Prof. Amira Ali El-Fallal

Ph. D. , Wales Univ., U. K.

Principles & Practices of Sterilisation

Aim

To describe how material and specific objects are practically sterilised, outlining the reasons why, how sterilisation is achieved, and the underlying principles by which this is achieved

Objectives, by the end of these lectures you should be able to:

- **Define Sterilisation and Sterility**
- **State the reasons why the above is necessary**
- **Give examples of materials and objects requiring sterilisation**
- **Describe the methods used to achieve sterility and the principles upon which they are based**
- **State the factors that have to be considered when setting up a sterilisation protocol**

1- How do bacteria develop resistance to antibiotics

2- Give examples to selective and differential media

3- If you have a sample of water how can you detect the contamination with alive bacteria

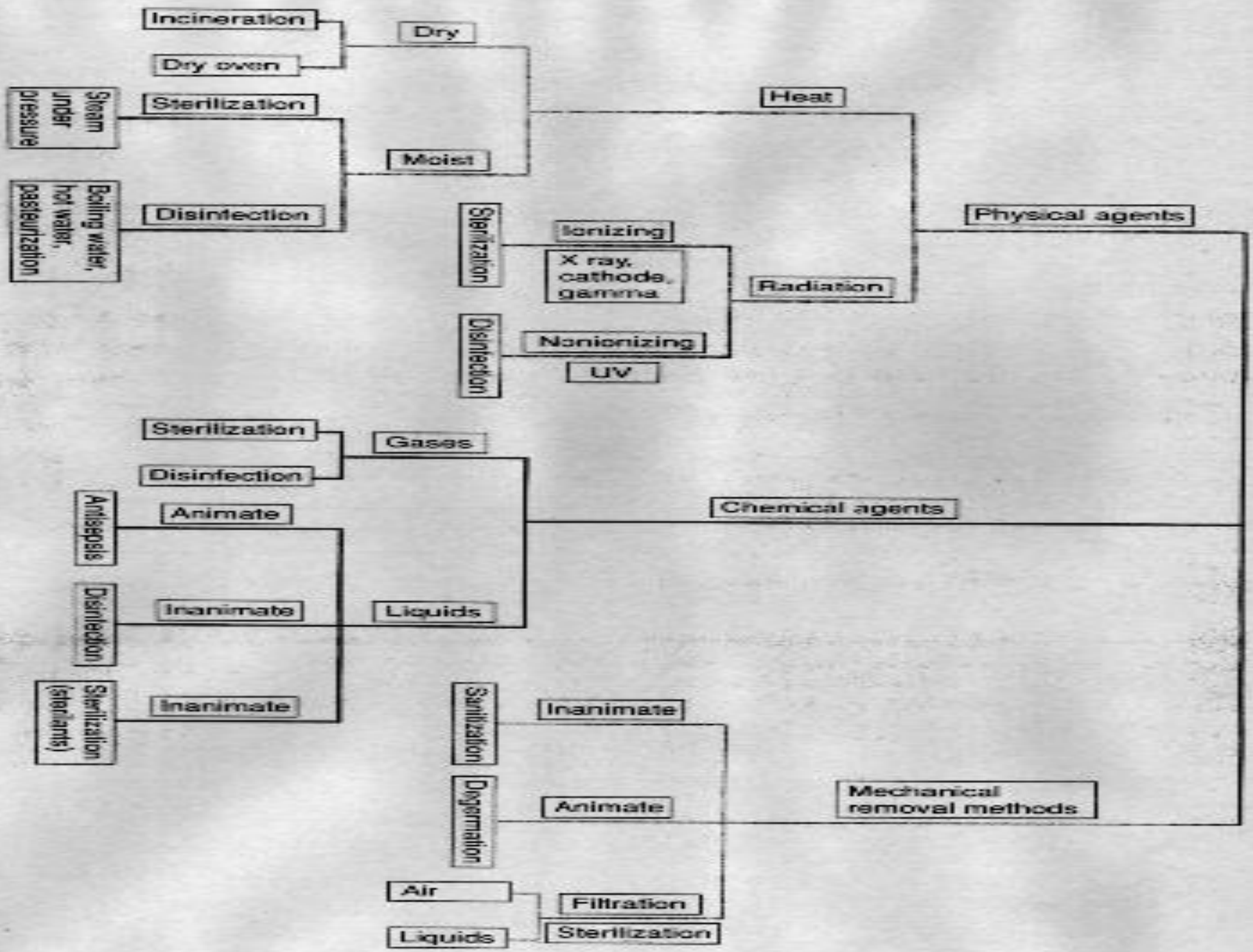
Sterility

A material is sterile if it contains no living or dormant organisms

Sterilisation

The process of killing or removing organisms

What has to be sterilised & why ?


Incineration
Dry
Dry oven
Steam under pressure
Sterilization
Moist
Boiling water, hot water, pasteurization
Disinfection
Heat
Physical agents
Sterilization
Ionizing
X ray, cathode, gamma
Radiation
Disinfection
Nonionizing
UV
Sterilization
Gases
Disinfection
Chemical agents
Antisepsis
Animate
Disinfection
Inanimate
Liquids
Sterilization (sterilants)
Inanimate
Sterilization
Inanimate
Disgermation
Animate
Mechanical removal methods
Air
Filtration
Liquids
Sterilization

Methods

Physical

Heat

Radiation

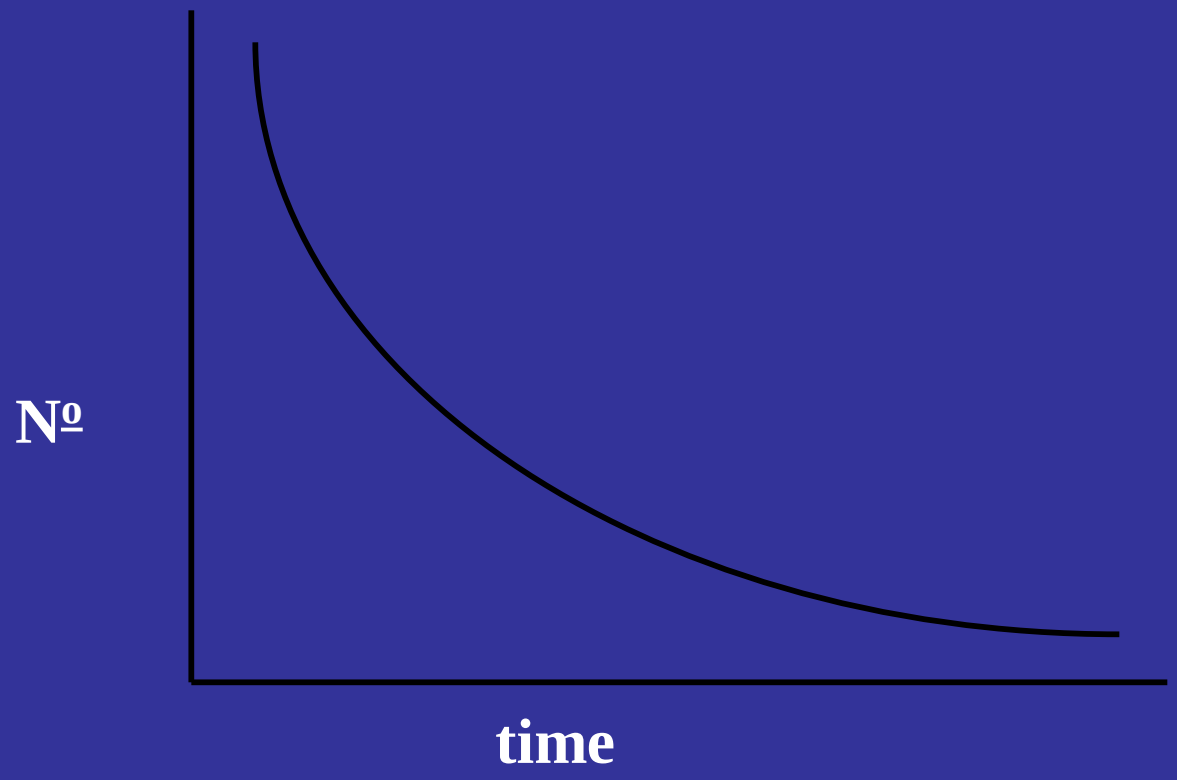
Filtration

Chemical

Antimicrobial / Chemical Agents

Combination

Survivor Curve



Decimal Reduction Time (D value)

The time taken under defined conditions to reduce a population by 90%

or ---------- for the survivor curve to traverse 1 log cycle

Written as:

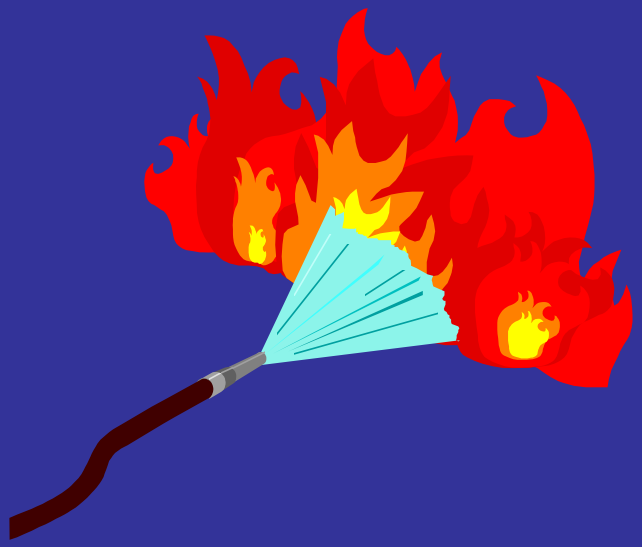
D_{temp}: x min

D_{121} : 2 min

D value as a function of temperature for Clostridium sporogenes PA 3679

Temp (°C)	D (min)
110	19.65
113	10.46
115	4.92
118	2.56
121	1.23

Heat



DRY

Red heat-Flaming-Hot air oven-Infrared radiation

MOIST

Under 100C-at 100C

PRESSURE

autoclave

Dry Heat: Kills by **oxidation** effects.

Direct Flaming: Used to sterilize inoculating loops and needles. Heat metal until it has a red glow.

Incineration: Effective way to sterilize disposable items (paper cups, dressings) and biological waste.

Hot Air Sterilization: Place objects in an oven. Require 2 hours at 170 ^{o}C for sterilization. Dry heat is transfers heat less effectively to a cool body, than moist heat.

Dry Heat

- Flaming
- Oven (170° C 2 hours)

Moist Heat: Kills microorganisms by **coagulating** their proteins.

In general, moist heat is much more effective than dry heat.

Boiling: Heat to 100^{o}C or more at sea level. Kills vegetative forms of bacterial pathogens, almost all viruses, and fungi and their spores within 10 minutes or less. Endospores and some viruses are not destroyed this quickly. However brief boiling will kill most pathogens.

