Diyala University
College of science/ Biotechnology department
Assist. Lecturer: Sabah Mahmood Hamad
Micro-phasology
Year : 2

LAB 1 : Safety Procedures

1. Laboratory coats are worn.
2. Long hair is tied back away from the shoulders.
3. Working areas are kept clear of all unnecessary materiales.
4. Hand and bench tops are kept clean with disinfectant.
5. Nothing is placed in the mouth such as fingers, pencils or any subject.
6. Do not smoke, eat or drink in the laboratory.
7. Unnecessary activities can cause accidents and promote contamination.
8. Before leaving the laboratory, carefully wash and disinfect your hands.

Tools , instrument

1-**Loop**: use for transfer of bacterial cells from medium to another (as colony or drop"0.01ml"), sterilized by the flame of burner after and before using.

2-Slide: use for the examination .placed on microscope stage.

3-**Cover- sips**: placed on the slide, the sample will be between the cover and the slide.

4-**Test tube**: use to place the broth, solid, or semi-solid medium for stabbing, or place as slant for bacterial culturing. The empty tubes or with uncultured broth sterilized by autoclave (15 min) but with cultured broth by autoclave (30 min).

Equipment

1-Hood:capin sterile maintain a free of contamination environment in the laboratory and contain a **burner** may be gaseous or alcoholic ,use for sterilization of the loop ,needle and other metal tools by the fame (dry heat sterilization)

2-Autoclave: equipment with high temperature, pressure and steam to sterilize the culture media and some of metal tools and glass wares.

Temperature=121 Cº

Pressure=1 atm (15 pound/inch2)

Time=10-30 min.

- 10 min. for media with sugar
- 15 min for uncultured media
- 30 min. for cultured media &contaminated tools &glass wares

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LAB2: Sterilization and Disinfection

Sterilization: *Sterilization* describes a process that destroys or eliminates all forms of microbial life including their spores and is carried out in health-care facilities by physical or chemical methods

Disinfection: *Disinfection* describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores.

Sepsis: Comes from Greek for decay or putrid. Indicates bacterial contamination.

Asepsis: Absence of significant contamination.

Aseptic techniques are used to prevent contamination of surgical instruments, medical personnel, and the patient during surgery.

Aseptic techniques are also used to prevent bacterial contamination in food industry.

There are many methods of sterilization:

But while the selection method requires not alter the sterilization of the physical and chemical properties of a substance which sterilized

1- Heat sterilization: is the best way to sterilization and it's easy of use and cost .But it cannot be applied to heat living tissue or tools that are affected by the high heat like rubber materials.

Heat sterilization are two ways: dry heat and moist heat

Dry heat: dry heat sterilization are concentrated on the oxidation mechanism of cell structure, and are exposing materials or items that have to be sterilized up to a temperature

ranging from 160-180 ° C for one hour. This method is suitable for the sterilization of glass and metal tools and powders, oils

nuclear acids of microorganism.

Moist heat: The best factor for sterilization is water vapor under pressure, as it is in the closed autoclave. Water vapor helps under pressure on the temperature influence in the things that are sterilize like clothes and there is a direct relationship between temperature and vapor pressure, rise in temperature to 121 degrees Celsius for 15 minutes is sufficient to eliminate the spores of Clostridium .

2- Radiation: gamma irradiation selected method for sterilizing a large number of small-sized tools like, syringes, gloves and other, also use this method to sterilize vaccines can also be invoked to save some foods; but the cost of this method is too high.

3- Filtration: is the separation of contaminants from the fluid, a method used for a long time, especially for water purification and other liquids. Consisting of filters of works by static electricity and fine pores.

4- Chemicals: There are still gases used formaldehyde and ethylene oxide and ethanol, both of which sterilizes by it's effect on protein and

Disinfection methods

A- Alcohols and Phenols , Aldehydes , Halogens and hydrogen peroxide 6%.

B-Physical methods:

Boiling & Pasteurization

UV(ultraviolet radiation)

why is 70% alcohol better than 100%?

Pure alcohol 100% coagulates protein in cell wall that it is contact. The alcohol 100% will go through the cell wall of the organism in all direction, coagulating the protein just inside the cell wall. The ring of the coagulated protein would then stop the alcohol from penetrating farther from the cell, and no more coagulation would take place. At this time the cell would become inactive but not dead. Under the favorable conditions the cell would then begin to function. If 70 percent of alcohol is poured to a single celled organism, the diluted alcohol also coagulates the protein, but at a slower rate, so that it penetrates all the way through the cell before coagulation can block it. Then the entire cell is coagulated and the organism dies.

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LAB 3:

Culture Media

Bacteria have to be grown (cultured) for them to be identified by appropriate procedures they have to be grown separately (isolated) on culture media and obtained as pure for study.

Culture Media

A substance containing nutrients in which

microorganisms are cultivated for scientific purposes.

Colony: macroscopically visible collection of millions of bacteria originating from a single bacterial cell .

TYPES OF CULTURE MEDIA

Media are of different types on consistency and chemical composition.

A. On Consistency: divided depending on agar substance which is '**Agar**' is most commonly used to prepare solid media.

Agar is polysaccharide extract obtained from seaweed. Agar is an ideal solidifying agent as it is : (a) Bacteriologically inert, i.e. no influence on bacterial growth, (b) It remains solid at 37°C, and (c) It is transparent.

1. Solid Media. 2% agar, Advantages of solid media: (a) Bacteria may be identified by studying the colony character, (b) Mixed bacteria can be separated. (C) Solid media is used for the isolation of bacteria as pure culture.
2. Liquid Media (broth) . no agar It is used for profuse growth and called broth , e.g. blood culture in liquid media and nutrient broth. Mixed organisms cannot be separated.

3- Semi solid medium – 0.5% agar. Eg: Motility medium.

3. INDICATOR (DIFFERENTIAL) MEDIA.

An indicator is included in the medium. A particular organism causes change in the indicator, e.g. blood, neutral red,. Examples: Blood agar and MacConkey agar are indicator media.

4. TRANSPORT MEDIA. These media are used when samples cannot be cultured soon after collection. Examples: Cary-Blair medium,

5. STORAGE MEDIA. Media used for storing the bacteria for a long period of time.

Examples: Egg saline medium, chalk cooked meat broth.

COMMON MEDIA IN ROUTINE USE

Nutrient Broth. 500 g meat, e.g. ox heart is minced and mixed with 1 liter water. 10 g peptone and 5 g sodium chloride are added, pH is adjusted to 7.3. Uses: (1) As a basal media for the preparation of other media, (2) To study soluble products of bacteria.

Nutrient Agar. It is solid at 37°C. 2.5% agar is added in nutrient broth. It is heated at 100°C to melt the agar and then cooled.

Peptone Water. Peptone 1% and sodium chloride 0.5%. It is used as base for sugar media and to test indole formation.

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Blood Agar. Most commonly used medium. 5-10% sheep or horse blood is added to melted agar at 45-50°C. Blood acts as an enrichment material and also as an indicator. Certain bacteria when grown in blood agar produce haemolysis around their colonies. Certain bacteria produce no haemolysis.

Types of heamolysis :

(a) beta, the colony is surrounded by a clear zone of complete haemolysis.

(b) Alpha (a) haemolysis. The colony is surrounded by a zone of greenish zone due to formation of biliverdin.

(c) Gamma (y) No haemolysis, there is no change in the medium surrounding the colony,

Chocolate Agar or Heated Blood agar. Prepared by heating blood agar. It is used for culture of pneumococcus, gonococcus, meningococcus and Haemophilus. Heating the blood inactivates inhibitor of growths.

MacConkey Agar. Most commonly used for enterobacteria. It contains agar, peptone, sodium chloride, bile salt, lactose and neutral red. It is a selective and indicator medium :

(1) Selective as bile salt does not inhibit the growth of enterobacteri but inhibits growth of many other bacteria.

(2) Indicator medium as the colonies of bacteria that ferment lactose take a pink colour due to production of acid. Acid turns the indicator neutral red to pink. These bacteria are called 'lactose fermenter', e.g. Escherichia coll. Colourless colony indicates that lactose is not fermented, i.e. the bacterium is non-lactose fermenter, e.g. Salmonella. Shigella, Vibrio.

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LAB4: Factors affecting bacterial growth

1. Oxygen requirement
2. Temperature requirement .
3. PH
4. Osmotic pressure (salt tolerance) .

1/ Oxygen requirement :

Bacteria are divided into four groups according to oxygen requirement :

1. Bacteria grow only in the presence of oxygen, called: strict aerobes .
2. Bacteria grow only in the absence of oxygen, called: strict anaerobe .
3. Bacteria grow in the presence or absence of oxygen, called: facultative anaerobe .
4. Bacteria grow in a little amount of oxygen, called: micro aerophilic

In our bodies :

-The aerobic places: skin, eye, mouth, throat, nose .

-The anaerobic places: deep in tissue, large intestine .

-The micro aerobic: upper part of stomach .

How to achieve anaerobic condition :

1. Anaerobic hood .
2. Anaerobic jar: use gas generating kit which contains chemicals to consume the oxygen present in the jar .

To check anaerobic condition we use :

1. Chemical indicator :

- Called: Redox dye .
- It is a yellow strip and it turns green or blue in the presence of O2 .

1. Biological indicator:

Use Strict aerobic bacteria, if it didn't grow that means= anaerobic condition .

Or we use :Strict anaerobe bacteria, if it grows that's mean = anaerobic condition

2/ Temperature requirement :

Bacteria are divided into three groups according to temperature requirement :

a. Bacteria grow at cold temp. (4-10 C), called: Psychrophilic .
b. Bacteria grow at 15- 40C, called: Mesophilic (pathogenic bacteria).
c. Bacteria grow at 45-90 C, called: Thermophilic

PH

1. Some bacteria grow at acidic PH (3-6), Called:

   Acidophilic .

2. Or grow at alkaline PH (8-10), Called:

   Alkelophilic /basophilic .

3. Most bacteria grow at neutral PH (7).

4/ Osmotic pressure

Bacteria vary in their tolerance to salt levels .

1. Some cannot tolerate high concentration of salt .
2. Other can tolerate medium concentration of salt .
3. Some can tolerate high concentration of salt, called: halophilic .

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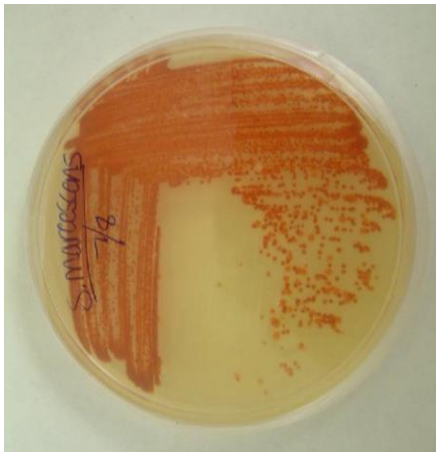
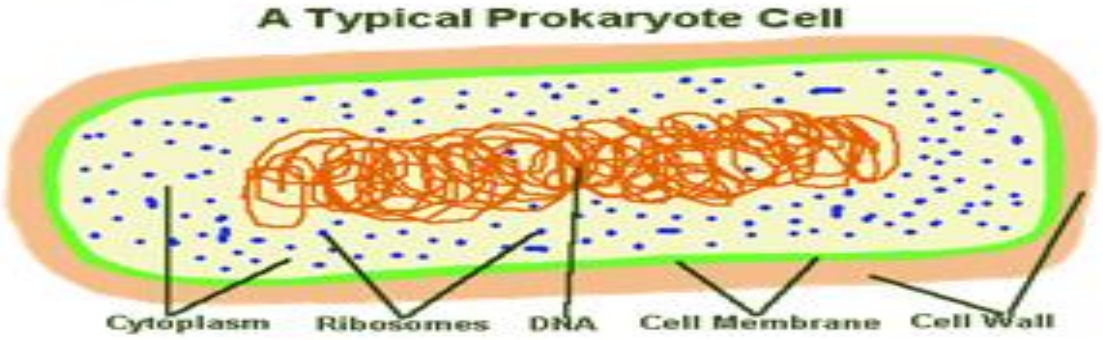


LAB5:CULTURE MEDIA & CULTURE METHODS

What are Bacteria?

- Bacteria are prokaryotes, meaning they are only **ONE** celled organisms. They are very small and can be harmful or beneficial.



- **Bacteria have to be grown (cultured) for them to be identified.**
- **(isolated) on culture media and obtained as pure for study.**

Colony – macroscopically visible collection of millions of bacteria originating from a single bacterial cell.

