

Microbiology Notes – Part Two  
From 1997

Chapter 6 – Microbial growth

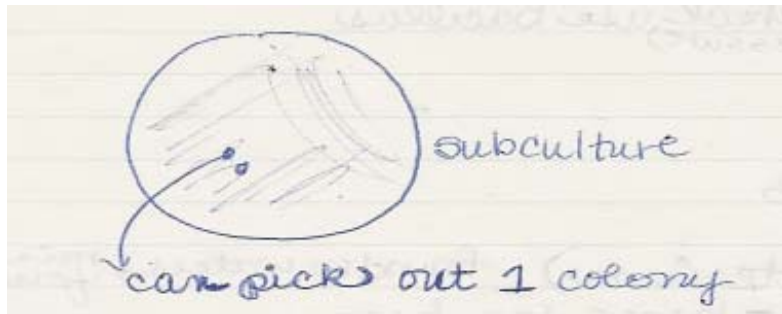
Cell – (1) The basic microscopic unit of structure and function of all living organisms.

Colony – group of cells you can see microscopically (A clone of bacterial cells on a solid medium that is visible to the naked eye.)

Culture – microorganisms that grow and multiply in a container of culture medium

Subculture

Pure culture – a population of one strain or species of bacteria.



Media

CVA – Crystal violet agar

Physical and chemical requirements.

Physical

- Temperature  
freezing 0 ° C  
Psychophile 2 – 10 ° C (Lysteria, fungal, cold bathroom tile, bread)  
thermophile can live up to 120 ° C (probably have spores and / or capsules)
- pH  
6-8 bacteria  
5-6 fungus
- O<sub>2</sub> -- terms
- Light  
most clinical pathogens are not light – sensitive, do not require light for growth.  
Halobacterium – environmental bacteria – does like light, found in waters, rain forests, and oceans.  
Another reason clinical pathogens do not need light because they are not photosynthetic.
- osmotic pressure  
hyper, iso, hypo

Chemical requirements are a little more complex.

- Nutrients—organisms need a certain number of nutrients
- 4 basic elements – Oxygen, carbon, nitrogen, hydrogen

- Micro nutrients  
Trace elements (like iron)  
vitamins  
Sometimes salt (to balance osmotic pressure).

\*\*Mesophile 37 ° C

Oligate, aerotolerate, and facultative terms you need to know for both lab and lecture.

Freezing 0 ° C

Psychrophile 2 – 10 ° C *Listeria* fungal cold bathroom tile, bread

Thermophile

- Probably have spores and or capsules
- Sauna, whirlpools,
- Check autoclaves
- 120 ° C – to check use *Bacillus* (steam under pressure)

Physical

- pH 6-8 bacteria
- 5-6 fungus
- O<sub>2</sub> – terms
- Light – (halobacterium) found in waters, rain forests
- Osmotic pressure – hyper, iso, hypo

Chemical

- Nutrients
- 4 basic elements O, C, N, H
- Micro nutrients – trace elements (like iron), vitamins, salt

\*\* Sometimes organisms are Fastidious. Means it is picky, needs a particular nutrient, needs to be provided with special stuff. Agars like egg yolk agar. Provides for fastidious because it has special nutrients.

Brain heart infusion agar is another one. Organism that causes Legionnaires disease needs charcoal. Will kill organism without charcoal. Always check when you are using something that may be fastidious because without the special requirements you will kill the organism transit.

Media – you can provide for these organisms with media.

- Solid – incorporation of agar which is algae derivative (Good thing is it is not degraded by bacteria)
- Liquid – no agar – broth – algae derivative
- Semi-solid—little agar, just enough to be thick.

Media is chemically defined which means it has all basic nutrients for growth.

- energy source (protein, carbohydrate)
- added salts, elements

Recipe book for media called Dipco Manual.

Different types of media:

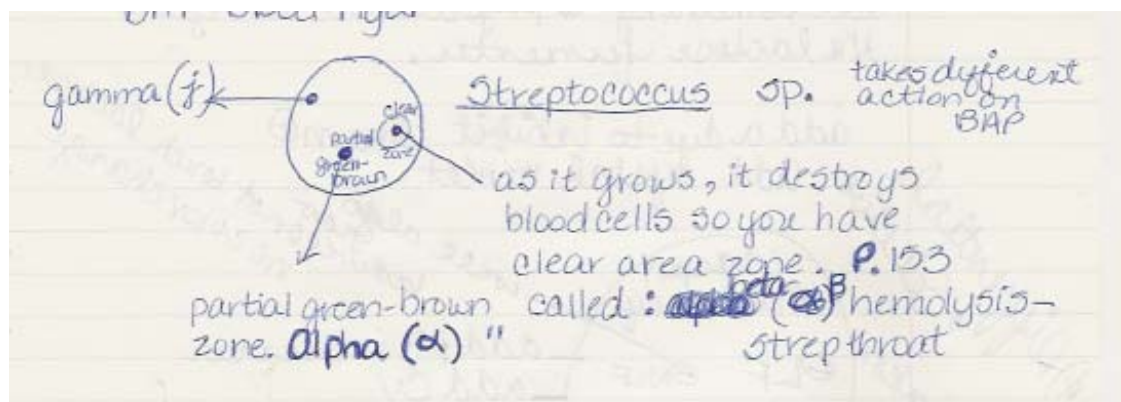
1. All purpose
  - \*\*TSA (trypticase soy agar)
  - \*\*TSP (trypticase soy broth)
  - \*\*Nutrient agar
2. Enriched – blood agar (BAP)  
TSA with 5% sheep blood.