Hepatitis virus: Can survive up to 30 minutes of boiling.

Endospores: Can survive up to 20 hours or more of boiling.

Reliable sterilization with moist heat requires temperatures above that of boiling water.

Autoclave: Chamber which is filled with hot steam under pressure. Preferred method of sterilization, unless material is damaged by heat, moisture, or high pressure.

Temperature of steam reaches **121ºC** at twice atmospheric pressure.

Most effective when organisms contact steam directly or are contained in a small volume of liquid.

All organisms and endospores are killed within 15 minutes.

Require more time to reach center of solid or large volumes of liquid.

Moist Heat (Continued):

Pasteurization: Developed by Louis Pasteur to prevent the spoilage of beverages. Used to reduce microbes responsible for spoilage of beer, milk, wine, juices, etc.

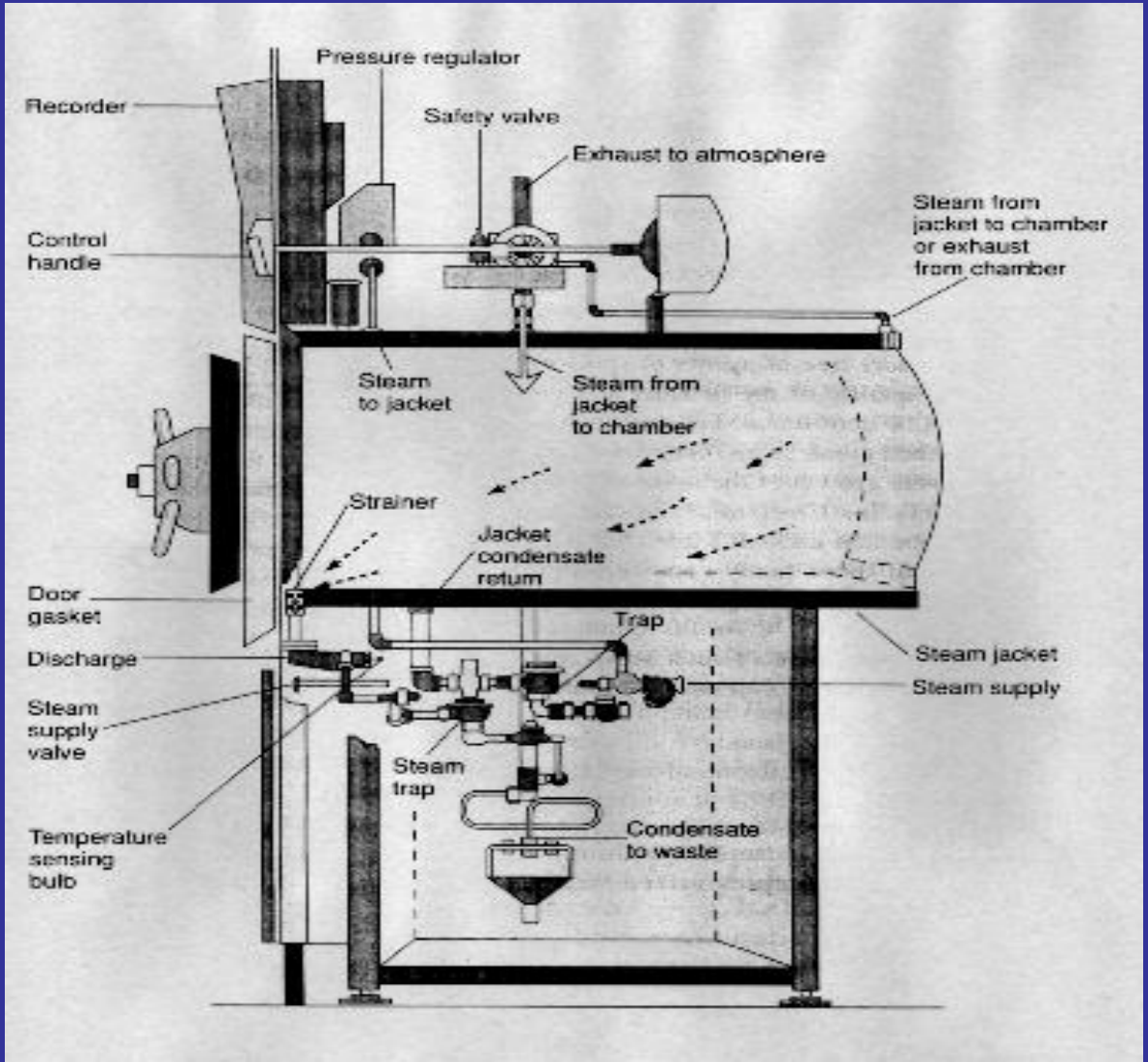
Classic Method of Pasteurization: Milk was exposed to 65^{o}C for 30 minutes.

High Temperature Short Time Pasteurization (HTST): Used today. Milk is exposed to 72^{o}C for 15 seconds.

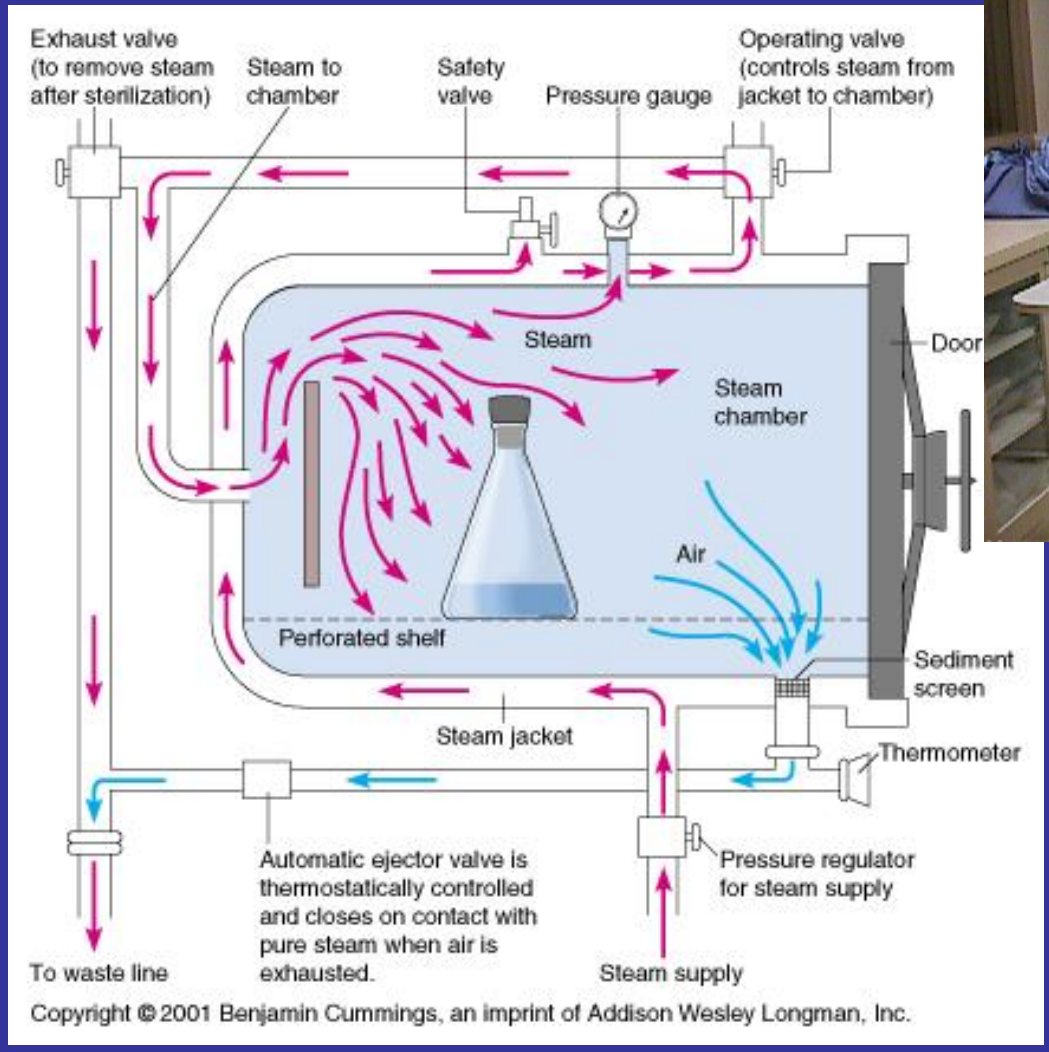
Ultra High Temperature Pasteurization (UHT): Milk is treated at 140^{o}C for 3 seconds and then cooled very quickly in a vacuum chamber.

Advantage: Milk can be stored at room temperature for several months.

The Autoclave



Autoclave (Fig 7.2)



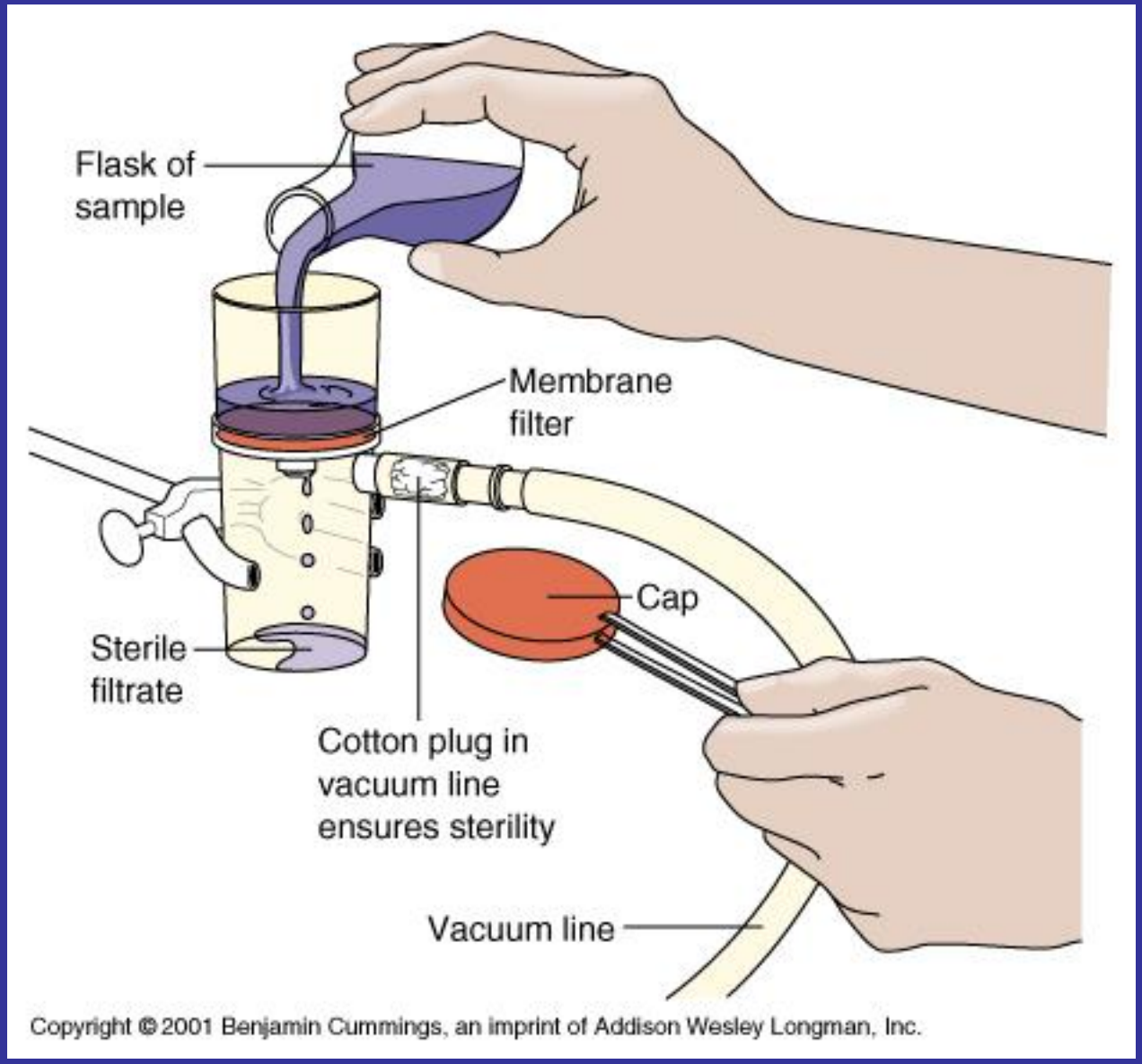
Filtration: Removal of microbes by passage of a liquid or gas through a screen like material with small pores. Used to sterilize heat sensitive materials like vaccines, enzymes, antibiotics, and some culture media.