Agar

- Obtained from seaweeds.
- Used for preparing solid medium
- No nutritive value
- Not affected by the growth of the bacteria.
- Melts at 98^{o}C & sets at 42^{o}C
- 2% agar is employed in solid medium

Types of culture media

I. Based on their consistency
   a) solid medium
   b) liquid medium
   c) semi solid medium
II. Based on the constituents/ ingredients
   a) simple medium
   b) complex medium
   c) synthetic or defined medium
   d) Special media

Special media

- Enriched media
- Enrichment media
- Selective media
- Indicator media
- Differential media
- Sugar media
- Transport media
- Media for biochemical reactions

III.Based on Oxygen requirement

- Aerobic media
- Anaerobic media

Solid media – contains 2% agar

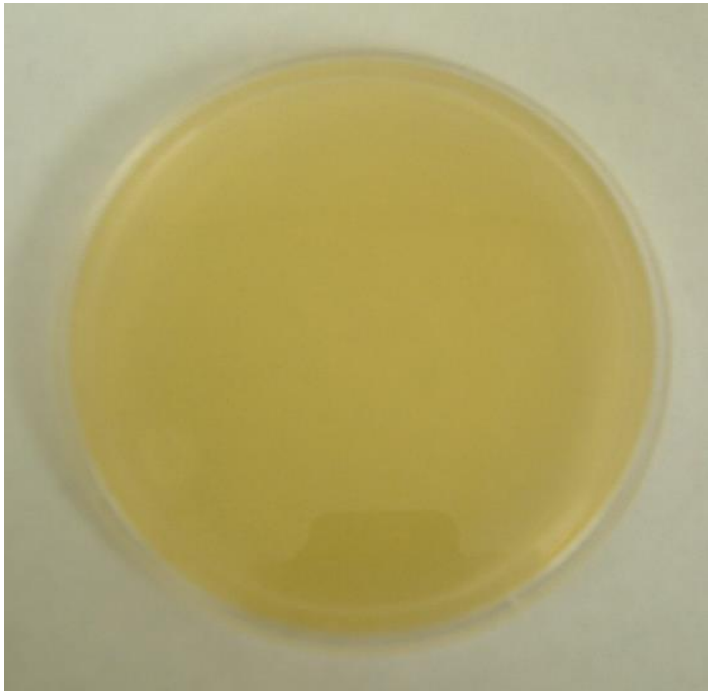
- Colony morphology, pigmentation, hemolysis can be appreciated.
- Eg: Nutrient agar, Blood agar

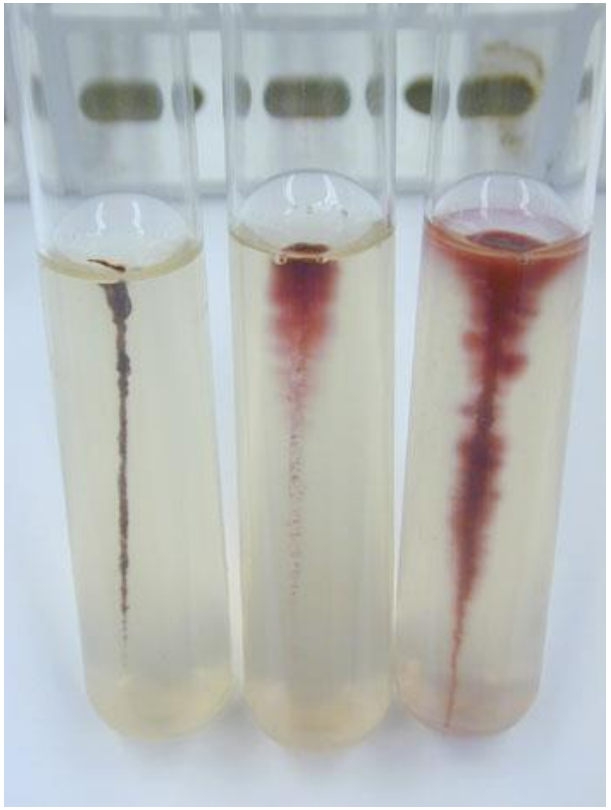
Liquid media – no agar.

- For inoculum preparation, Blood culture, for the isolation of pathogens from a mixture.
- Eg: Nutrient broth

Semi solid medium – 0.5% agar.

- Eg: Motility medium





Simple media / basal media

- Eg: NB, NA
- NB consists of peptone, meat extract, NaCl,
- NB + 2% agar = Nutrient agar

Complex media

- Media other than basal media.
- They have added ingredients.
- Provide special nutrients

Synthetic or defined media

- Media prepared from pure chemical substances and its exact composition is known
- Eg: peptone water – 1% peptone + 0.5% NaCl in water

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LAB 6

Enriched media

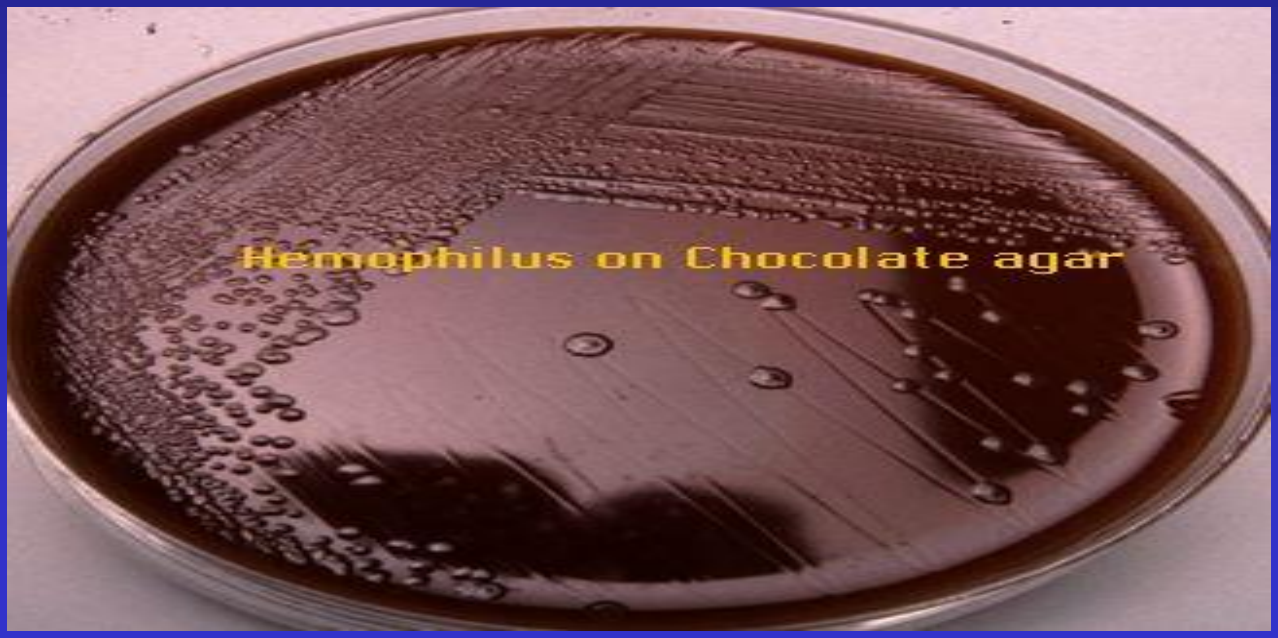
- Substances like blood, serum, egg are added to the basal medium.
- Used to grow bacteria that are exacting in their nutritional needs.
- Eg: Blood agar, Chocolate agar

Blood agar

Chocolate agar

Media

Enriched Media



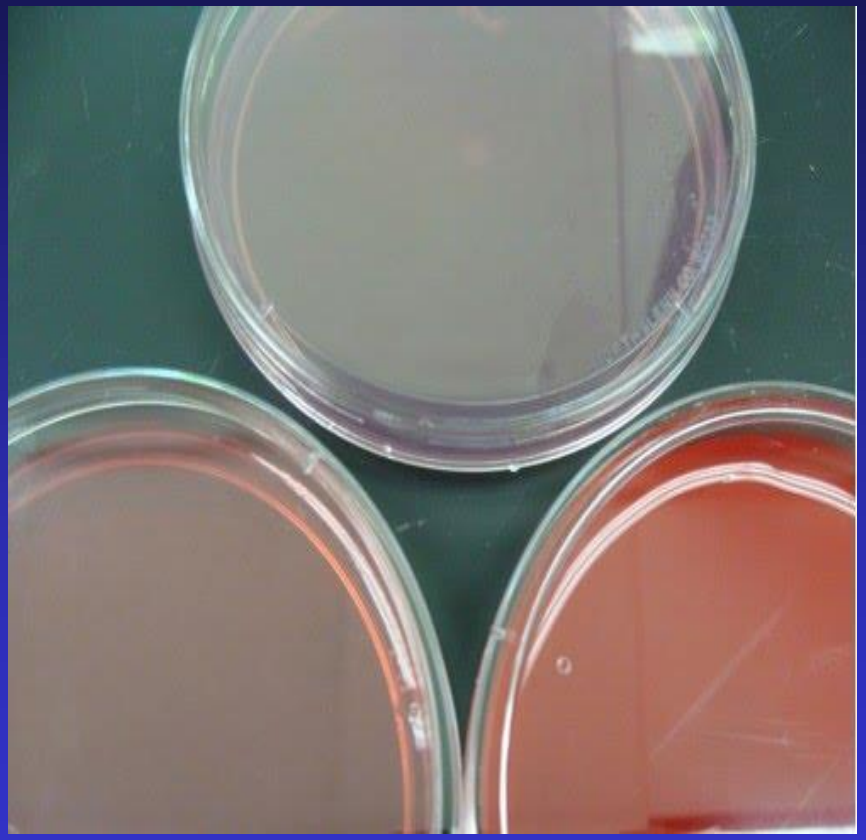
Media

Selective Media

Selects form a microorganism while inhibiting most others

Phenol Ethanol Agar

Deoxycholate Agar



Differential Media

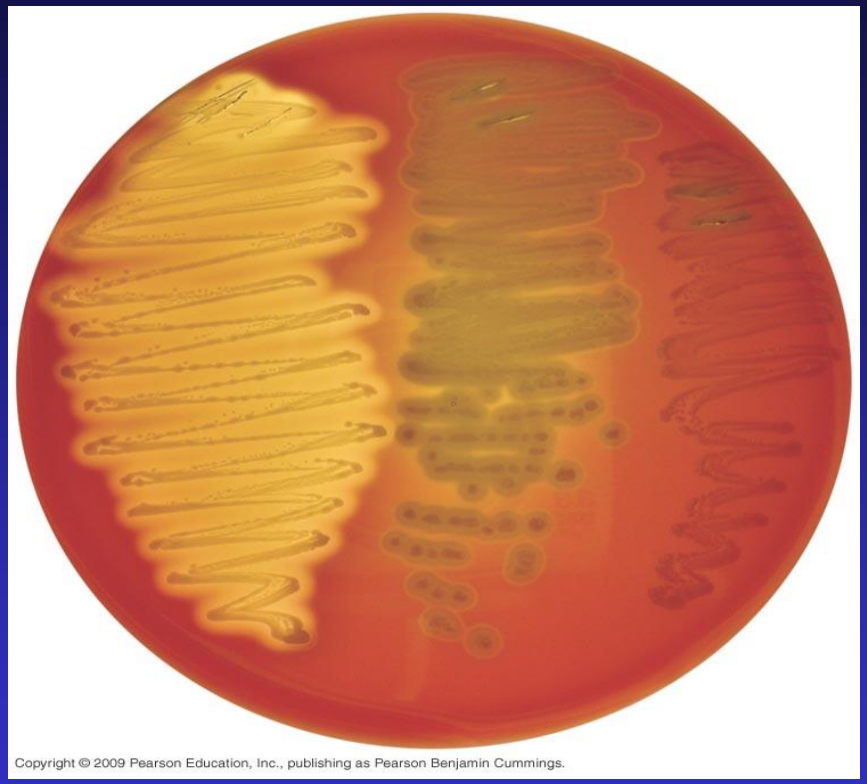
Allow for the differentiation of microorganisms based on action that occurs on the media or a color change within the media that is based on a pH change

Mannitol Salt Agar

MacConkey Agar

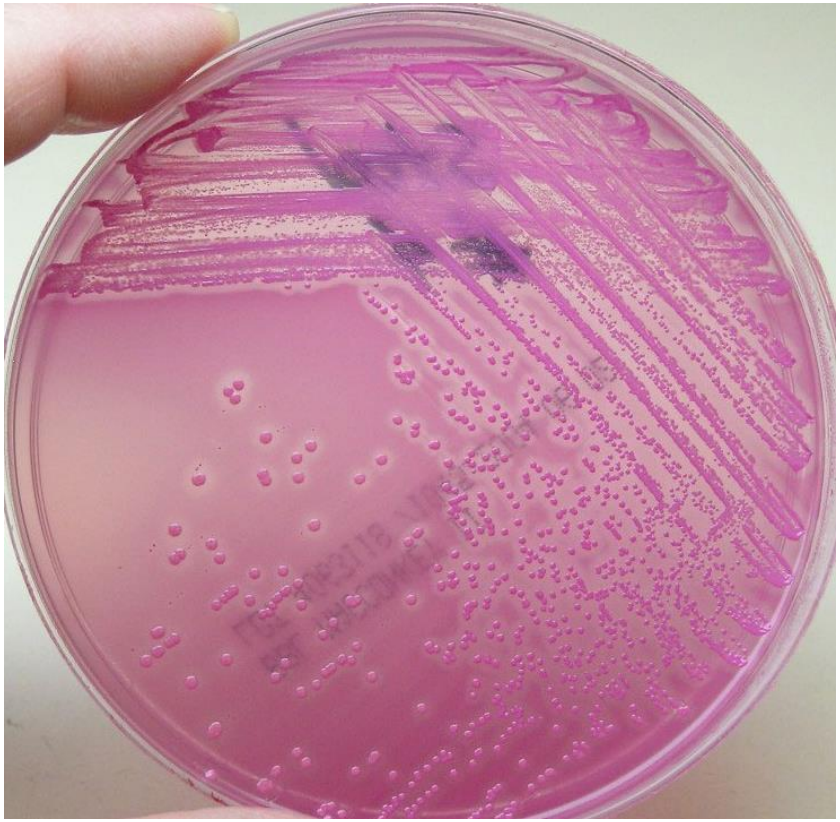
Blood Agar considered?

