients.
Understand the logic of each layer.

Explanation of Results
Typically the lower portions of the column are colonized by photoautotrophic green and purple sulfur bacteria. The aerobic surface of the column is occupied by oxygenic cyanobacteria. Just below the surface phototrophic purple non-sulfur bacteria predominate.

Over time gradients of different nutrients should have formed in the Winogradsky columns. These gradients affect where different microbes grow within the columns. For example, over time there's more oxygen at the top of a column that at the bottom, and this means that microbes that can tolerate or make oxygen will be at the top. Microbes that cannot tolerate free oxygen (called anaerobic bacteria) will be further down. Similarly, microbes that need light to make energy (via photosynthesis or a similar process) will need to live where they can get light in the column.





Media

Selective - contains dyes or toxics substances, which inhibit the growth of certain microbes, but support the growth of others.

Differential- distinguishes between different types of microorganisms based on differences in appearance of growth or color changes.

Blood agar Plate

Blood agar is a differential medium on which bacteria can be classified on the degree to which they are able to metabolize hemoglobin.

Aim of Test: blood agar plates allow for the growth of fastidious organisms (species that do not grow easily) and the differentiation of cells according to three hemolytic activities.

The Three Types of Hemolytic Activities:

- a clear zone around bacterial growth -RBC hemolyzed (Beta-hemolysis)
- a greenish zone around growth -RBC partially hemolyzed (Alpha-hemolysis)
- no change around growth -blood is not hemolyzed (Gamma-hemolysis)

Mannitol Salt Agar

Mannitol Salt Agar (MSA) is a **selective** and **differential** medium. The high concentration of salt (7.5%) selects for members of the genus Staphylococcus, since they can tolerate high saline levels. Organisms from other genera may grow, but they typically grow very weakly.

MSA also contains the sugar mannitol and the pH indicator phenol red. If an organism can ferment mannitol, an acidic byproduct is formed that will cause the phenol red in the agar to turn yellow.

Eosin Methylene Blue Agar

Eosin methylene blue agar (EMB) is a selective and differential medium used to isolate fecal coliforms.

EMB contains dyes that are toxic for Gram-positive bacteria and bile salt, which is toxic for Gram-negative bacteria other than coliforms.

It can distinguish between organisms that are capable of fermenting lactose from those that cannot.

O/F Glucose Media

Whether an organism can <u>respire or ferment glucose</u> can be tested with <u>Glucose O/F Medium</u>. A small amount of acid production can be associated with glucose respiration and can be distinguished where a strict aerobe is growing – i.e., at the top of the medium. If the organism were a facultative anaerobe, this acid formation would not be distinguishable due to the large amount of acid associated with fermentation, which would be diffusing throughout the medium.

Micropipettes

Look at the first number on the instrument. What is the maximum amount for the micropipette?

RULE OF THUMB

: Always select the SMALLEST size pipet that will handle the volume you wish to move to achieve the greatest accuracy. Accuracy decreases as you use unnecessarily large pipets for small volumes.

How to use

Micropipette- Most molecular biology experiments include procedures that require extremely small and precise volumes of solutions and reagents. The adjustable micropipette does just that.

To draw liquid into the micropipette tip:

- Depress the control button to the FIRST STOP and hold it in that position.
- The SECOND stopping point can be found when the plunger is depressed beyond the initial resistance until it is in contact with the body of the micropipette. The second stopping point is used for the complete discharging of solutions from the plastic tip.
- You should not reach this second stop when drawing liquid into the micropipette, only when expelling the last drop.
- Dispose of the used micropipette tip by pressing the eject button on the micropipette.

1000 microliters= 1 ml