High Efficiency Particulate Air Filters (HEPA): Used in operating rooms and burn units to remove bacteria from air.

Membrane Filters: Uniform pore size. Used in industry and research. Different sizes:

0.22 and 0.45um Pores: Used to filter most bacteria. Don't retain spirochetes, mycoplasmas and viruses.

0.01 um Pores: Retain all viruses and some large proteins.



Copyright © 2001 Benjamin Cummings, an imprint of Addison Wesley Longman, Inc.

FILTRATION (Pore diameter 0.75µ)

1-Berkefeld filters made up of fossil diatomaceous earth.

2- Chamberland filters made up of porcelain.

3- Seitz filters made up of asbestos disk.

4- Sintered glass filters made up of finely ground glass

5- Cellulose membrane filters made up of cellulose nitrate

Low Temperature: Effect depends on microbe and treatment applied.

Refrigeration: Temperatures from 0 to 7°C.

Bacteriostatic effect. Reduces metabolic rate of most microbes so they cannot reproduce or produce toxins.

Freezing: Temperatures below 0°C.

Flash Freezing: Does not kill most microbes.

Slow Freezing: More harmful because ice crystals disrupt cell structure.

Over a third of vegetative bacteria may survive 1 year.

Most parasites are killed by a few days of freezing.

Low Temperature

- Refrigeration is <u>bacteriostatic</u>
  - Most pathogens do not grow –
- Freezing: slow freezing creates ice crystals

Dessication: In the absence of water, microbes cannot grow or reproduce, but some may remain viable for years. After water becomes available, they start growing again.

Susceptibility to dessication varies widely:

Neisseria gonnorrhea: Only survives about one hour.

Mycobacterium tuberculosis: May survive several months.

Viruses are fairly resistant to dessication.

Clostridium spp. and ***Bacillus spp***.: May survive decades.

Osmotic Pressure: The use of high concentrations of salts and sugars in foods is used to increase the osmotic pressure and create a **hypertonic** environment.

Plasmolysis: As water leaves the cell, plasma membrane shrinks away from cell wall. Cell may not die, but usually stops growing.

Yeasts and molds: More resistant to high osmotic pressures.

Staphylococci spp. that live on skin are fairly resistant to high osmotic pressure.

Radiation: Three types of radiation kill microbes:

1. Ionizing Radiation: Gamma rays, X rays, electron beams, or higher energy rays. Have short wavelengths (less than 1 nanometer).

Dislodge electrons from atoms and form ions.

Cause mutations in DNA and produce peroxides.

Used to sterilize pharmaceuticals and disposable medical supplies. Food industry is interested in using ionizing radiation.

Disadvantages: Penetrates human tissues. May cause genetic mutations in humans.

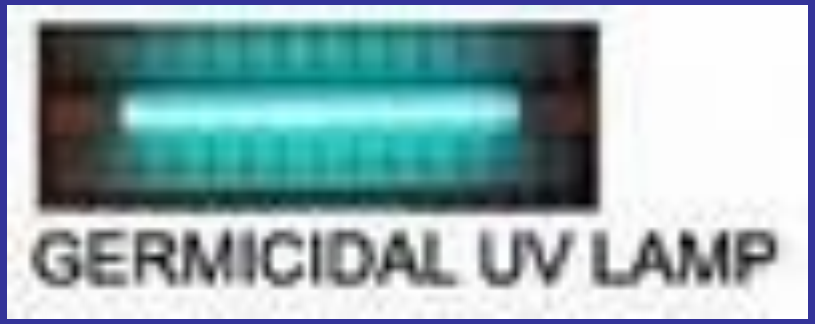
2. Ultraviolet light (Nonionizing Radiation): Wavelength is longer than 1 nanometer. Damages DNA by producing thymine dimers, which cause mutations.

Used to disinfect operating rooms, nurseries, cafeterias.

Disadvantages: Damages skin, eyes. Doesn't penetrate paper, glass, and cloth.

UV Radiation

- Thymine dimers in DNA
- Germicidal lamps, vaccine disinfection
- Not penetrating
- Can damage eyes



3. Microwave Radiation: Wavelength ranges from 1 millimeter to 1 meter.

Heat is absorbed by water molecules.

May kill vegetative cells in moist foods.

Bacterial endospores, which do not contain water, are not damaged by microwave radiation.

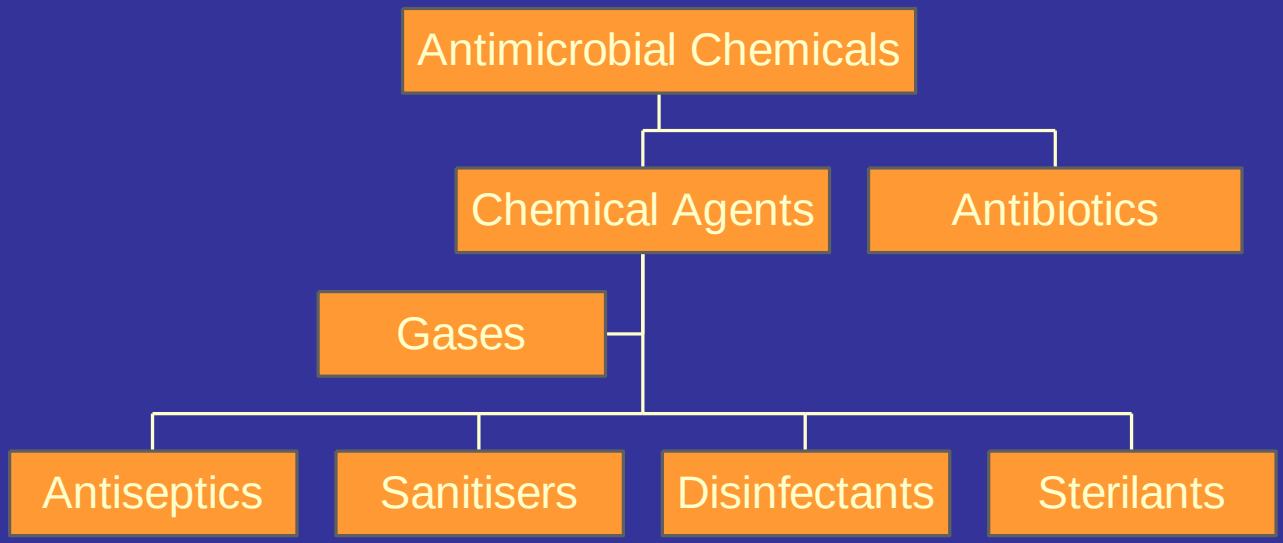
Solid foods are unevenly penetrated by microwaves.

Trichinosis outbreaks have been associated with pork cooked in microwaves.

Microwaves

- Very little effect on microbes
- Microwave ovens kill vegetative pathogens by heating
- Solid foods heat unevenly