A. Enriched
B. Selective
C. Differential

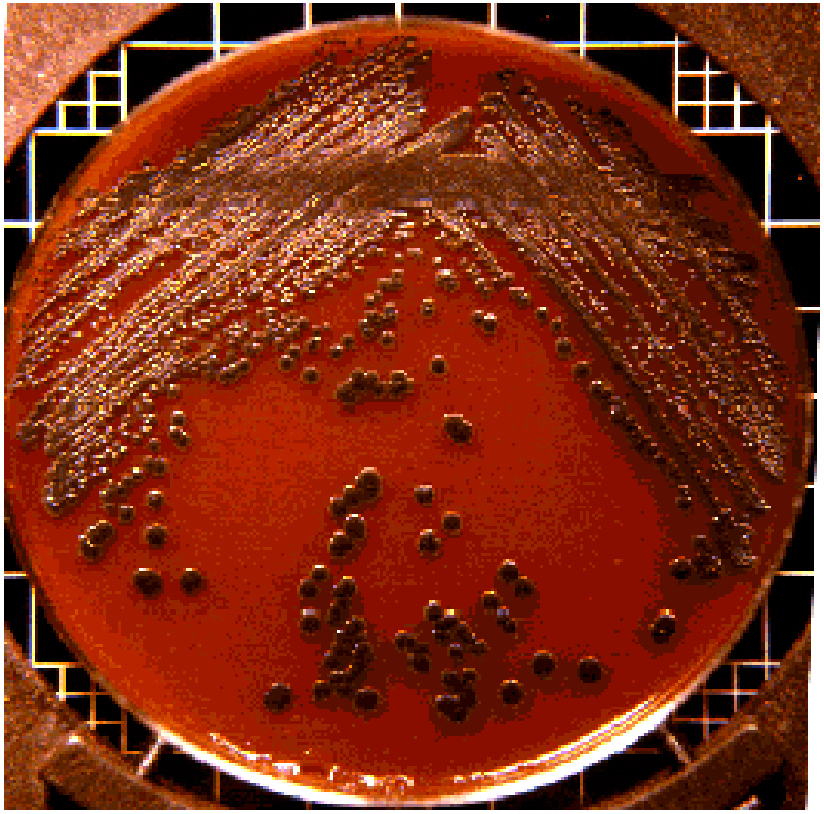


Enrichment media

- Liquid media used to isolate pathogens from a mixed culture.
- Media is incorporated with inhibitory substances to suppress the unwanted organism.



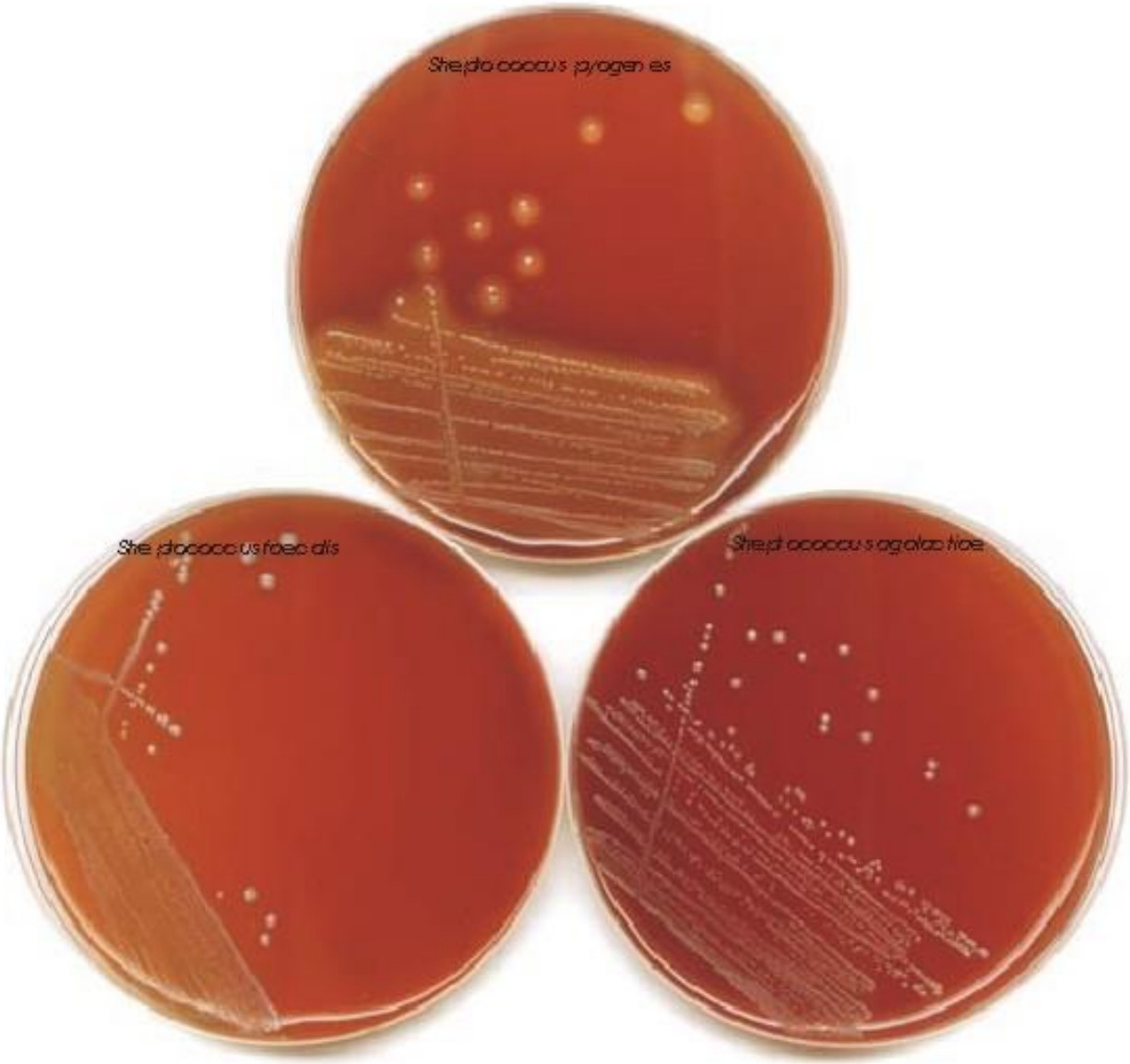
Mac Conkey’s medium



Potassium Tellurite media

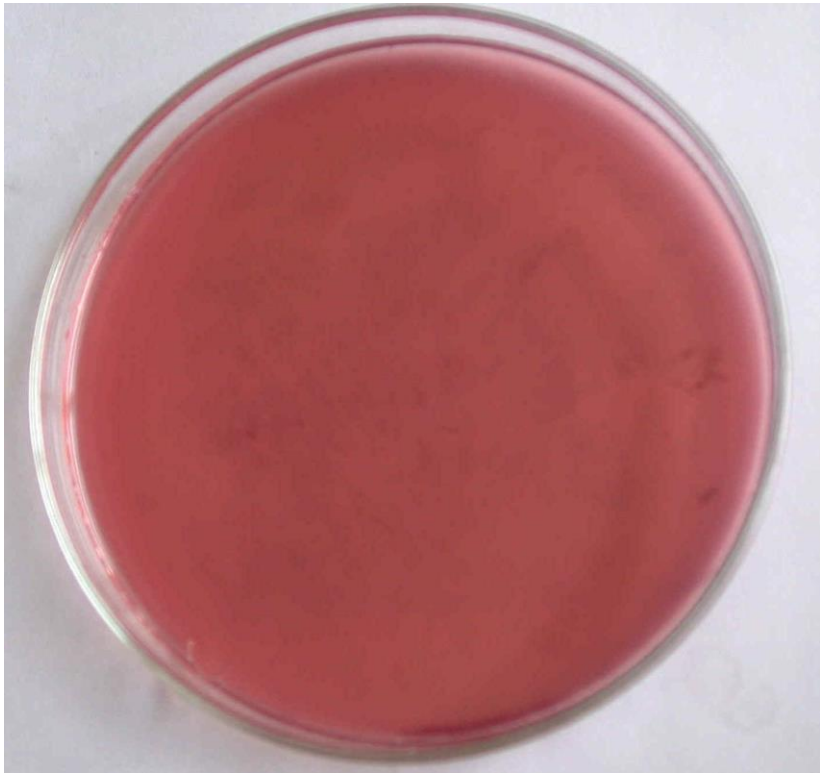
Indicator media

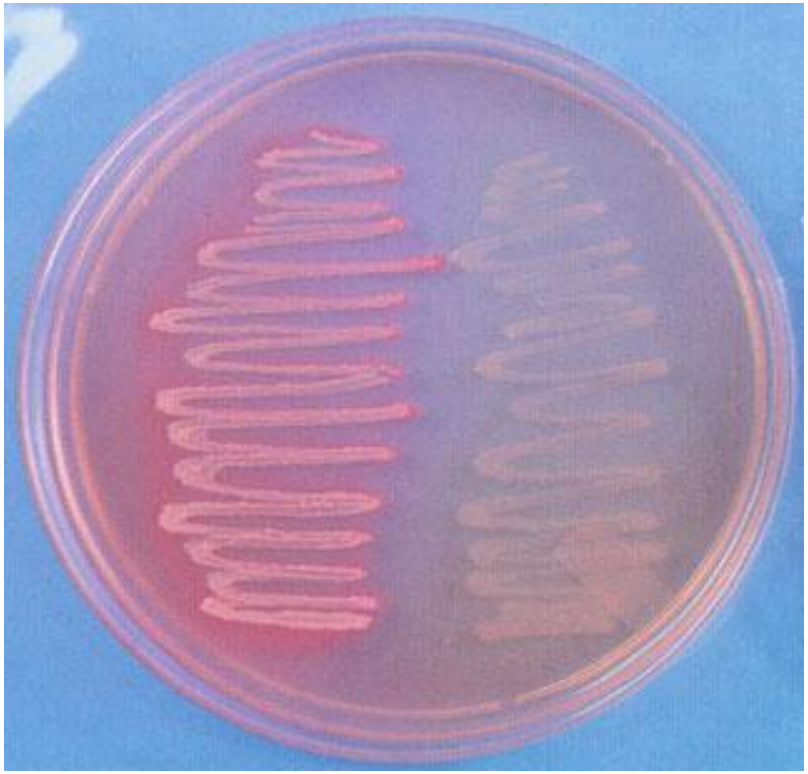
- These media contain an indicator which changes its colour when a bacterium grows in them.
- Eg:
  - Blood agar
  - Mac Conkey's medium
  - Christensen's urease medium


Streptococcus pyogenes
Streptococcus faecalis
Streptococcus agalactiae

Urease medium

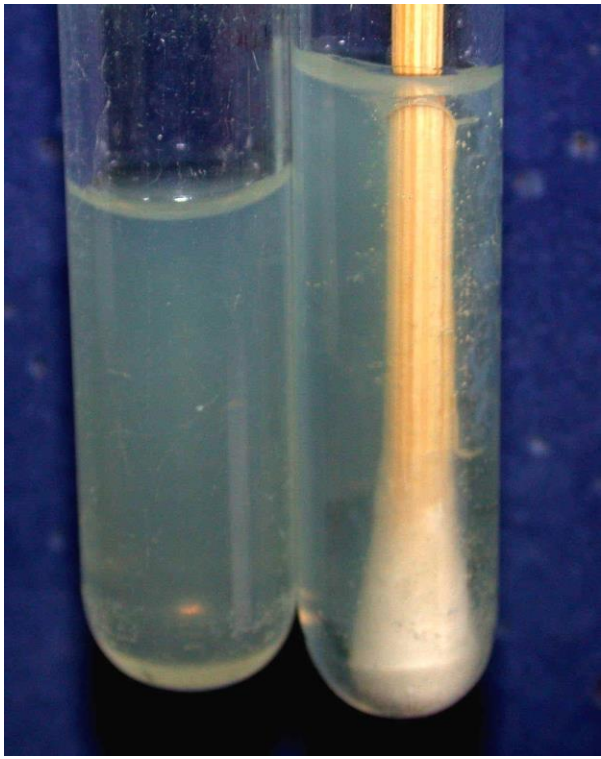
- Lactose fermenters – Pink colonies
- Non lactose fermenters – colourless colonies