Basically an all purpose agar with an added something.

Grows everything that the all purpose grows plus things that need blood. Very rich.

3. Differential media – tells a difference.  
BAP – Blood agar

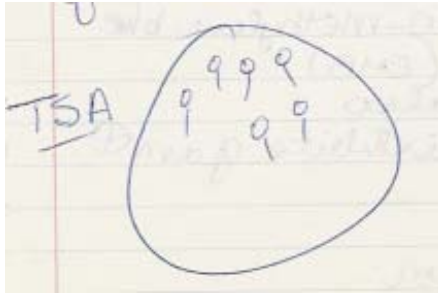
If you take a throat sample from a patient, looking for organism that causes strep throat, when you take that throat sample, you are going to have many types of bacteria in that sample. You are going to have normal flora, Staphylococcus (on mucous membranes), strep, corynebacterium. A rich blend of organisms which makes it a pain to find the organism that causes strep throat. To make it easier, you use differential media.



4. Coliform – indicator of raw sewage.  
Organism that ferments lactose, or uses lactose that is an indicator of fecal matter, raw sewage. When you hear coliform count is very high, it means that there is a lot of raw material, usually there is E. coli. E. coli is found in humans

so it is a good indicator of fecal matter.  
E. coli (used to check coliform count). Gram – Bacilli.

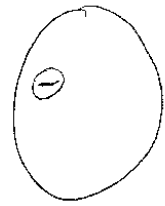
E. Coli  
GNB  
Lactose fermenter (LF)  
Found in intestines



TSA – everything will grow on this  
many different kinds of organisms.

You will have

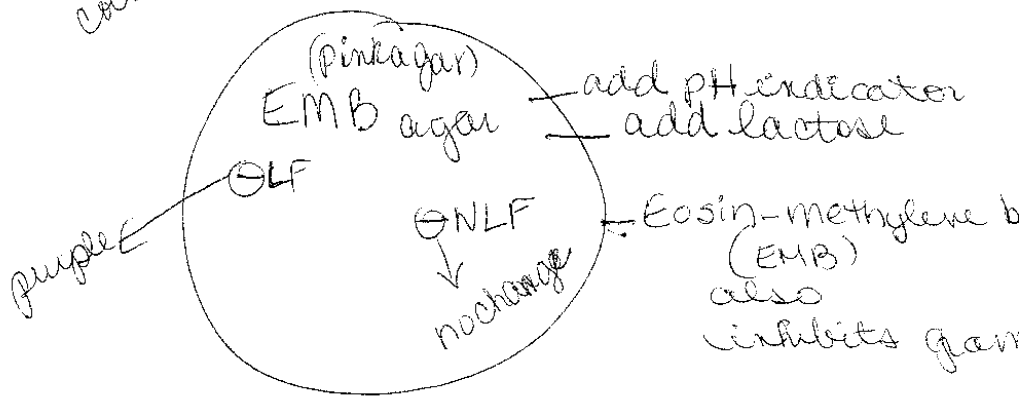
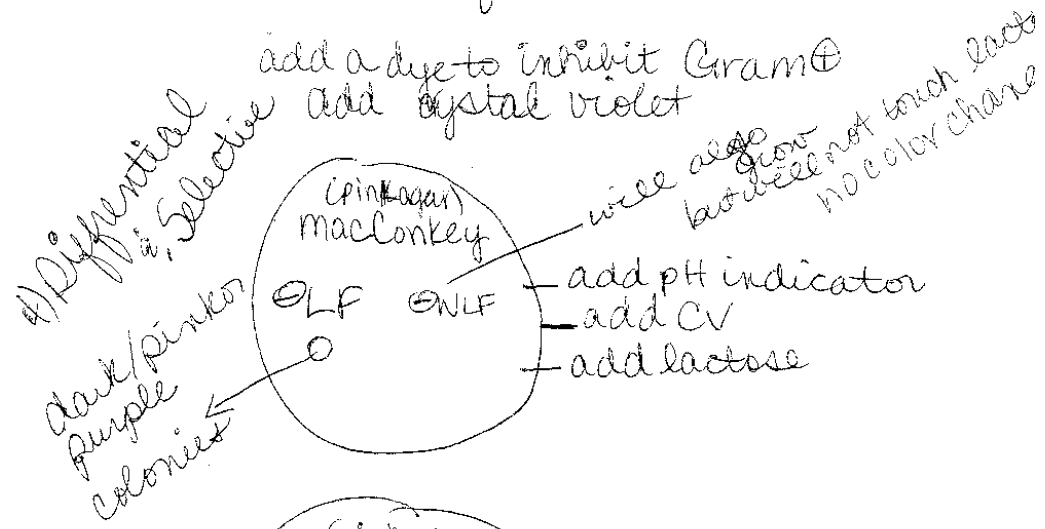
easier way on