Antimicrobial Chemicals

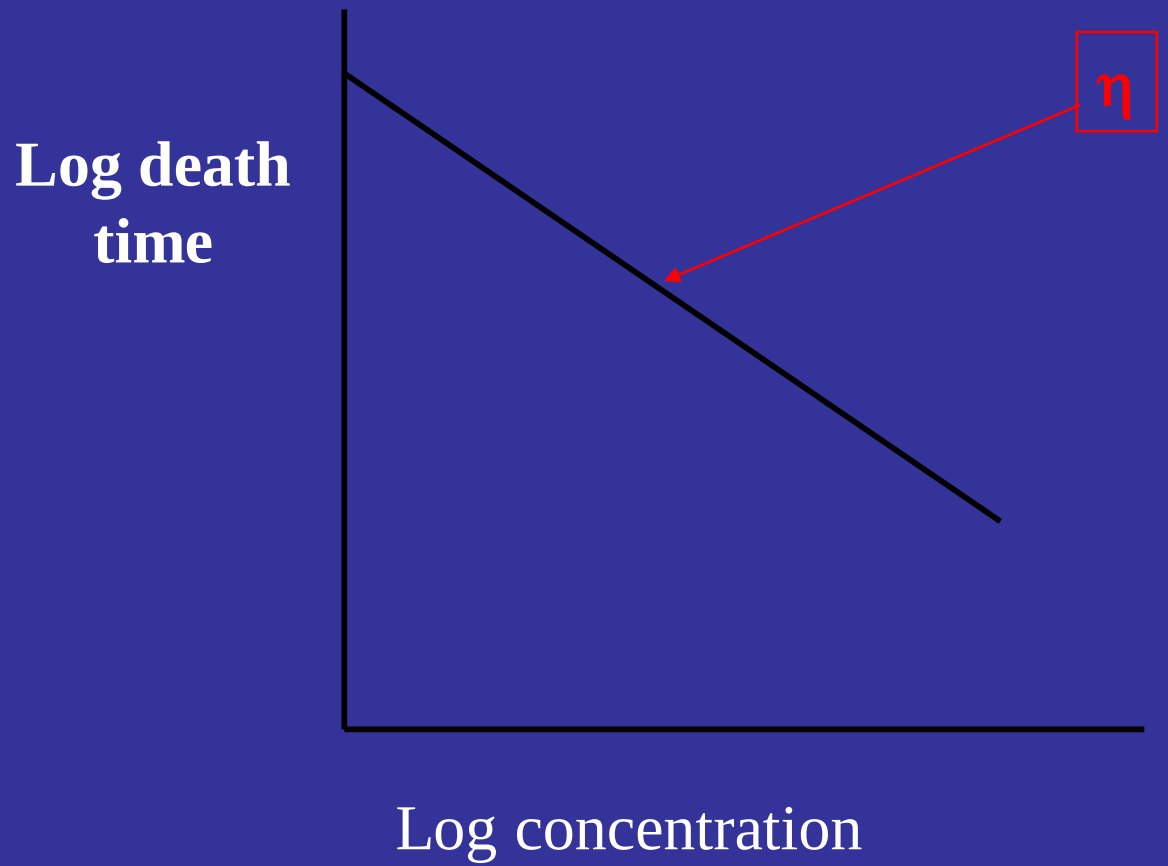


Bacteriostatic

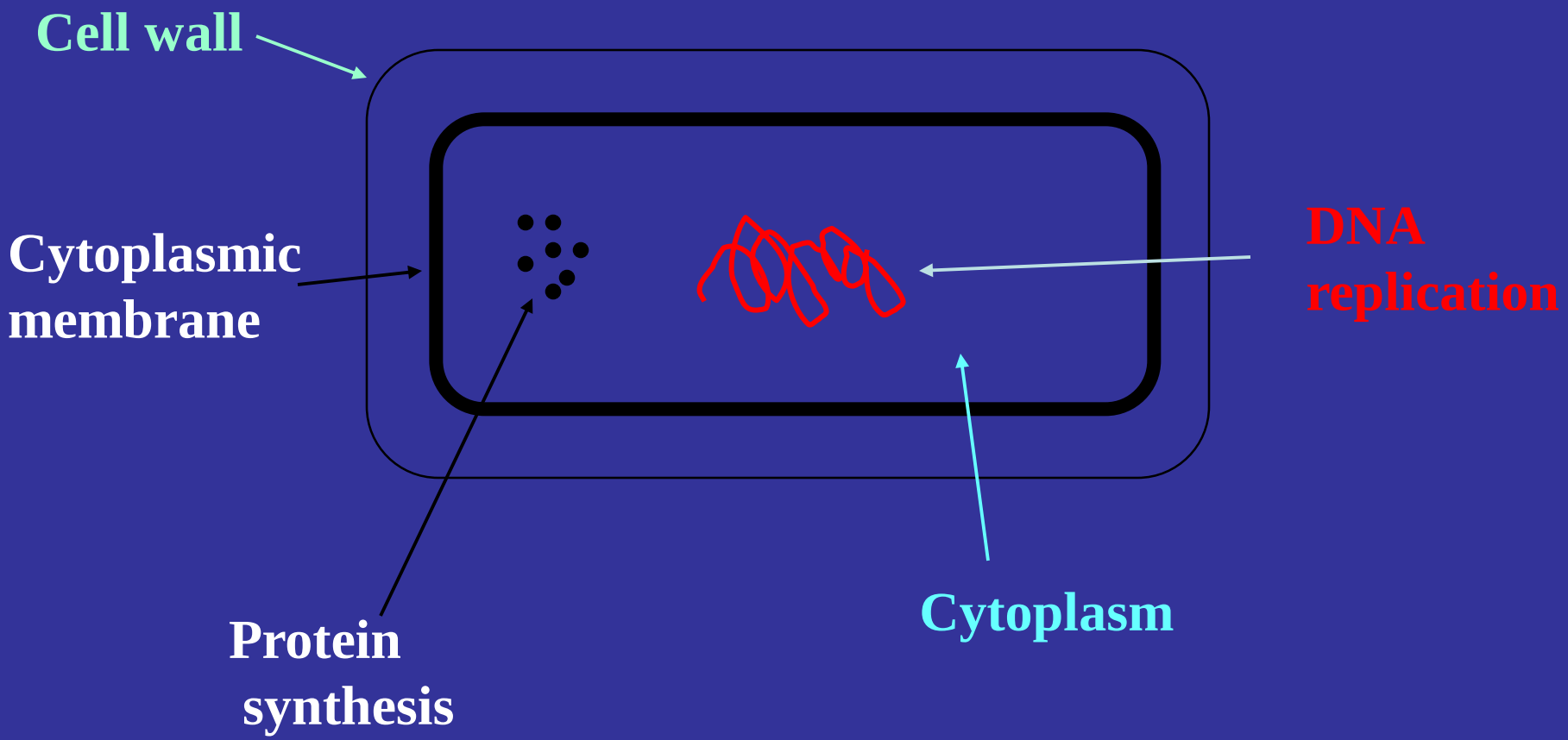
Vs

Bacteriocidal

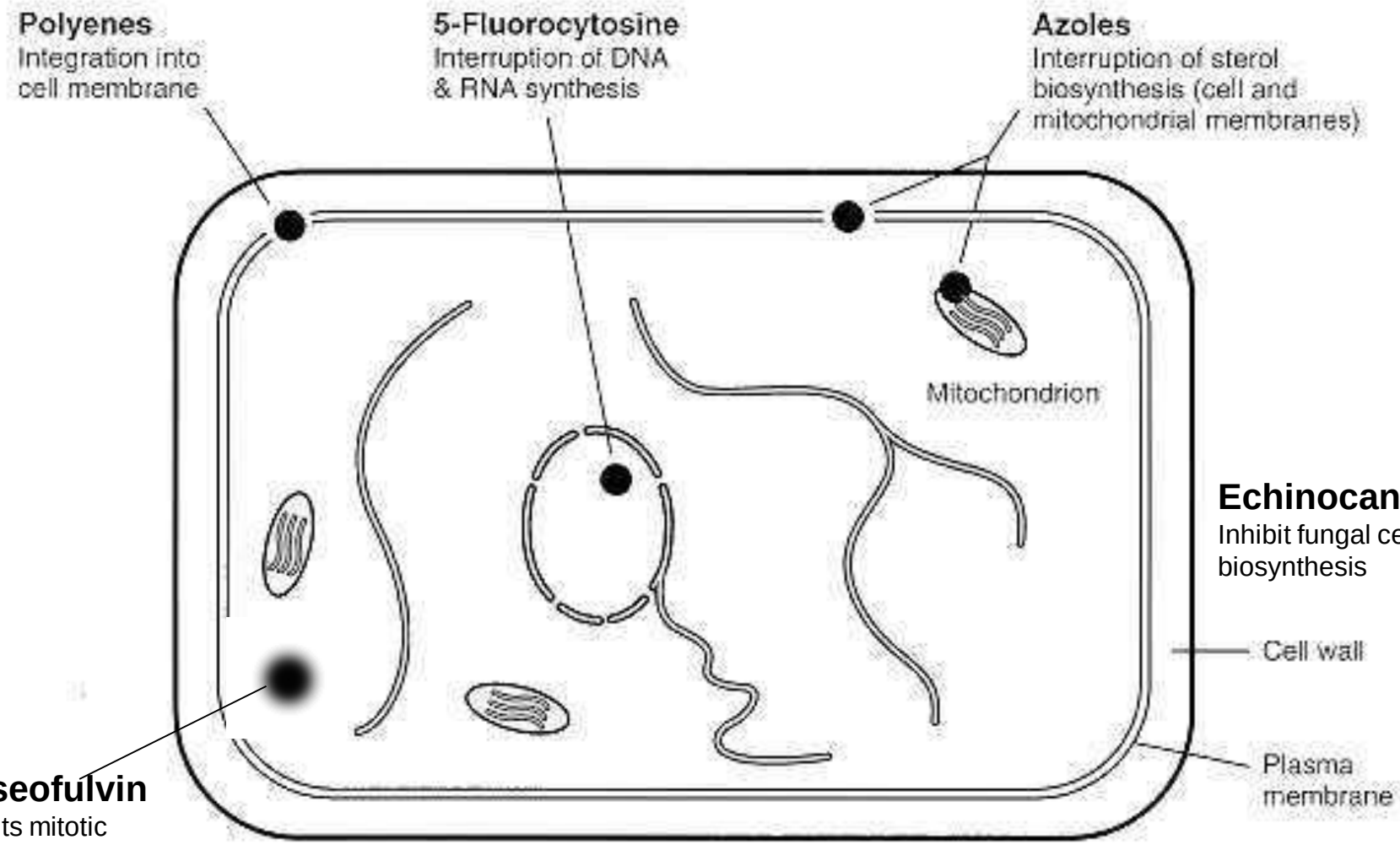
The Concentration Exponent(η)



SITES OF ANTIMICROBIAL ATTACK



Antifungal Agents- Sites of action



Disinfection: Reducing the number of pathogenic microorganisms to the point where they no longer cause diseases. Usually involves the removal of **vegetative** or **non-endospore forming** pathogens.

May use physical or chemical methods.

- **Disinfectant**: Applied to inanimate objects.

  Antiseptic: Applied to living tissue (***antisepsis***).
- **Degerming**: Mechanical removal of most microbes in a limited area. Example: Alcohol swab on skin.
- **Sanitization**: Use of chemical agent on food-handling equipment to meet public health standards and minimize chances of disease transmission. E.g: Hot water & soap.

Disinfection is the destruction of pathogenic and other kinds of microorganisms by physical or chemical means. **Disinfectants** are chemical substances used to destroy viruses and microbes (germs), such as bacteria and fungi, as opposed to an **antiseptic** which can prevent the growth and reproduction of various microorganisms, but does not destroy them.

The ideal disinfectant would offer complete **sterilization**, without harming other forms of life, be inexpensive, and non-corrosive. Unfortunately ideal disinfectants do not exist. Many disinfectants are only able to partially sterilize. The most resistant pathogens are bacteria spores but some viruses and bacteria are also highly resistant to many disinfectants.

antiseptics disinfect skin. **Antibiotics** either kill or interfere with the life cycle of bacteria inside the body. Substances which kill bacteria are said to have a bactericidal effect, while those which interfere with cell growth and reproduction are said to be bacteriostatic.

Disinfectants and antiseptics are bactericidal (some disinfectants are bacteriostatic at low concentrations): antibiotics can be either bactericidal or bacteriostatic.

Sanitation refers to killing 99+ % of germs in applicable situations. **Sanitisers** are compounds that sanitise.

Sanitizers contain chemicals that reduce, but do not necessarily eliminate, microorganisms such
as bacteria, viruses and molds from surfaces.
Public health codes may require cleaning with the use of sanitizers in certain areas, like toilets and food preparation areas..