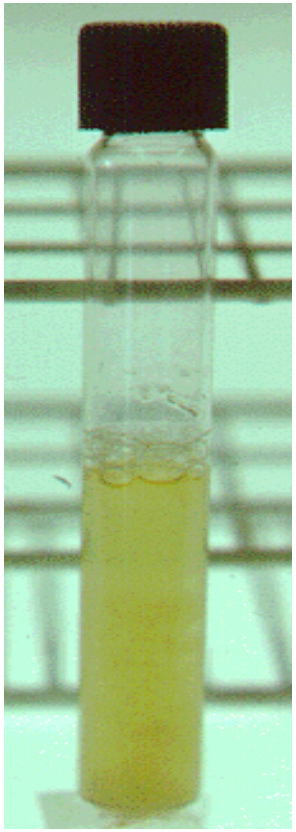
Transport media

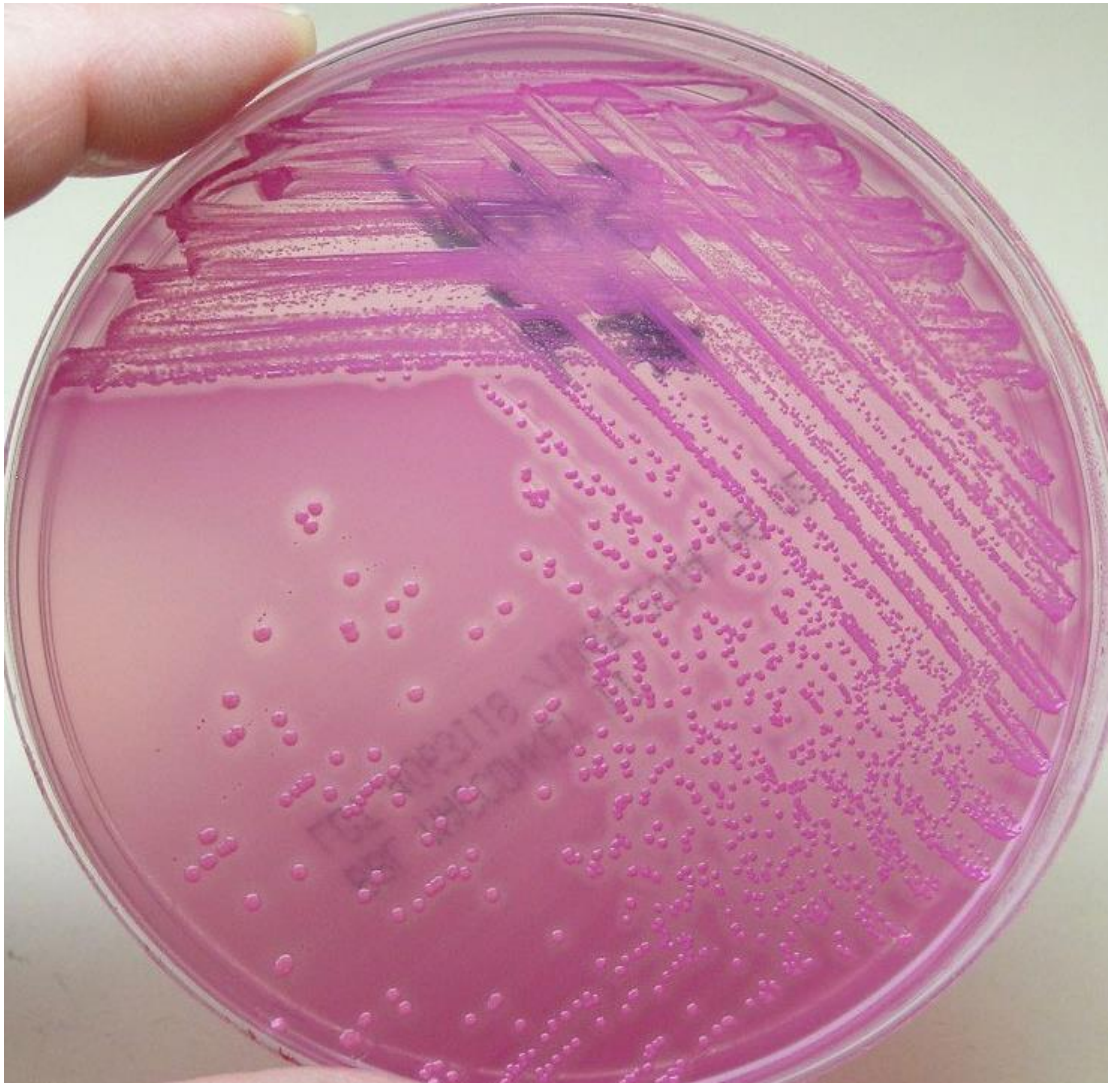
- Media used for transporting the samples.
- Delicate organisms may not survive the time taken for transporting the specimen without a transport media.
- Eg:
  - Stuart's medium – non nutrient soft agar gel containing a reducing agent
  - Buffered glycerol saline – enteric bacilli

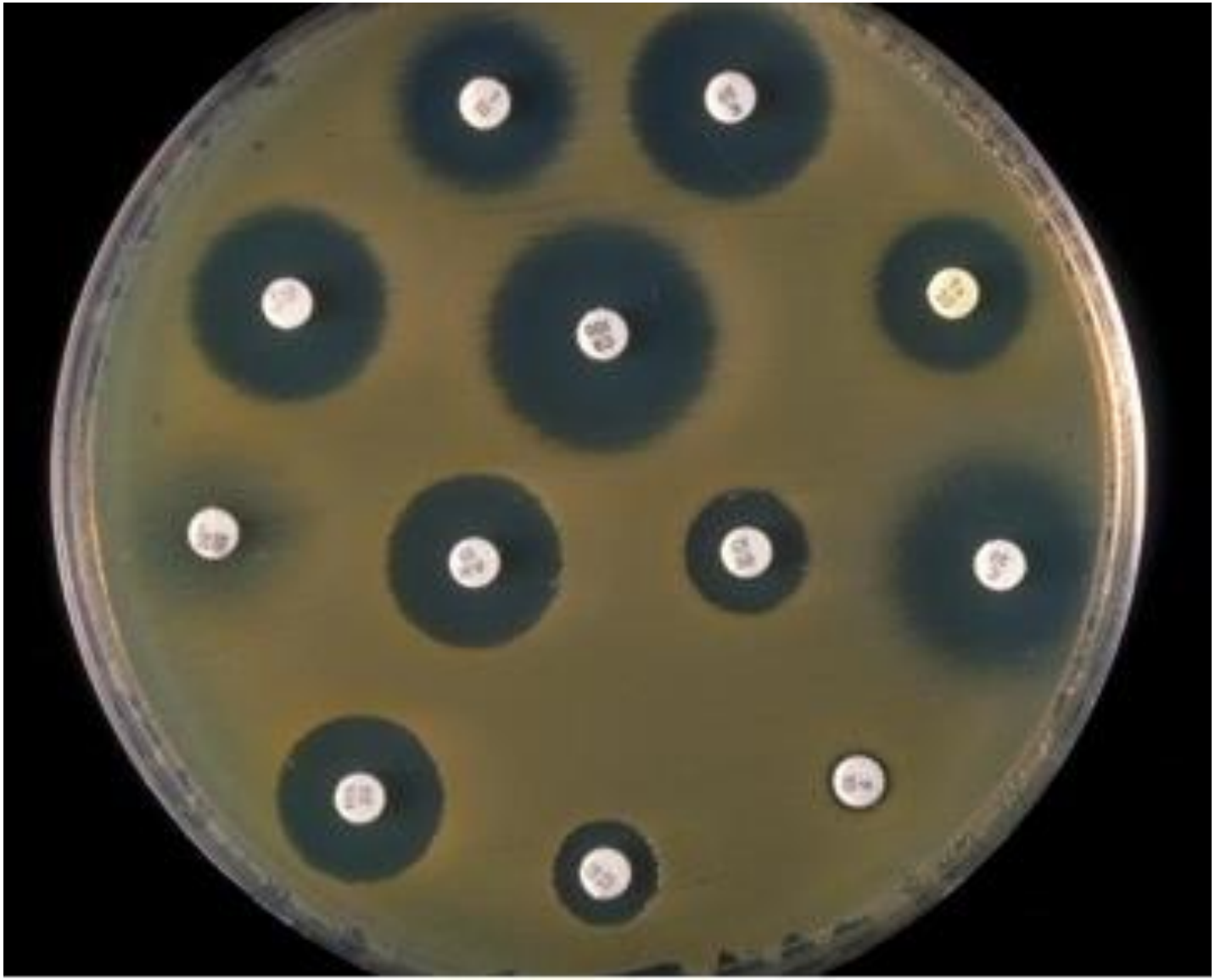


Anaerobic media

- These media are used to grow anaerobic organisms.
- Eg: Robertson's cooked meat medium, Thioglycolate medium.







Antibiotic sensitivity testing

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LAB7:
Growth of Microbes

- Control of growth is important for
  - infection control
  - growth of industrial and biotech organisms

Factors Regulating Growth

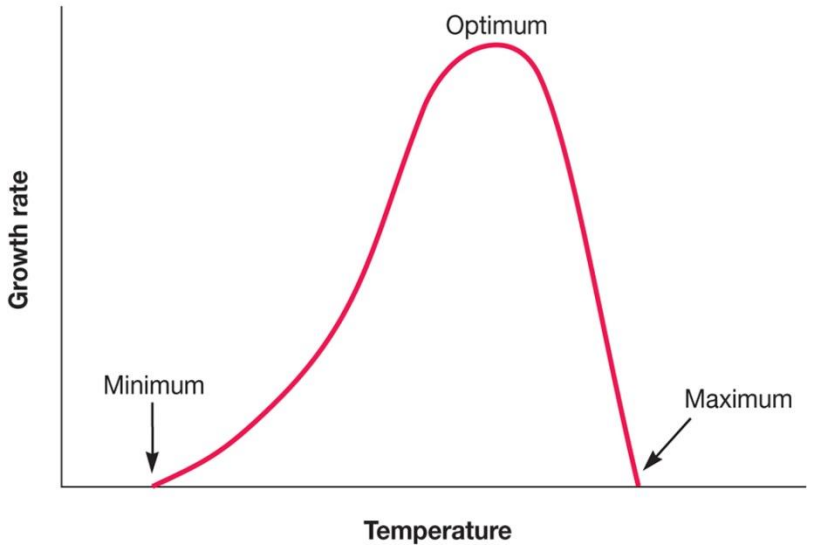
- Nutrients
- Environmental conditions: temperature, pH, osmotic pressure
- Generation time

Environmental Factors Influencing Growth

- Temperature
- O_2
- pH
- Osmotic Pressure
- Others: radiation, atmospheric pressure

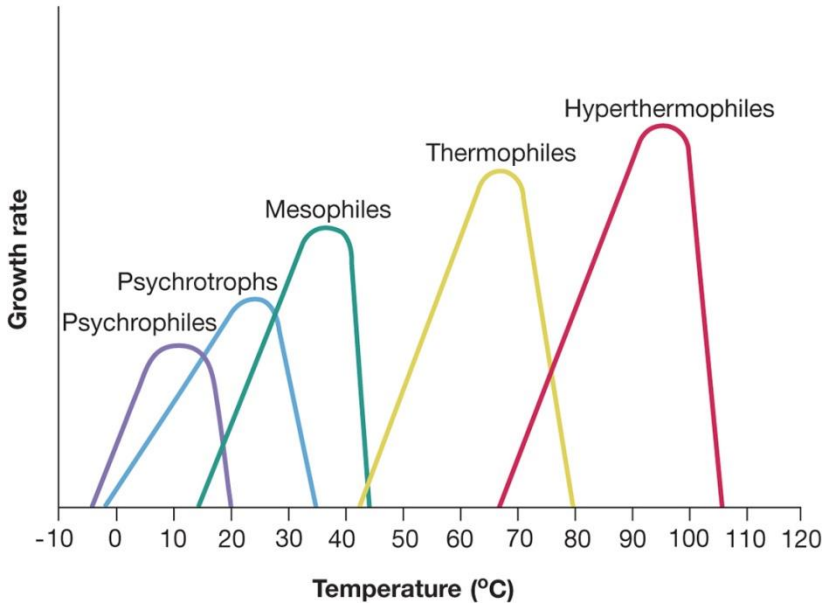
Temperature

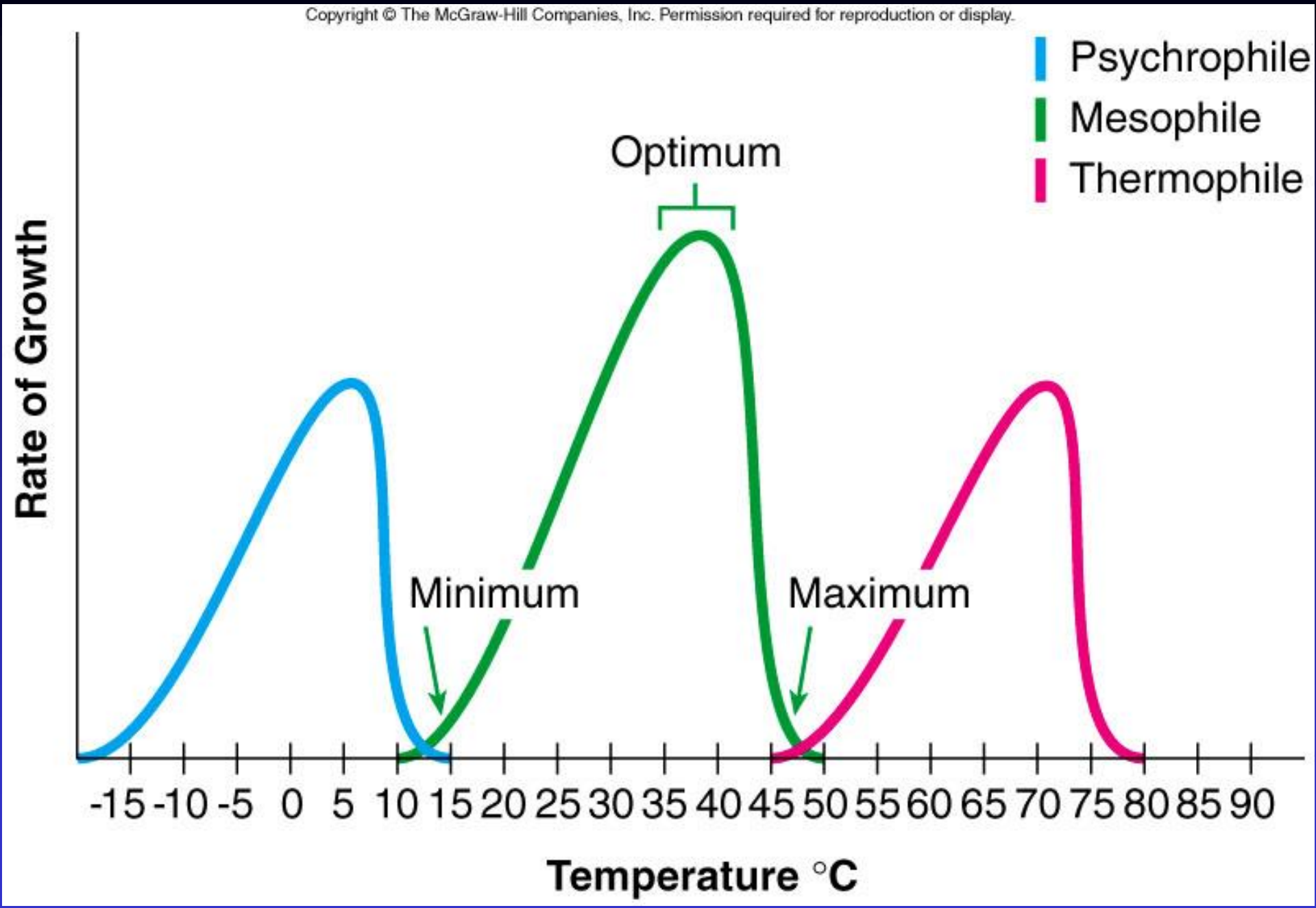
- Microbes cannot regulate their internal temperature
- Enzymes have optimal temperature at which they function optimally
- High temperatures may inhibit enzyme functioning and be lethal
  - minimal
  - maximal
  - optimal