only gram- will grow.

CVA

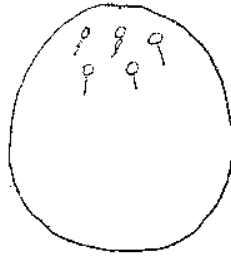
add something to suppress or let grow the lactose fermenter.



will know by color.

BAP is not selective  
CVA is selective

TSA



everything will grow on this.  
you will have many  
different kinds of  
organisms.

Easier way to do this:

4) Differential & Selective - selects for gram<sup>⊖</sup>



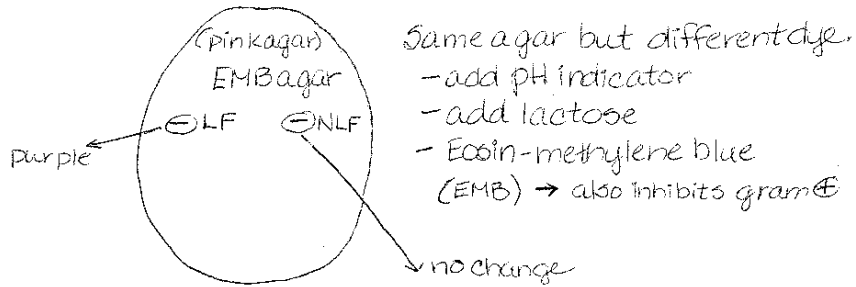
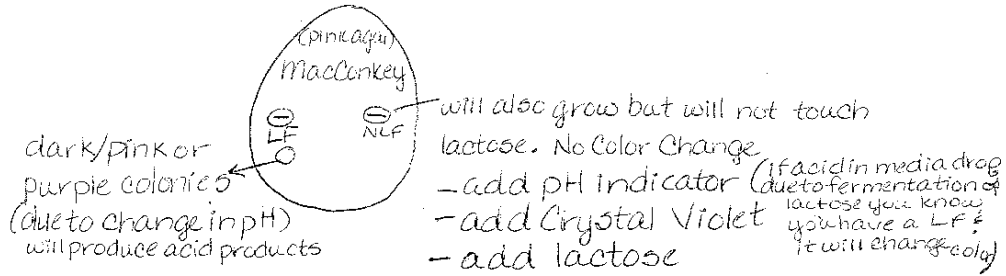
Only gram<sup>⊖</sup> organisms will  
grow on this.

CVA  
Crystal Violet Agar

Need to add something to suppress or let  
grow the lactose fermenter.

You can add milk, there is milk agar.

add a dye to inhibit gram<sup>+</sup>s. Add Crystal Violet, also Eosin methylene blue.

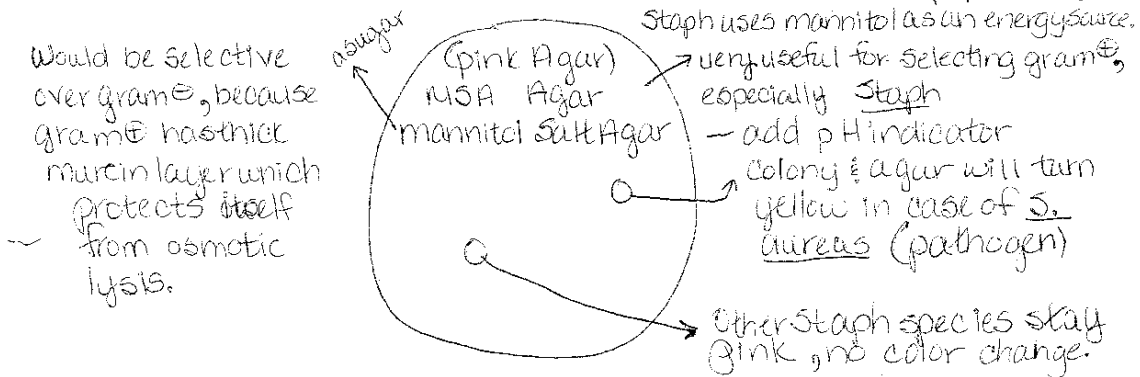


Will know by color.