Disinfectants contain chemicals that destroy or inactivate microorganisms that cause infections. Disinfectants are critical for infection control in hospitals and other healthcare settings.

Triclosans. Triclosans are hand sanitizers prevent the growth of bacteria, but they may do much more harm than good. The FDA (Food and Drug Administration) says they're contributing to making bacteria resistant to antibiotics, and helping create superbugs such as MRSA

.

Parabens. Hand sanitizers often contain chemicals called parabens that prevent the growth of bacteria. They've been linked to a host of dangerous health problems including cancer, neurotoxicity, endocrine disruptions, and skin irritation. Avoid labels that include these common butylparaben, methylparaben, and propylparaben parabens: ethylparaben,

Alcohol. Hand sanitizers contain about 65 percent ethyl alcohol — pure alcohol — and some of it is absorbed into the skin. Research has found alcohol in the blood stream of people who used hand sanitizers, and children have been known to lick enough from their hands to become drunk. Alcohol from the sanitizers can also be absorbed through inhaling its vapors. Many hand sanitizers also contain isopropyl alcohol (rubbing alcohol) which is a petrochemical that's a known neurotoxin. It is also absorbed through the skin and is toxic even in small doses.

Antibiotic resistance. Hand sanitizers and similar products are increasing the development of bacteria that are resistant to antibiotics. If you still want to use a hand sanitizer, choose one that isn't labeled "antibacterial

Immune system. Hand sanitizers may affect your immune system by killing good bacteria which keep disease-causing bacteria at bay

Site of action of fungicide inhibitor:

1- Intracellular action:

There is reason to believe that the majority of antifungal compounds act within the cell by the inhibition of vital processes. In order to do this, they must be able to penetrate the cellular membrane and gain access to the subcellular components where these processes occur.

This cellular membrane consist of • lipoprotein. as in other organisms and a degree of lipoid solubility is therefore a necessary property to enable a compound to penetrate into the cell. Examples of antifungal compounds of high lipoid solubility are captan and dichlone.

- The uptake of fungicides has been well demonstrated by the use of radioactive tracers, in particular C’’. Thus it was shown that **spores** of *Neurospora sitophila* accumulated a 10,000—fold concentration of glyodin from an aqueous solution of 2 µg/ml, and similar, though slightly lower, rates of uptake were recorded for other fungi and other fungicides.

- it seems that the uptake is not a normal diffusion process, but rather an active transport mechanism.

2- Action at the cell surface

A number of compounds of relatively low lipoid solubility are also effective fungicides, however, and evidence is accumulating that these may be acting at the cell surface, It now appears that the toxic effect of metal ions may be related with their clectronegativity.

Surface-active compounds are believed to act at the cell surface: the toxicity of dodine acetate to *Monilinia [Sclerotinia fructicola]* appears to be due in part to its effects in blocking vital anionic sites at the cell surface

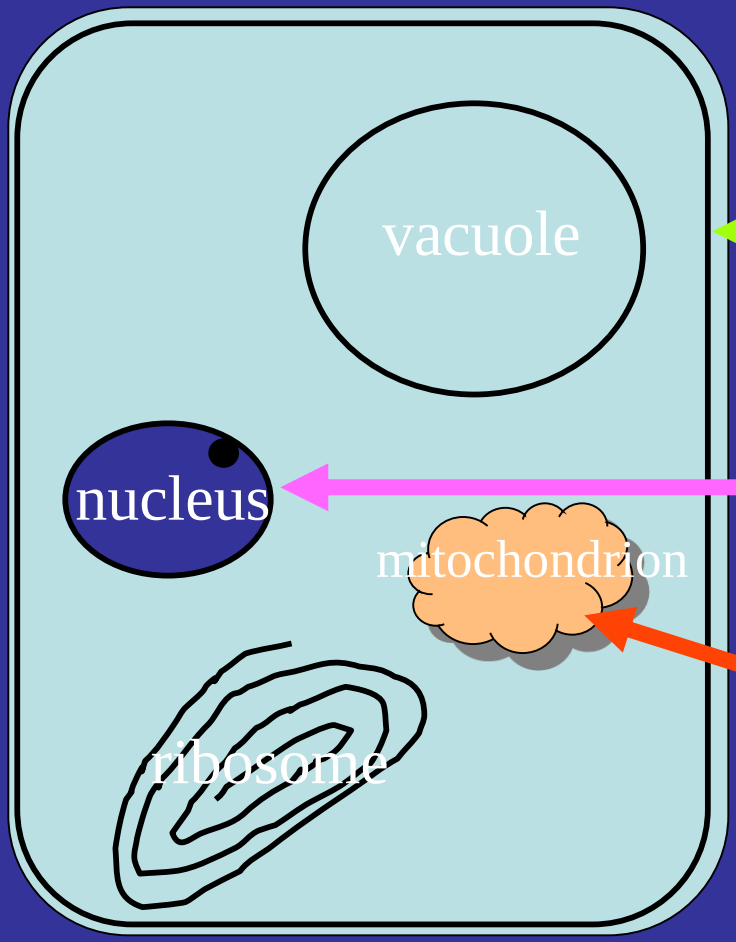
This compound, and also S-triazine fungicides, also gives rise to leakage of essential nutrients from the cell, presumably ***as*** a result of disturbed permeability.

3- Extracellular action

An aliphatic amine, which was effective against the fungus *Lentinus lepideus* when grown with a cellulose carbon source, but which was inactive when the fungus was grown with glucose as a carbon source. The effect appeared to be due to the inactivation of an extracellular cellulase enzyme upon which the fungus relied for its ability to utilize cellulose.

In a similar manner a number of naturally occurring polyphenols, in an oxidized and polymerized form, were shown to inhibit extracellular pectolytic and plant tissue macerating enzymes necessary for infection of apples by a plant pathogenic fungus, *Sclerotinia fructigena* such compounds were much less active in conventional spore germination toxicity tests against the fungus.

Site-specific inhibitors target individual sites within the fungal cell.



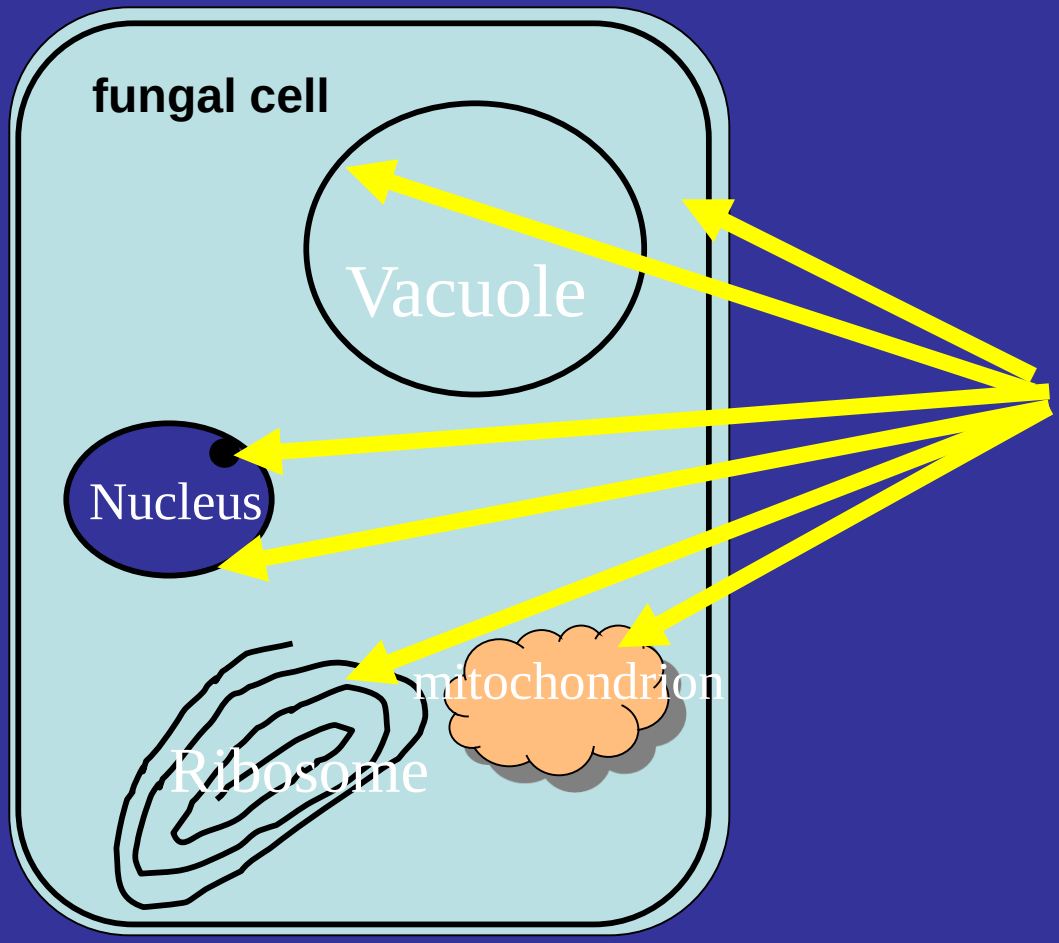
Examples

DMI fungicides – inhibit sterol synthesis in membranes

benzimidazoles – inhibit DNA Synthesis

carboxamides – inhibit respiration

Multisite inhibitors target many different sites in each fungal cell.



Examples

nitrile fungicides
dithiocarbamates
peroxides

Site-specific inhibitors target individual sites within the fungal cell.

1-The **DMI** (DeMethylation Inhibitors) or SBI (Sterol Biosynthesis inhibiting) fungicides which include the triazoles and imidazoles..

2-benzimidazoles – inhibit DNA synthesis

3-carboxamides – inhibit respiration

4-Tricyclazole inhibit melanin biosynthesis.

Comparison of Methods

Method	Spores	Vegetative Cells
X rays	0.4 MRads	0.1 Mrads
U.V.	1.5 hr	10 min
Ethylene oxide	1200 mgl^{-1}	700 mgl^{-1}
2% Glutaraldehyde	3 hr	10 min

- 1928 – Fleming discovered penicillin, produced by *Penicillium*.
- 1940 – Howard Florey and Ernst Chain performed first clinical trials of penicillin.