Temperature Ranges for Microbial Growth

- psychrophiles – 0° C to 20° C
- psychrotrophs – 0° C to 35° C
- mesophiles – 20° C to 45° C
- thermophiles – 55° C to 85° C
- hyperthermophiles – 85° C to 113° C





pH

- Many bacteria and viruses survive low pH of stomach to infect intestines
- *Helicobacter pylori*

lives in stomach under mucus layer

pH

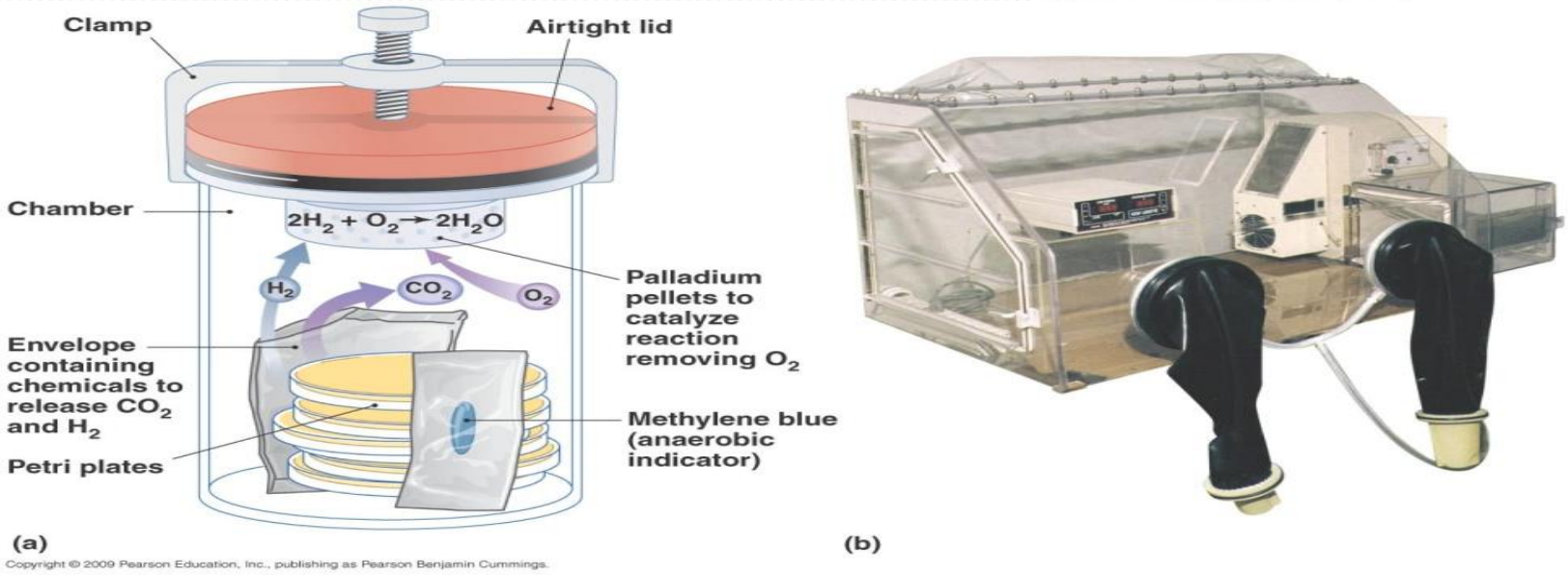
- Acidophiles
  - growth optimum between pH 0 and pH 5.5
- Neutrophiles
  - growth optimum between pH 5.5 and pH 7
- Alkaliphiles (alkalophiles)
  - growth optimum between pH 8.5 and pH 11.5
- Most microbes maintain an internal pH near neutrality
- Many microorganisms change the pH of their habitat by producing acidic or basic waste products

Oxygen and Bacterial Growth

- **Aerobe** -grows in presence of atmospheric oxygen (O_2) which is 20% O_2
- **Obligate aerobe** –requires O_2
- **Anaerobe** -grows in the absence of O_2
- **Obligate anaerobe** -usually killed in presence of O_2
- **Microaerophiles** -requires 2–10% O_2
- **Facultative anaerobes** -do not require O_2 but grow better in its presence –prefer O_2
- **Aerotolerant anaerobes** -grow with or without O_2

Culturing Microorganisms

- **Special Culture Techniques**
  - Techniques developed for culturing microorganisms
    - Animal and cell culture
    - Low-oxygen culture
    - Enrichment culture



Coy Laboratory Products

Figure 6-26b Brock Biology of Microorganisms 11/e

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LAB7:
Bacterial Division

- Bacteria divide by binary fission
- Alternative means
  - Budding
  - Conidiospores (filamentous bacteria)
  - Fragmentation

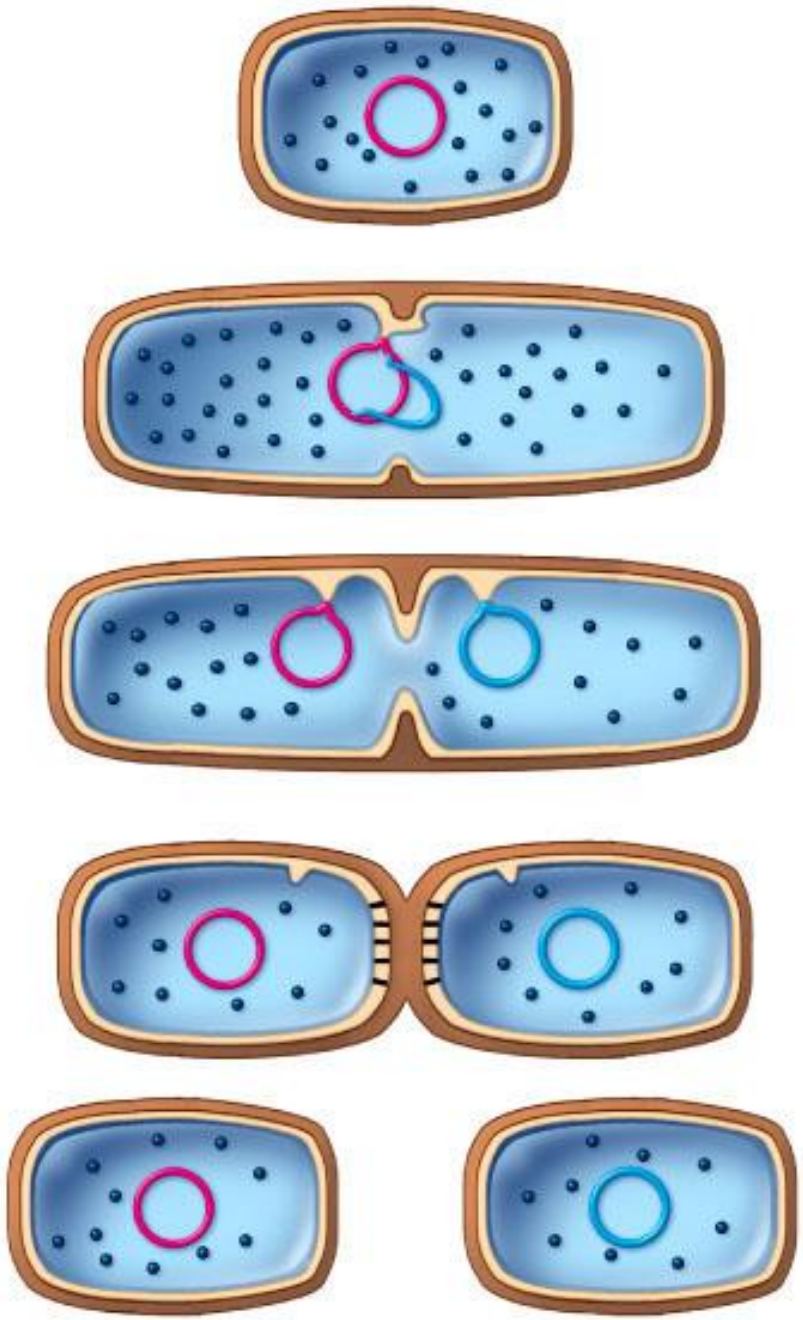
(a) A young cell at early phase of cycle.

(b) A parent cell prepares for division by enlarging its cell wall, cell membrane, and overall volume. Midway in the cell, the wall develops notches that will eventually form the transverse septum, and the duplicated chromosome becomes affixed to a special membrane site.

(c) The septum wall grows inward, and the chromosomes are pulled toward opposite cell ends as the membrane enlarges. Other cytoplasmic components are distributed (randomly) to the two developing cells.

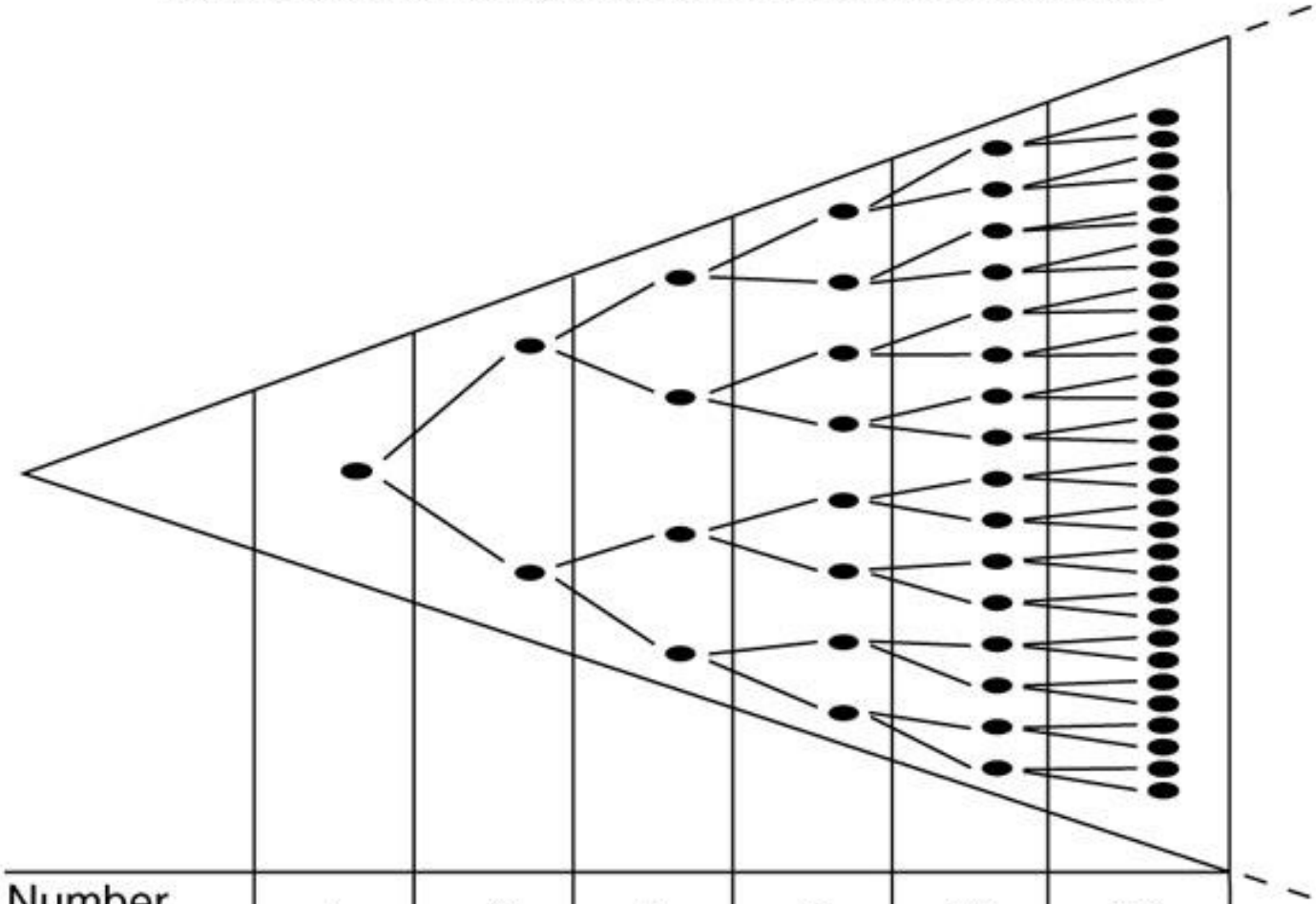
(d) The septum is synthesized completely through the cell center, and the cell membrane patches itself so that there are two separate cell chambers.

(e) At this point, the daughter cells are divided. Some species will separate completely as shown here, while others will remain attached, forming chains or doublets, for example.