Blood Agar Plate (BAP) is not selective, grows everything, it is enriched.

Crystal Violet Agar (CVA) is selective, it selects for gram<sup>-</sup>. It is not differential.

Another Differential & Selective: (one of the most popular agar)



What to know from the previous section:

- All these different types of media.
- Definitely need to know how blood agars are differential, what makes MSA, EMB, and MacConkey selective and differential
- Know the differences are between differential vs. differential and selective.
- You can save time and money using these agars.
- You need to include enough clinical information as you can.

It helps to take the time to include as much information as we can about a patient, it can make a big difference. If it is known that a patient has cholera, cholera would do nicely in a 10% salt, it will not grow in anything else. Clinical history is very important, if it is not written down about cholera, the lab might use the wrong media and if 1-% salt is not used, the cholera will not grow and the media will not produce normal flora.

5. Reduced media – anaerobes

2 most common --

\*\*Brewer's yeast

\*\*Fluid thioglycollate medium

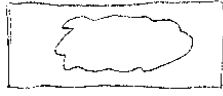
6. Special – Fastidious

Ex. Legionella needs charcoal to grow.

Whooping cough (Bordetella) needs charcoal also.

PCR will replace all of this eventually, will make all these obsolete.

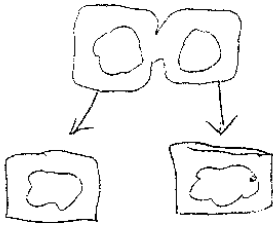
## Growth - Division



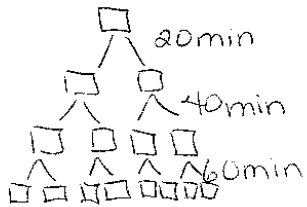
Bacteria divide by:  
binary **fission** - (no sex, no sperm,  
no egg, no mitosis, no meiosis).  
Simply split - Asexual reproduction.

Bacteria has 1 chromosome that is circular. It has to duplicate it's chromosome (DNA) before it divides. Second step is splitting of the cytoplasm.

- 1) DNA replication
- 2) Splitting of cytoplasm.



Sometimes this process is very quick, in the case of E. coli it happens every 20 minutes. Every 20 minutes you double. In an hour you will have 8 cells



$10^3$  Exponential Growth.

Parent cell is gone (lost), it is split. Time taken to go through this process is called Generation (doubling time).

Sometimes it takes a lot longer. Fungus takes a long time to grow. Doesn't grow overnight. Tuberculosis can take up to 6 weeks to grow. Generation time is very long.

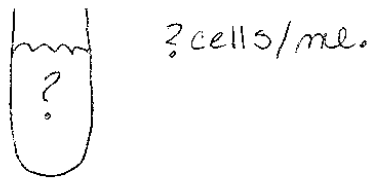
E. coli grows so fast, that's why it is used as the #1 in genetic studies.

Q.157 Figure 6.12  
Bacterial Growth curve

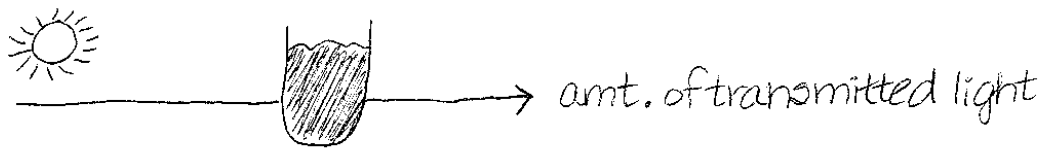
- 4 phases of microbial growth:
- 1) Lag Phase (Preparatory stage - preparing to divide, DNA replication).
  - 2) Log, or exponential growth, phase - period of rapid growth. Cells divide (most effective stage to test antibiotic)
  - 3) Stationary phase (level of growth, nutrients running out, wastes buildup)
  - 4) Death, or logarithmic decline phase.

Enclosed system - not feeding anything.

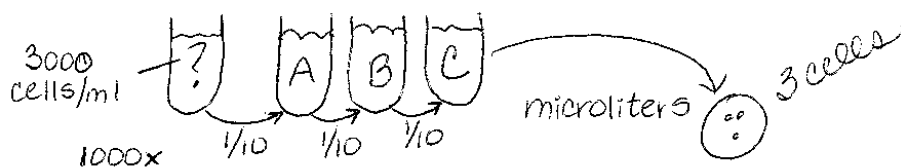
Ways to measure Population growth.



- 1) Direct Count - costs a lot of money.
- 2) Turbidity - Spectrophotometer



3) Serial Dilution (P.159)



Know for lab

How does turbidity relate to serial dilution?

Section on population count.

Statistical method (MPN) = most probable #