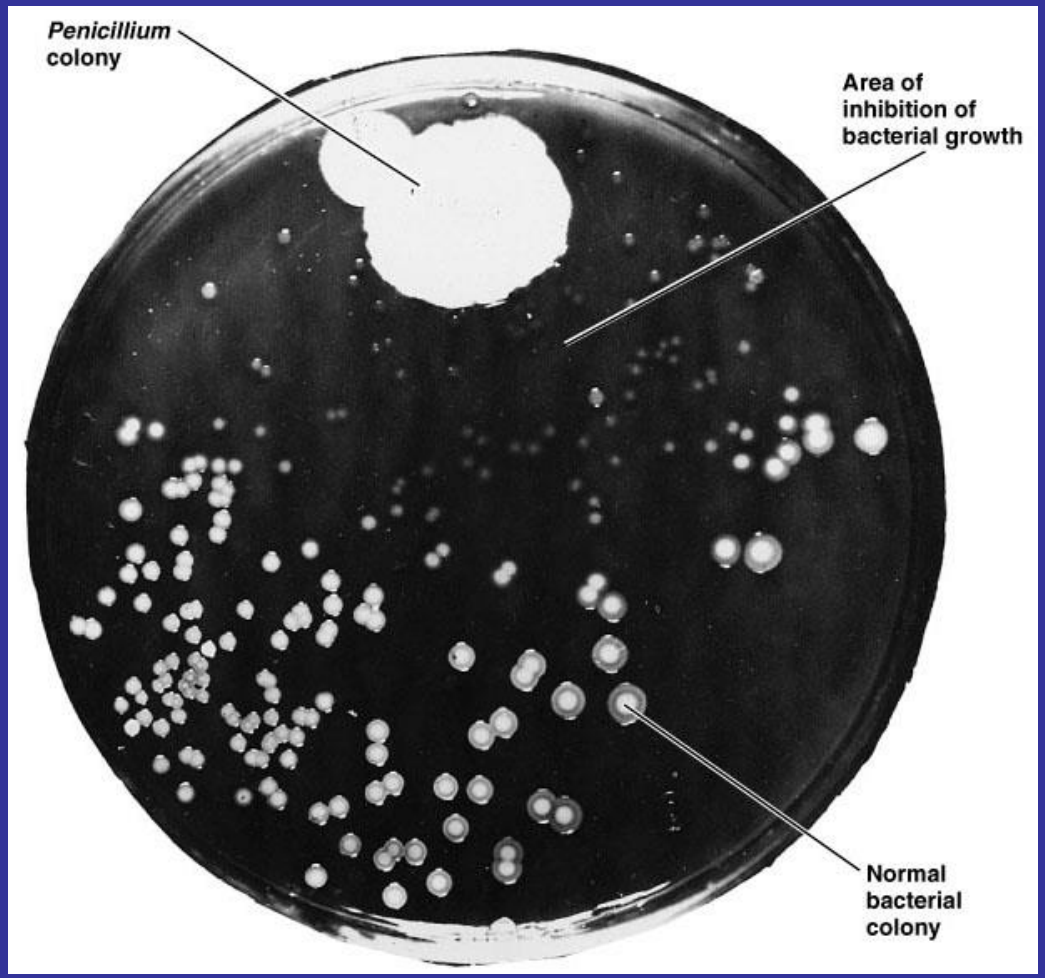
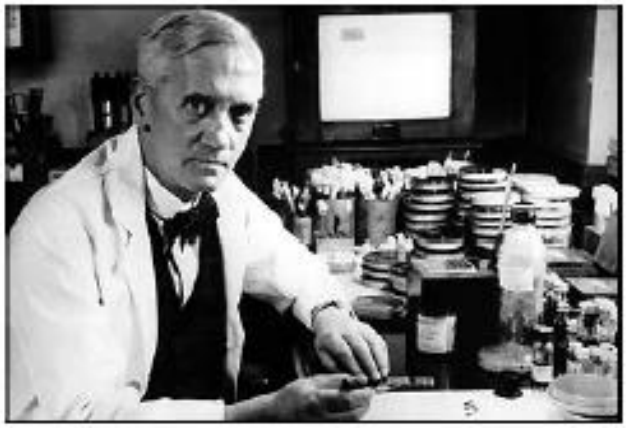


Figure 20.1

Penicillin

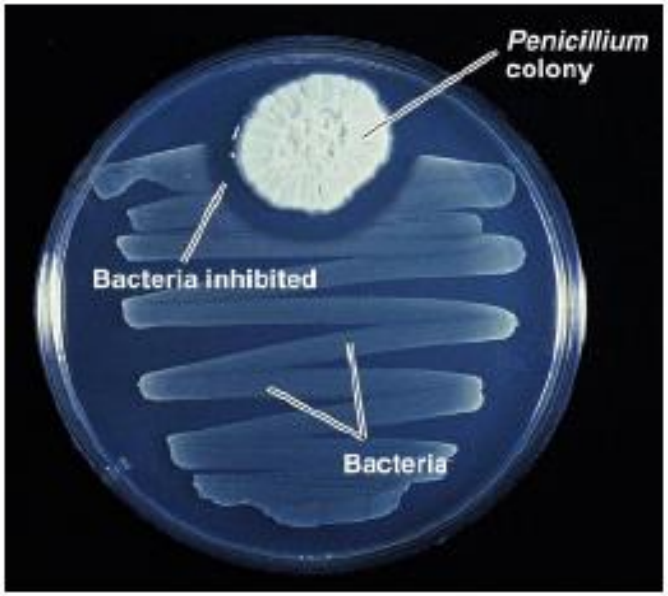
Antibacterial activity in *Penicillin notatum* by Alexander Fleming in 1928



Research continued by Howard Florey and Ernst Chain

Mass production of penicillin in 1940s

Nobel Prize 1945



Inhibition of bacterial growth by a contaminating colony of *Penicillium notatum*

What are antibiotics?

- Drugs that prevent the growth of bacteria
- Attack prokaryotic cellular processes
- Do not affect eukaryotic cells
  - Do not harm human cells
  - Cannot be used for fungal or parasitic diseases
- Are not effective against viruses
- Characterised based on target specificity
  - Narrow or broad spectrum

Modes of action

- Disruption of cell membrane function
  - Polymyxins –
- Inhibition of protein synthesis
  - Chloramphenicol –
  - Erythromycin –
  - Tetracycline –
  - Streptomycin –

Inhibitors of cell wall Synthesis

Ampicillin

Cephalosporin

Bacitracin

Vancomycin

- Inhibition of nucleic acid synthesis
  - Rifamycin –
- Inhibitors of enzymatic function of primary metabolism
  - Competitive inhibition –
  - Noncompetitive inhibition –

How Do Bacteria Develop Resistance?

- Presence of antibiotics provides selection pressure for spontaneous mutants (1 in 10^6) with increased resistance
- High population density → efficient gene transfer
- Short generation time → rapid evolution

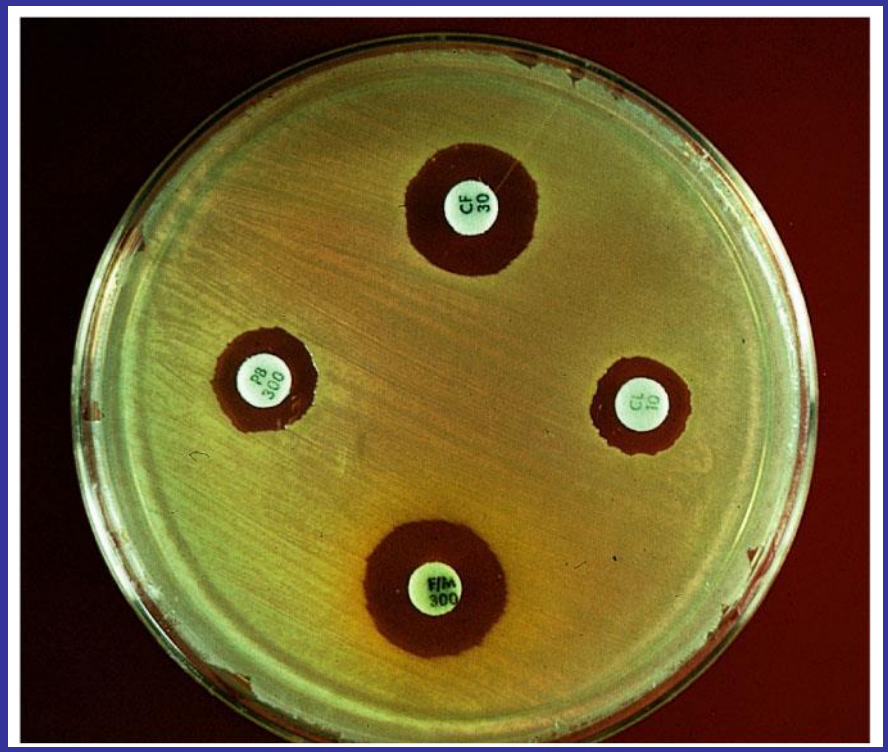
How Does it work?

- Inactivating enzymes
- Alter antibiotic target
- Pump antibiotics out of the cell

Antibiotic Resistance

- A variety of mutations can lead to antibiotic resistance.
- Mechanisms of antibiotic resistance
  1. Enzymatic destruction of drug
  2. Prevention of penetration of drug
  3. Alteration of drug's target site
  4. Rapid ejection of the drug
- Resistance genes are often on plasmids or transposons that can be transferred between bacteria.

- We humans will always have to find or create new antibiotics as microbes become resistant



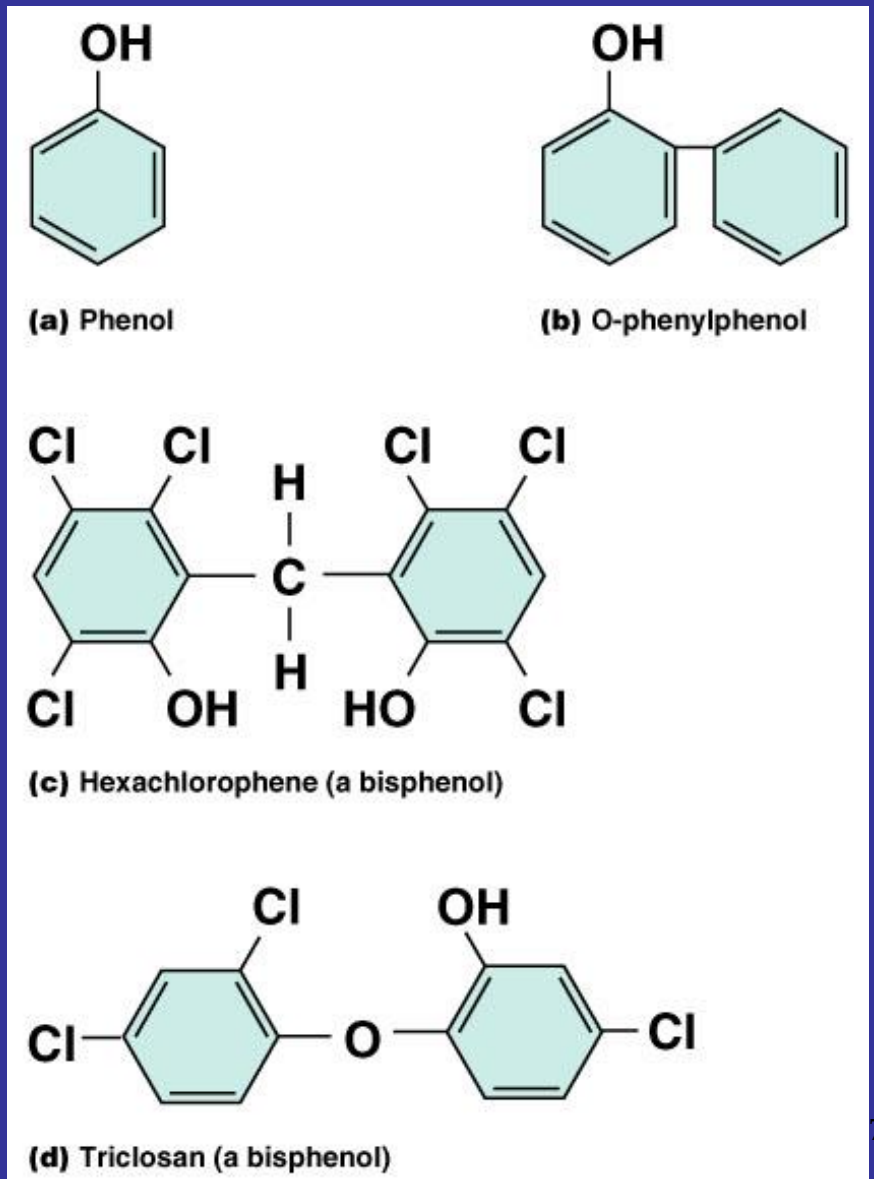
Attributes of an ideal antimicrobial agent