LAB8:
Generation Time

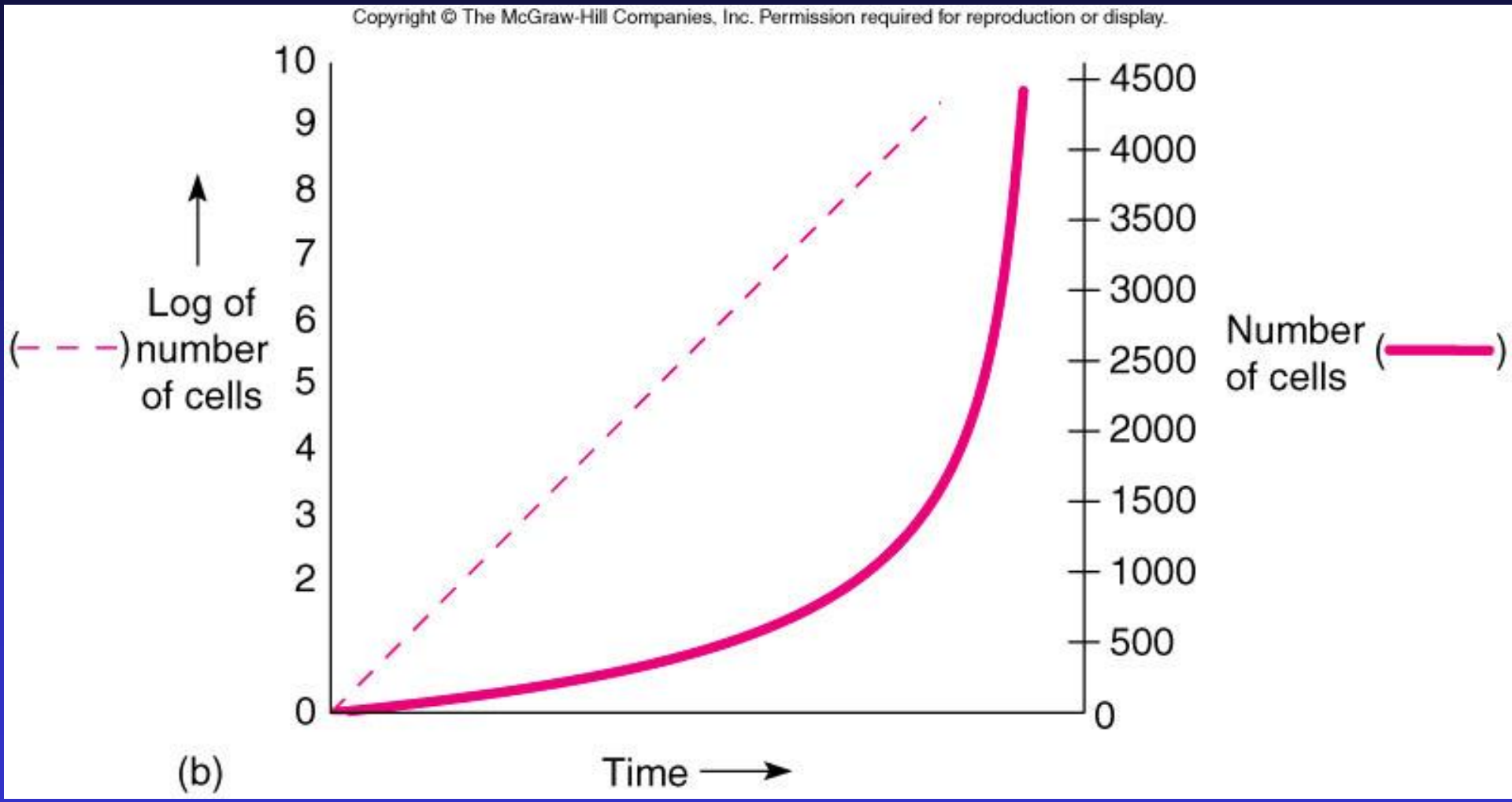
- Time required for cell to divide/for population to double
- Average for bacteria is 1-3 hours
- *E. coli* generation time = 20 min
  - 20 generations (7 hours), 1 cell becomes 1 million cells!



Number of cells	1	2	4	8	16	32
Number of generations		1	2	3	4	5
Exponential value		2^1 (2×1)	2^2 (2×2)	2^3 (2×2×2)	2^4 (2×2×2×2)	2^5 (2×2×2×2×2)

(a)

growth on graphs

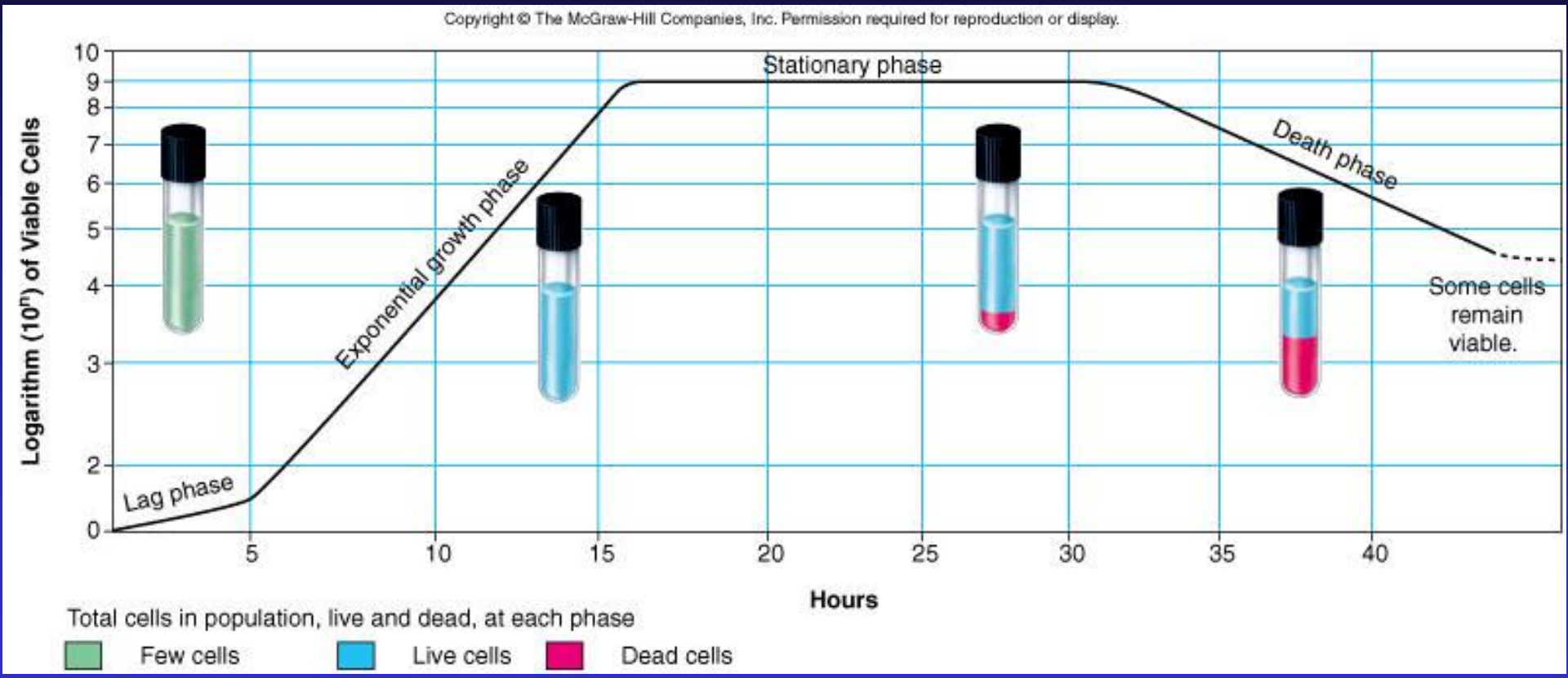


Stages in the Normal Growth Curve

Data from an entire growth period typically produce a curve with a series of phases

- Lag Phase
- Exponential Growth Phase
- Stationary Growth Phase
- Rapidly Declining Phase
- Death Phase

Standard Growth Curve



Phases of Growth

- Lag phase – making new enzymes in response to new medium
- Log phase – exponential growth
  - Desired for production of products
  - Most sensitive to drugs and radiation during this period

Phases of Growth

- Stationary phase –
  - –nutrients becoming limiting or waste products becoming toxic
  - – death rate = division rate
- Death phase – death exceeds division

Possible Reasons for Stationary Phase

- Nutrient limitation
- Limited oxygen availability
- Toxic waste accumulation
- Critical population density reached
- Bacteria die off and liberate some nutrients

LAB11:
Measuring Growth

- Direct methods – count individual cells
- Indirect Methods – measure effects of bacterial growth

Direct Counting

• Growth is measured by the change in the number of cells over time. Cell counts done microscopically measure the total number of cells in a population, whereas **viable** cell counts (plate counts) measure only the living, reproducing population.

