

## Microbial Growth

Growth rate = change in cell # over time  
Binary fission – mediated by divisome, Fts  
Growth cycle in closed system media...  
Understand Lag, Log, Stationary, Death

## Culture/Growth Media

Defined - precise recipe of chemicals  
Complex - extracts (beef, yeast, milk, blood...)  
Selective - inhibit some microbes, not others  
Differential - shows different phenotypes

## Culturing Microbes

Pure Culture - single kind of microorganism  
Solid/agar media immobilizes cells - colonies  
Aseptic Technique - methods to keep sterile  
Many rules for tools - loop, tubes, plates...

## Viable Count – Plate-Based

1 colony = 1 starting bacterium  
Unknown samples - dilute/plate; target 30-300  
Pros - only counts live cells  
Cons - not rapid, selective (right media?)

## Total Cell or Direct Microscopic Count

Uses microscope plus counting chamber...  
Allows putting a defined, small volume on slide  
Pros – relatively rapid, nonselective  
Cons - dead=alive, clumps or small cells difficult

## ACTIVITIES - SESSION ONE

### Loop Transfer With Test Tube - Work Individually, Incubate 37°

Label 1 tube of nutrient broth - any time you label glass tubes, use BLUE Sharpee pens  
Flame loop and wire until red hot; cool 10-15 seconds  
Pick up tube and remove cap, holding with little finger - NEVER SET CAPS DOWN!  
Flame tube mouth 2-3 seconds; place loop into tube and touch media  
Withdraw loop of media and re-flame mouth of tube; replace cap  
Flame loop contents and repeat procedures above TWO more times as described above  
**Notebook records – indicate if there were problems, contamination!**

#### Nutrient Media

Beef Extract: 3 g  
Peptone: 5 g  
NaCl: 8 g

Prepared in liquid (no agar) or agar forms (15 g agar/L). Nonselective media for propagation of most controls and food/body derived samples in this course.

### Streak-Plating and Pure Cultures - Work Individually, Incubate RT°

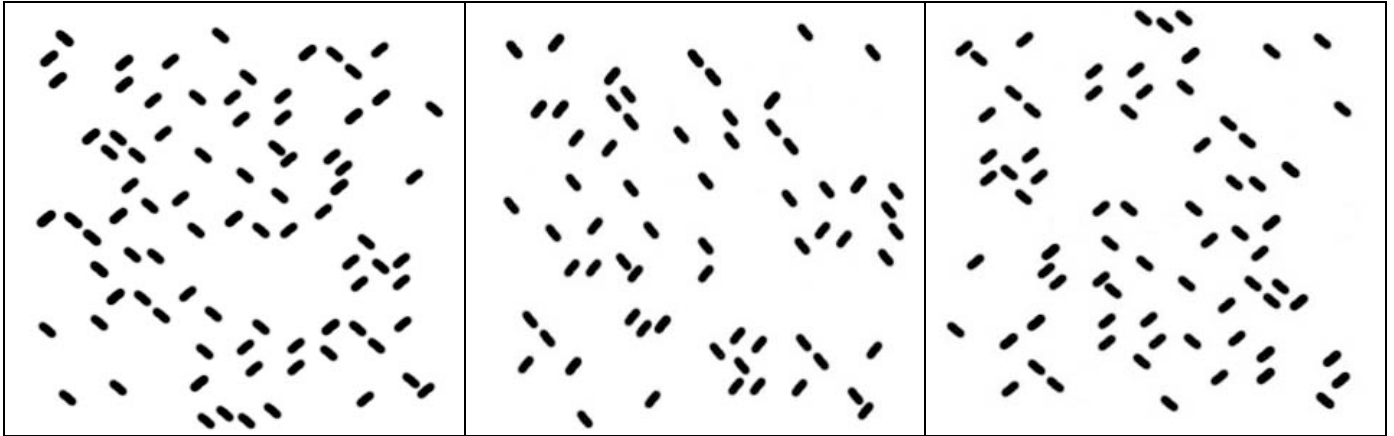
Label 1 nutrient agar plate on the media-containing side  
Flame and cool your loop before collecting a loop of Serratia  
Follow the diagram on the board - which can be drawn on the plate  
Apply bacteria to first region and flame/cool loop  
Touch first region three times and then spread beyond; flame/cool loop  
Touch second region three times and then spread beyond; flame/cool loop  
**Notebook records – in-color drawing, indicate if there were problems, contamination!**

### Viable Counting Using Plates - Work In Pairs, Incubate RT°

Obtain 6 test tubes, each with 9 ml water; label 1-6; 1 will be the least dilute, 6 the most  
Using a sterile transfer pipette, remove 1 ml Serratia, add to test tube 1, mix  
Using a new pipette, remove 1 ml from tube 1, add to tube 2, mix - repeat down the line to tube 6  
Obtain 4 nutrient plates and label with your name and #3-6, reflecting 4 most dilute tubes  
Spread 0.1 ml liquid from tube 1 to plate 1 - repeat with all test tubes onto correct plates  
Next time, count colonies and back-calculate how many cells in each dilution tube, and original  
**Notebook records – labeled table of raw data, calculations (cells/ml in original tube)**

### Direct/Microscopic Total Counting PROBLEM – DRY LAB

The counting grid schematics below show three replicates from a MOCK patient urine sample  
Each square = 0.1 mm X 0.1 mm X 0.02 mm; note - this differs from Cell Biology hemocytometers  
Use this information to calculate a single value for bacteria cells/ml in original patient sample  
**Notebook records – labeled table of raw data, calculations (cells/ml in original tube)**



### LAB MATERIALS TURN-IN

13 pts. Informal Notebook records: make sure all guidelines in syllabus and above are followed;  
Math calculations (viable, direct counting) will EACH represent 5 pts. ALL OR NOTHING.

2 pts. PAIR TURN-IN: Each pair will turn in the ONE viable count plate they used for enumeration.  
This must be properly labeled and clearly indicate the dilution.