1- Solubility in body fluids
2- Selectively toxic
3- Toxicity not easily altered
4- Not allergenic
5- Stability in body
6- Resistance not easily acquired
7- Long shelf life
8- Reasonable cost

Types of Disinfectants

- Phenol
- Phenolics. Lysol
- Bisphenols. Hexachlorophene, Triclosan

Disrupt plasma – membranes



For example for phenol η is 6

Therefore if concentration halved

then contact time is increased

2^6 or 64 times

One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly. Phenol is the standard, and the corresponding rating system is called the "**Phenol coefficient**". The disinfectant to be tested is compared with phenol on a standard microbe (usually *Salmonella typhi* or *Staphylococcus aureus*). Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective have a coefficient < 1.

Phenol and other phenolics – The active ingredient in most bottles of "household disinfectant". It is also to found in some mouthwashes and in disinfectant soap and handwashes. Phenol is probably the oldest disinfectant (used by Lister) and was called carbolic acid in the early days of antiseptics. Phenol is rather corrosive to the skin and sometimes toxic to sensitive people, so the somewhat less corrosive substitute phenol.

- *o*-phenylphenol is often used as part of a disinfectant formula. Hexachlorophene is a phenolic which was once used as a germicidal additive to some household products but was banned due to suspected harmful effects.

Common disinfectants

Chlorine – Used to disinfect swimming pools, and is added in small quantities to drinking water to reduce waterborne diseases.

Chloramine – Used in drinking water treatment instead of chlorine because it produces less disinfection byproducts.

Chlorine dioxide – Used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts.

- Sodium chlorite, sodium chlorate, and potassium chlorate have little disinfection effect but are used as precursors for generating chlorine dioxide.

- Ozone – a gas that can be added to water for sanitation.

Dettol –**Chloroxylenol**, Used to disinfect surfaces at home. It kills the majority of bacteria. It is one of the few disinfectants useful against viruses.

Alcohol – Usually ethanol or isopropanol – Wiped over benches and skin and allowed to evaporate for quick disinfection. Alcohols are more effective combined with water, 70% alcohol is more active than 95% alcohol. Alcohol is not effective against bacterial spores.

Hydrogen peroxide – Used in hospitals to disinfect surfaces. It is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.

When hydrogen peroxide comes into contact with the catalase enzyme in cells it is broken down into water and oxygen. It is the oxygen that kills bacteria. However, as recent studies have show hydrogen peroxide to be toxic to growing cells as well as bacteria, its use as an antiseptic is no longer recommended.

Iodine – Usually dissolved in an organic solvent or as Lugol's iodine solution. It is used in the poultry industry. It is added to the birds' drinking water. Iodine is rapidly neutralised by the presence of organic material, so surfaces must be cleaned prior to disinfection. Although no longer recommended because it increases scar tissue formation and increases healing time, tincture of iodine has also been used as an antiseptic for skin cuts and scrapes.

Povidone-iodine

Potassium permanganate – Formula $KMnO_4$. Red Crystalline powder. Colours everything it touches. Used to disinfect aquariums. It is also used widely in community swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of $KMnO_4$/water solution is kept near the pool ladder.

Participants are required to step in the basin and then go into the pool. It is also used widely to disinfect community water ponds and wells in Tropical countires. It is also used to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution. $KMnO_4$ is a very useful Disinfectant.

Quaternary ammonium salts (quats) such as benzalkonium chloride are a large group of related compounds. Some have been used as a low level disinfectant. They are effective against bacteria, but not against spores or viruses. Nor are they effective against some species of Pseudomonas bacteria. Quats are biocides which also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth.

Hypochlorites – Sodium hypochlorite, often in the form of common household bleach, is used in the home to disinfect drains, and toilets. A dilute form is used under the brand name Milton to disinfect baby bottles. Other hypochlorites such as calcium hypochlorite are also used, especially as a swimming pool additive. Hypochlorite gives off free chlorine and it is the chlorine that is the true disinfectant. Hypobromite solutions are also sometimes used.

Objectives, by the end of this lecture you should be able to:

- **Define the growth**
- **Methods of measuring the growth of microorganisms their advantages and disadvantages**
- **Detection the pollution with microorganisms**

The growth

The growth of a cell is culmination of all of the physiological activities of the cell.

It is a complex process involving:

1- Entrance of basic nutrients into the cell.

2- Conversion of these compounds into energy and vital cell constituents.

3- Replication of the chromosomes.

4- Increase in size & mass of the cell.

5- Division of cell into 2 daughter cells each containing a copy of genome and other vital components

Microbial Growth

Microbial growth = increase in number of cells, not cell size

Measurement of Growth

- Change in cell number
  - Microscopic counts
  - Viable plate counts
- Change in turbidity or light scattering
  - Spectrophotometer
- Change in the amount of a cell component
  - Dry weight
  - DNA/RNA
  - Protein

Direct Measurements of Microbial Growth

Breed method:

1-Spread a known volume of the suspension (0.01 ml) over a $1cm^2$ of slide

2-Dry, fix & stain

3-Count the number in many microscopic field. (calculate the mean)

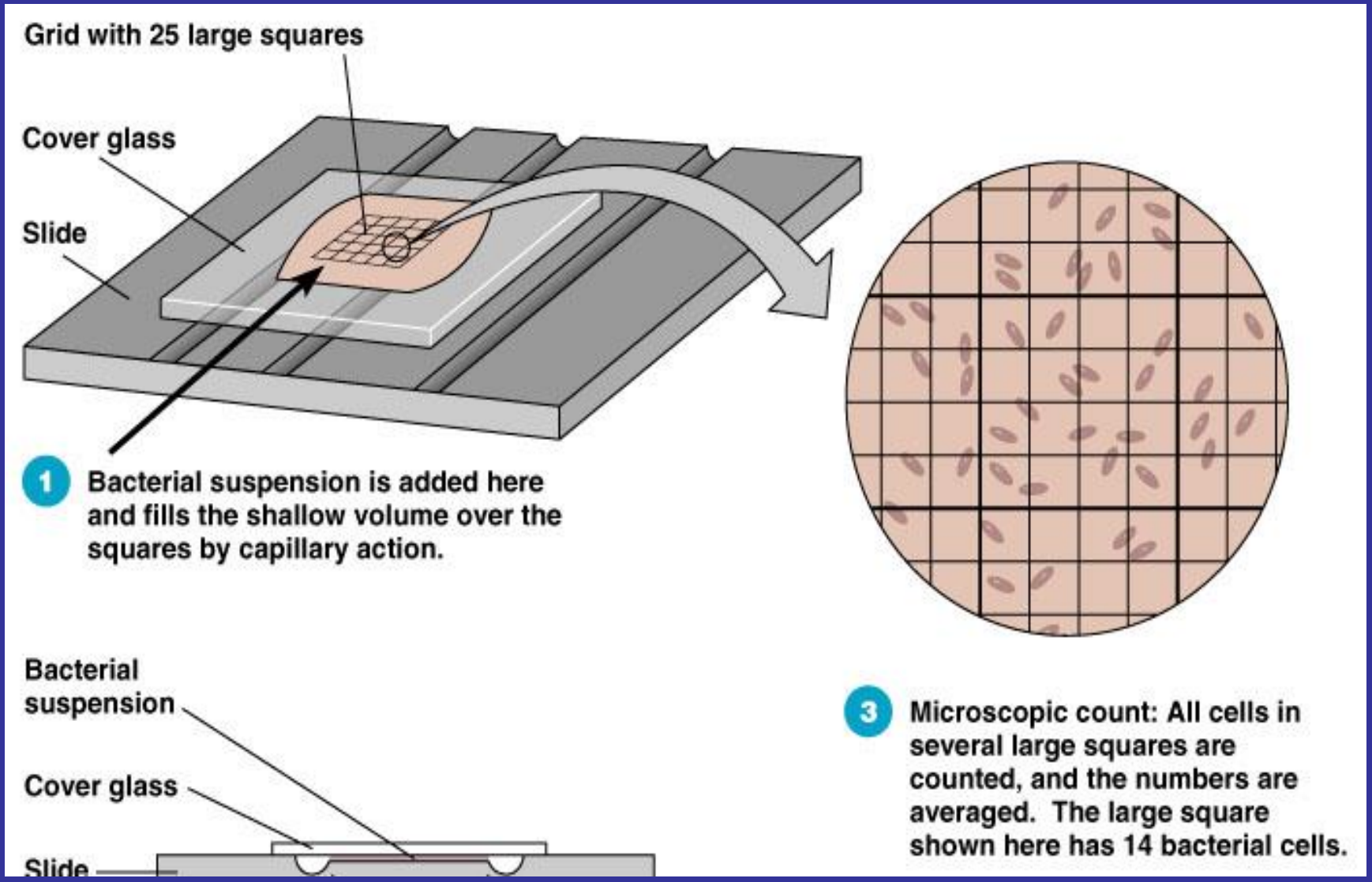
4-Calculate the area of microscopic field πr^2

5- The number of fields = 1/field area

Number of cells in 1 ml =mean number of cells in one field x number of fields x 100