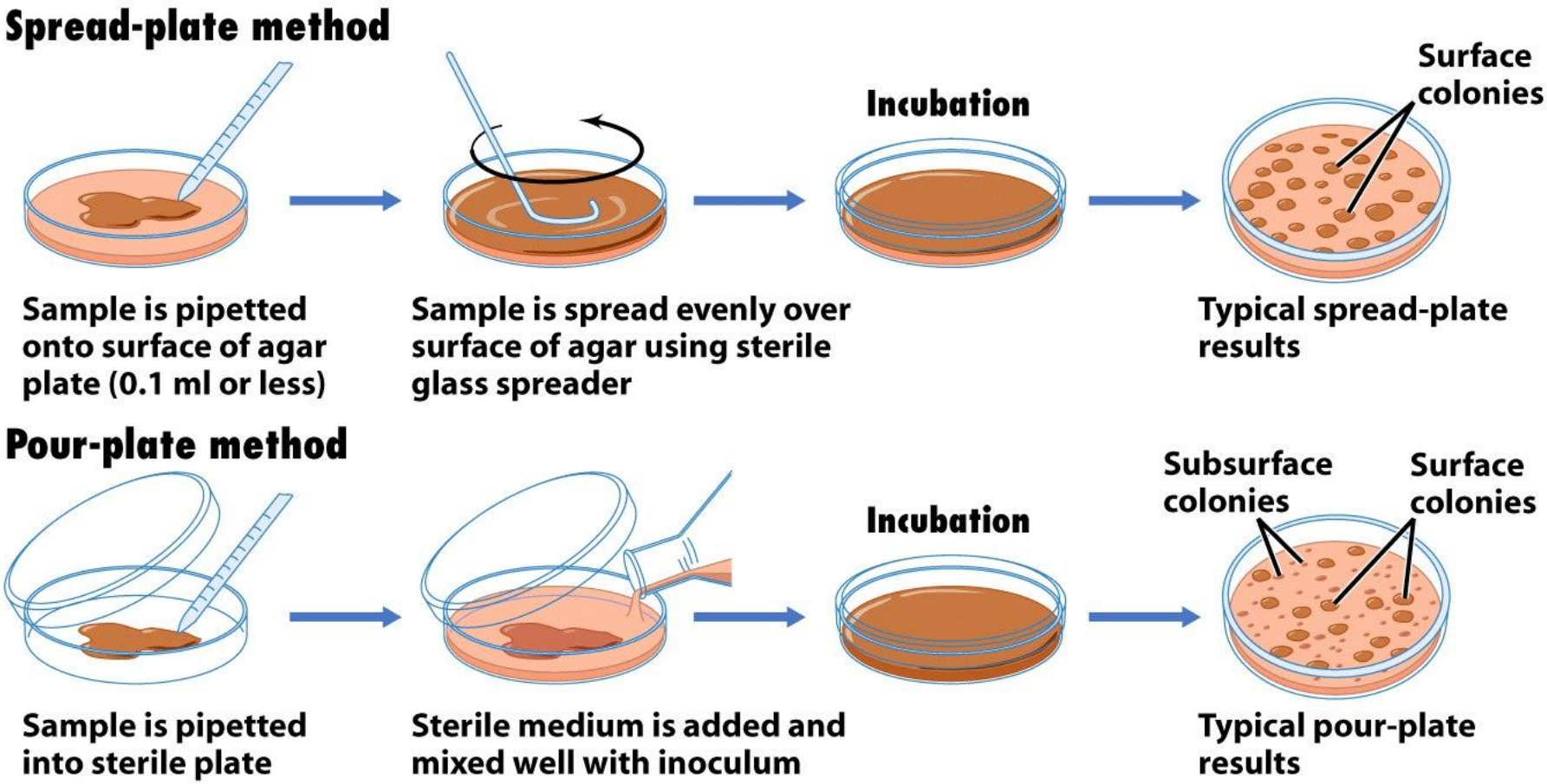


Figure 6-10 Brock Biology of Microorganisms 11/e

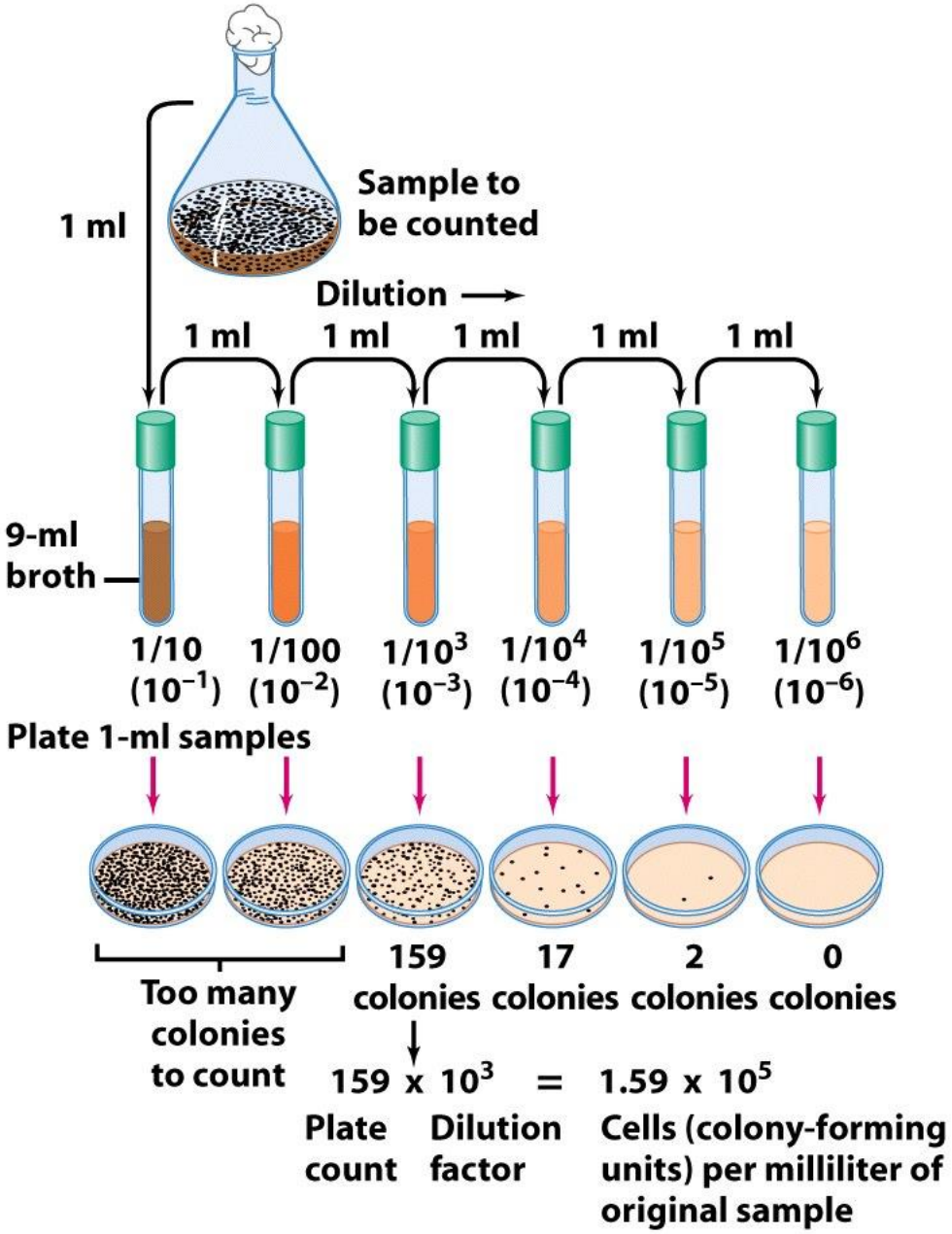


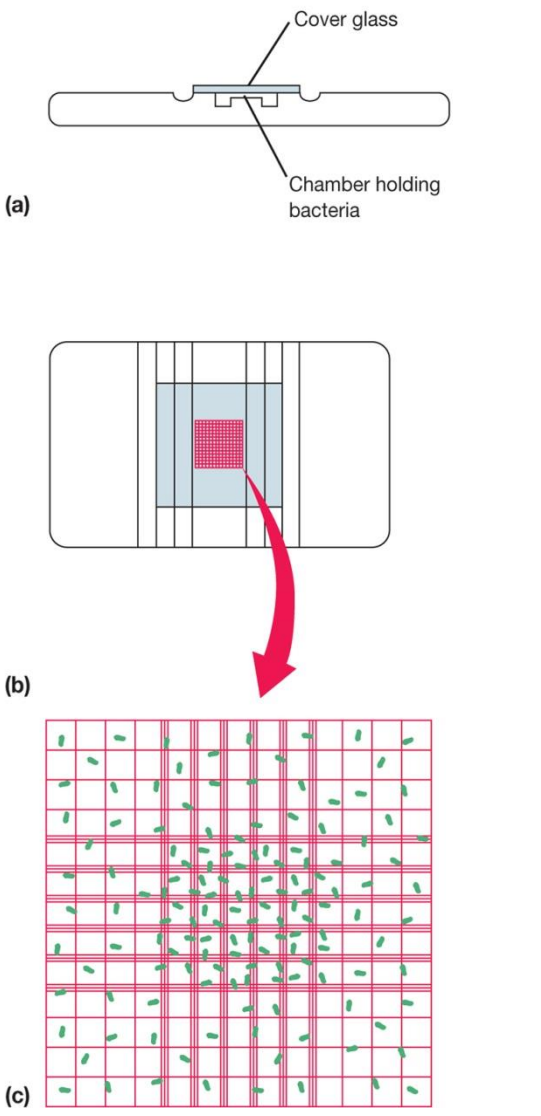
Figure 6-11 Brock Biology of Microorganisms 11/e

Viable Counting Methods

- Spread and pour plate techniques
  - diluted sample of bacteria is spread over solid agar surface or mixed with agar and poured into Petri plate
  - after incubation the numbers of organisms are determined by counting the number of colonies multiplied by the dilution factor
  - results expressed as colony forming units (CFU)

Counting Chambers

- Easy, inexpensive, and quick
- Useful for counting both eukaryotes and prokaryotes
- Cannot distinguish living from dead cells



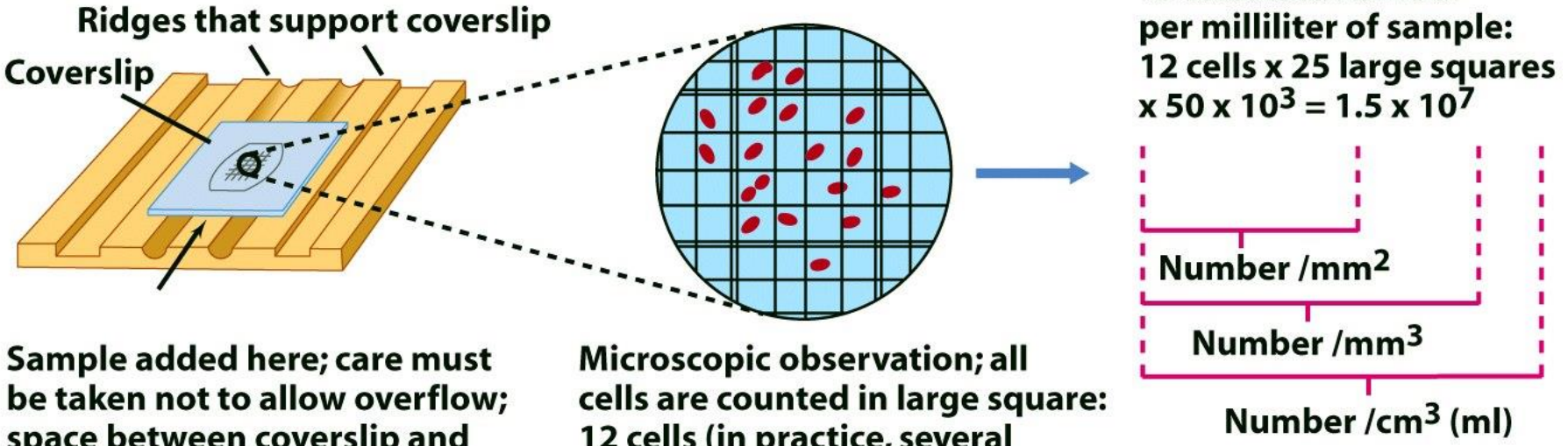


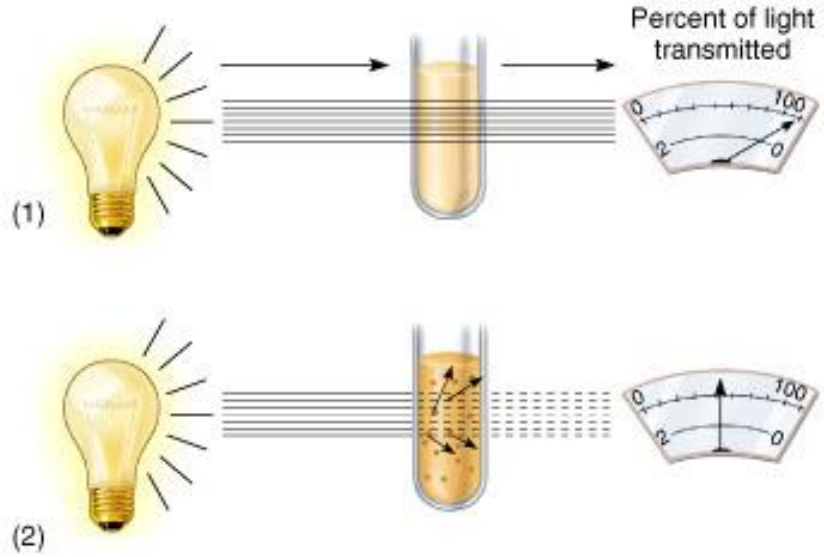
Figure 6-9 Brock Biology of Microorganisms 11/e

Indirect Counting

• Turbidity measurements are an indirect but very rapid and useful method of measuring microbial growth. However, to relate a direct cell count to a turbidity value, a standard curve must first be established.

Turbidity

(a)



(b)

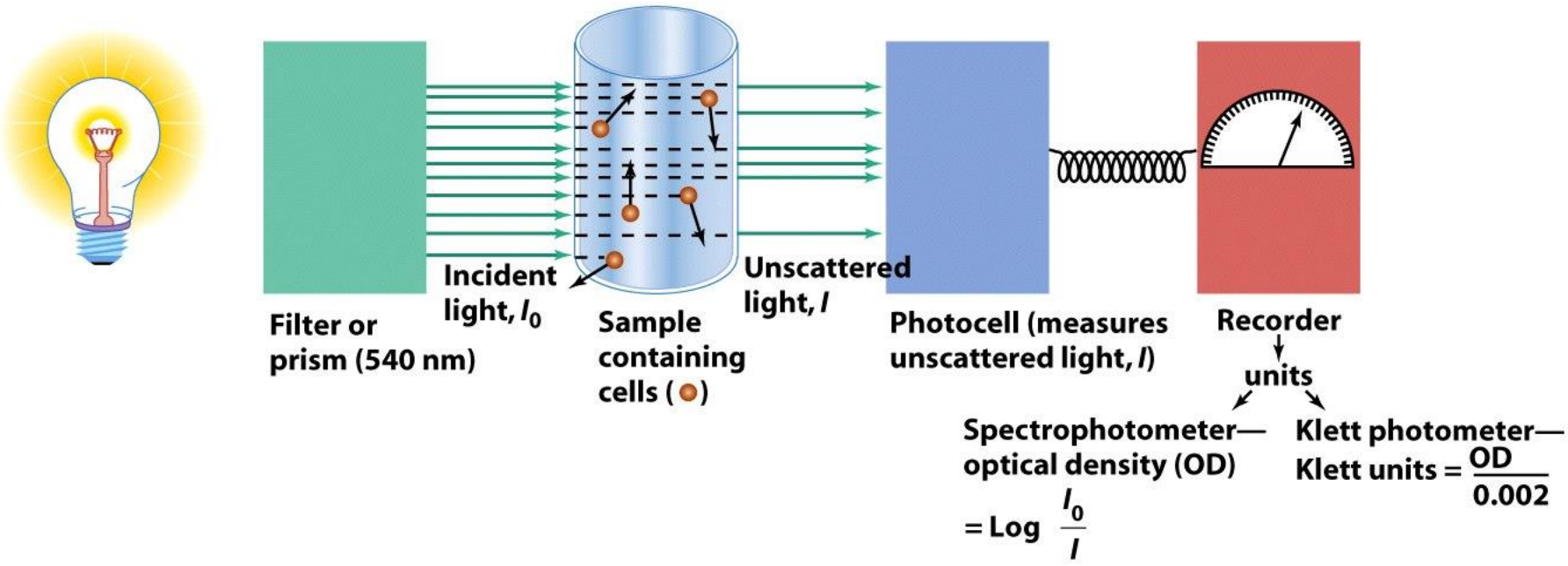


Figure 6-12a Brock Biology of Microorganisms 11/e

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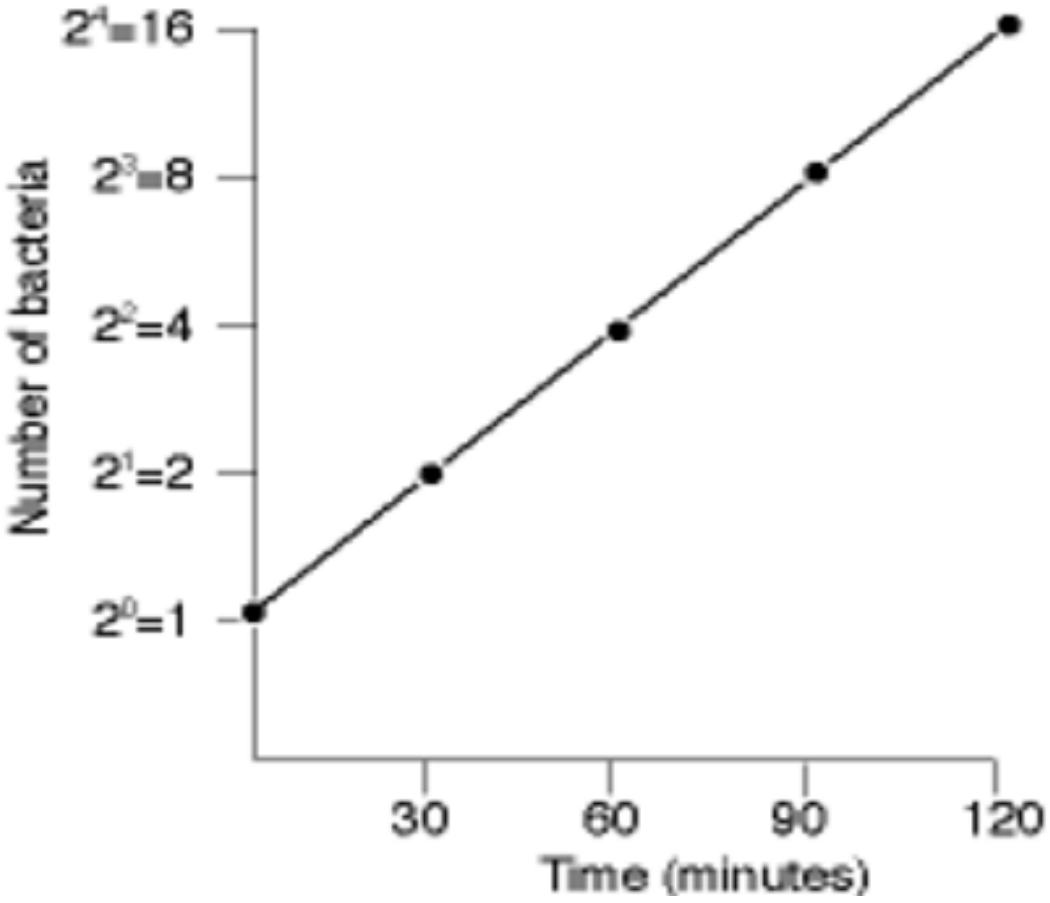
Micro-phasology
Year : 2

LAB 12
Bacterial growth

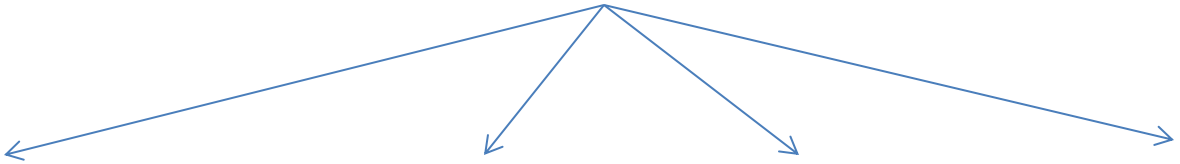
- The mathematics of bacterial growth is fairly simple, since each original cell divides to form two new cells, with the loss of the original parent.
- the mathematical series describing growth is: 1, 2, 4, 8, 16, … .
- This can be written as 2^0, 2^1, 2^2, 2^3, 2^4, 2^5, ..

The generation time (time needed for the cell to divide into two) differs according to species and prevailing conditions. For example, a bacterium that divides every 30 min has a generation time of 30 min.



The bacterial growth curve

- Bacterial growth over time can be graphed as cell number versus time.
- This is called a growth curve.
- This curve typically has 4 distinct phases:



Lag phase

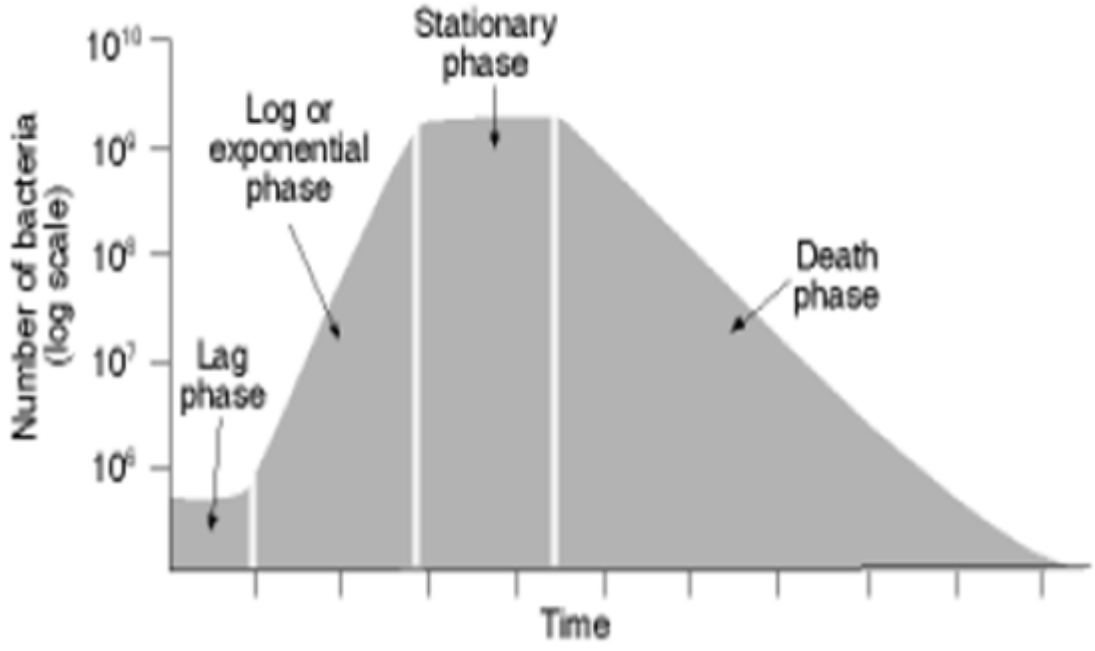
Exponential (log) phase

Stationary phase

Death phase

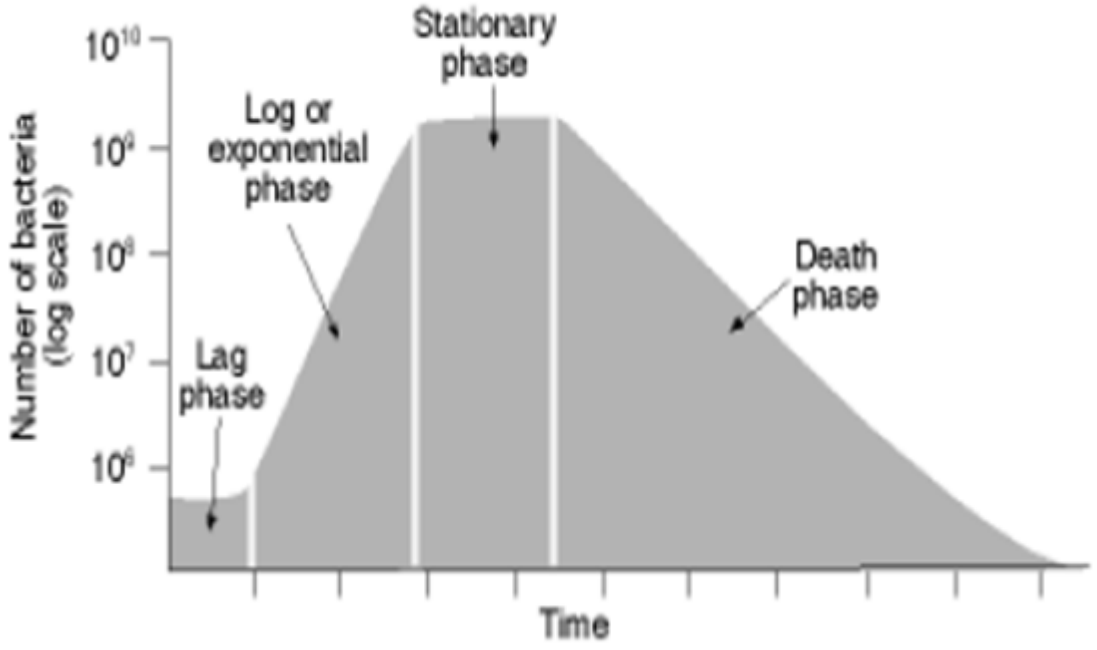
Lag phase:

* **Is** the first phase.
* No increase in cell number
* Cells are actively metabolizing, in preparation for cell division.
* It may be short or very long, according to the growth medium.



Exponential or log phase

* Is the second phase.
* called the exponential or log phase.
* This is the period in which the cells grow most rapidly, doubling at a fairly constant rate.
* The time it takes the culture to double is called the generation time.
* The generation time depends on several factors:.
    1. the organism . 2. the growth medium 3. temperature



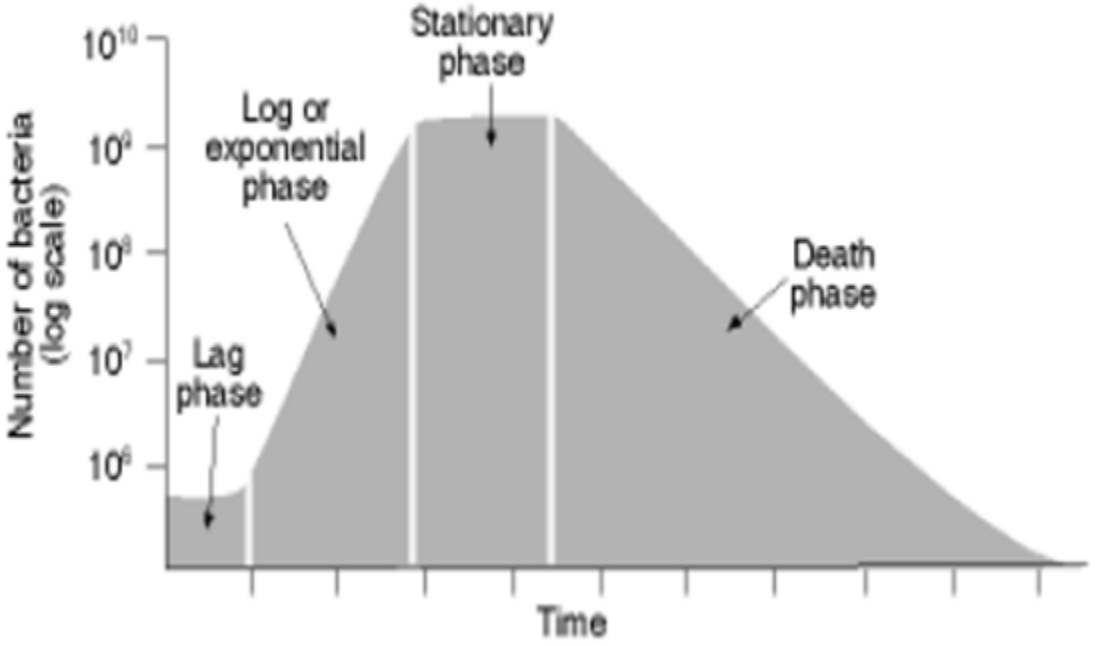
Stationary phase:

* **Is** third phase . * metabolism slows. * cells cease rapid cell division.

Why?

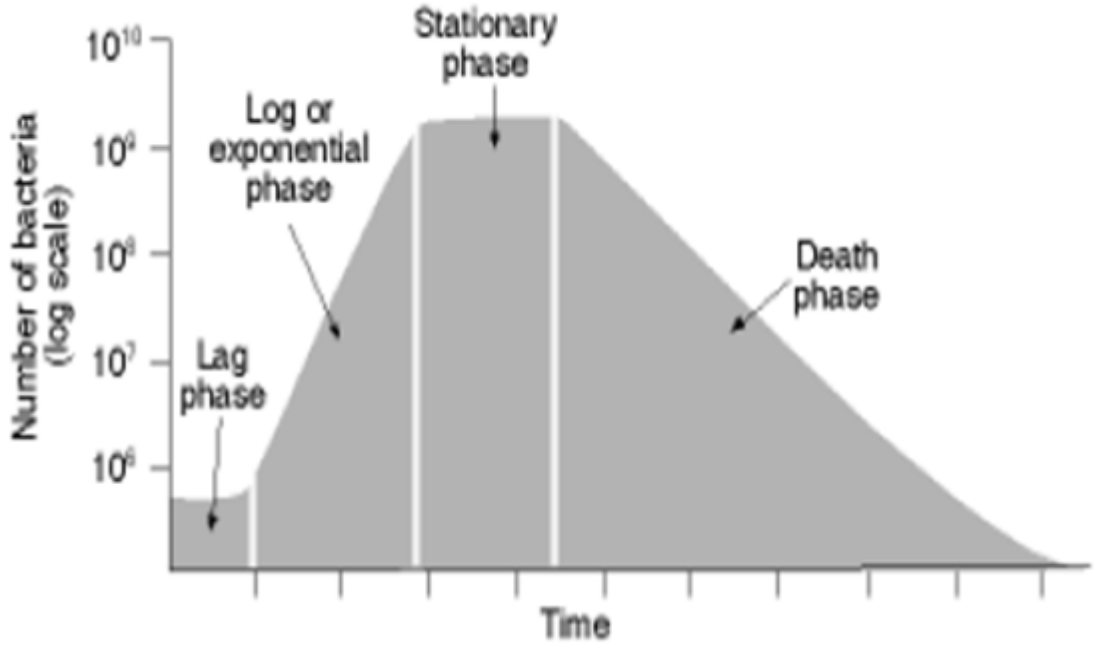
Due to :

* high cell density.
* depletion of nutrients .
* accumulation of waste products.



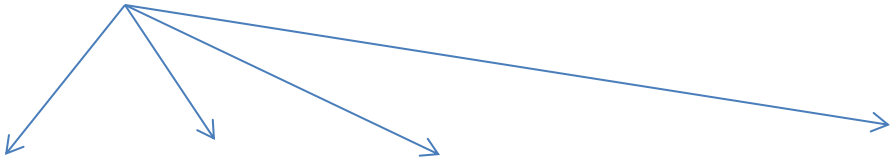
Death phase:

* **Is** the final phase .
* cells quickly lose the ability to divide.
* exponential death.



LAB 10
Factors affecting bacterial growth

Many factors affect the generation time of the bacterium:



Temperature pH oxygen salt concentration and nutrients

While most bacteria grow best when these parameters are optimum

Temprature

According the temperature degree that bacteria survive, they can classified to :

psychrophiles

The bacteria that grow best from about 0 - 20 ºC

mesophiles

grow best at ambient temperatures 20 -40 ºC

thermophiles

The bacteria that have an optimum temperature above 40 ºC

extramophiles.

that grow well at 100- 120 ºC