- Diameter of fields=0.16
- Radius=0.08
- Area=3.14x0.08x0.08=0.02mm^2 =0.0002cm^2
- Fields number in 1 $cm^2$1/0.0002=5000
- 1cell represents 500000/ml

Direct Measurements of Microbial Growth



Direct Measurements of Microbial Growth

- Direct Microscopic Count

$$\text{Number of bacteria/ml} = \frac{\text{number of cells counted}}{\text{volume of area counted}}$$

$$\frac{14}{8 \times 10^{-7}} = 17{,}500{,}000$$

- Advantages:
- simple
- Quick method
- Morphology of cells is observed
- Disadvantages:
- Dead cells are not distinguished from viable cells.
- Fatigue for eyes
- It is difficult in condensed suspension

Direct Measurements of Microbial Growth

1- Plate Counts: Perform serial dilutions of a sample

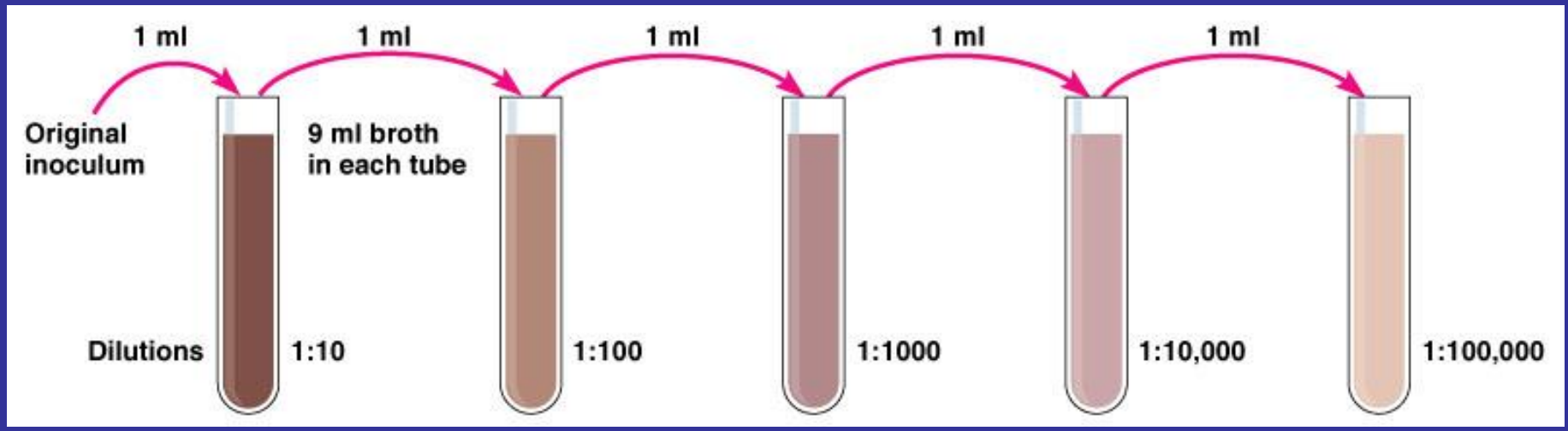


Plate Count

Inoculate Petri plates from serial dilutions

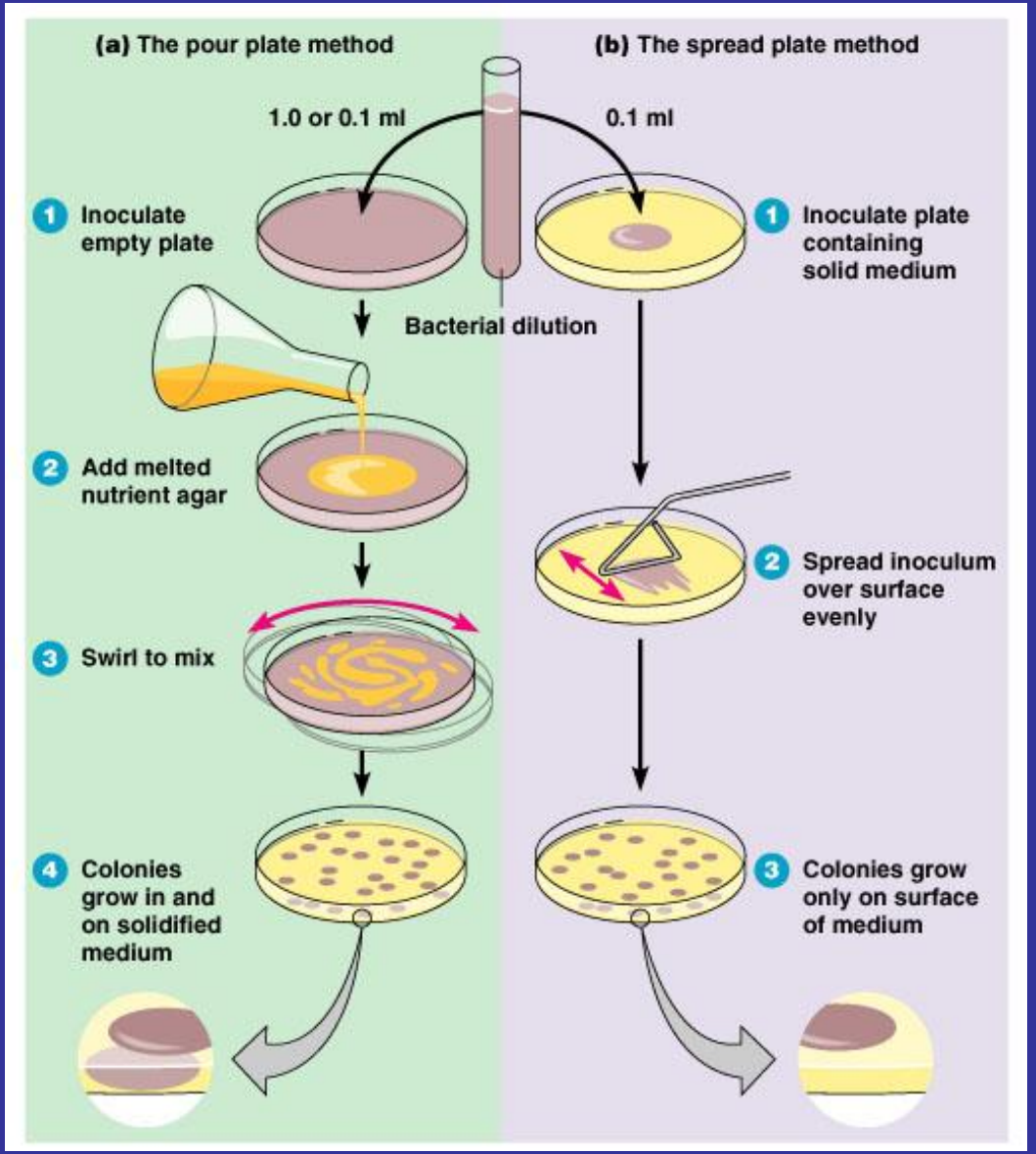
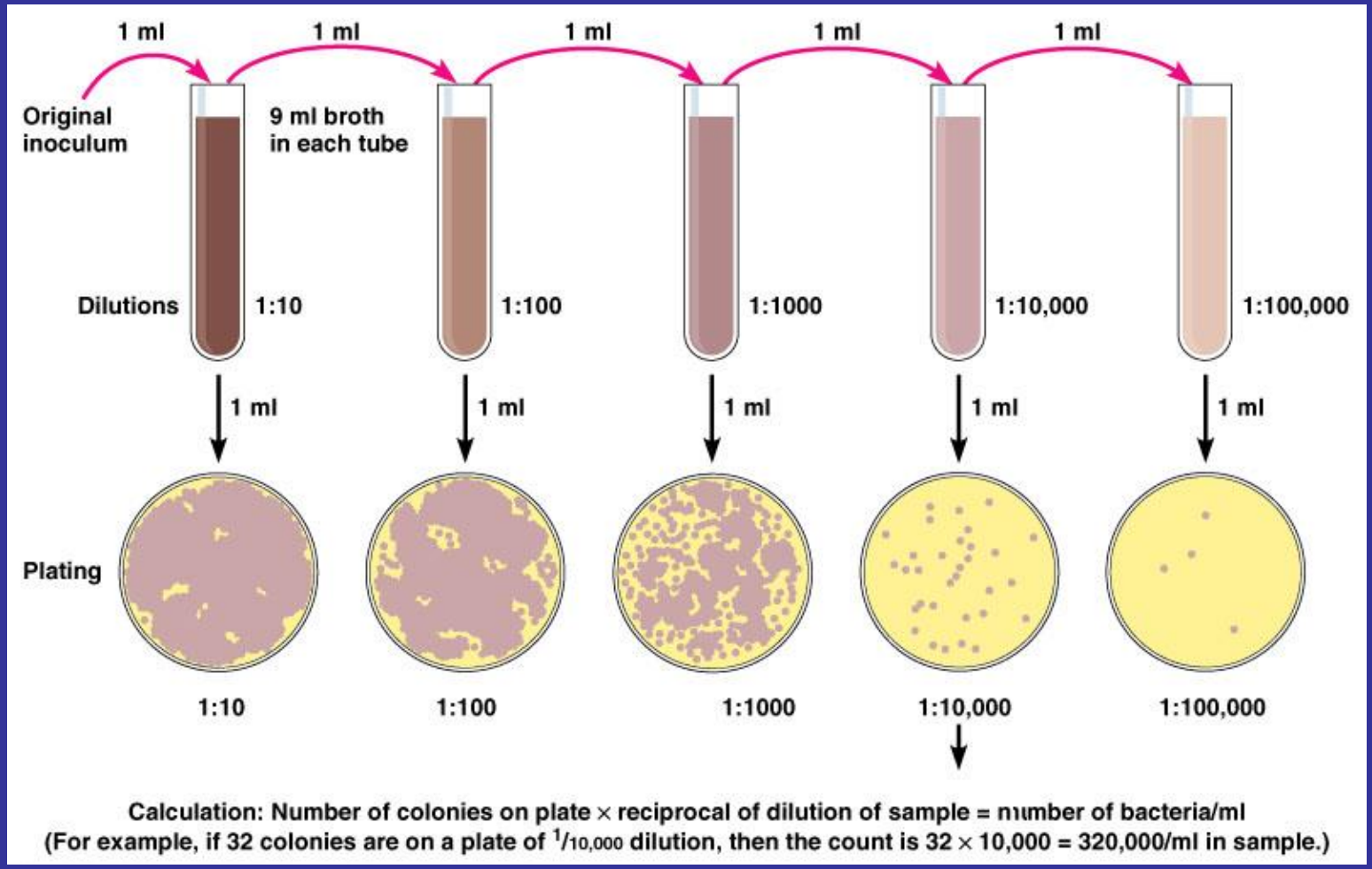
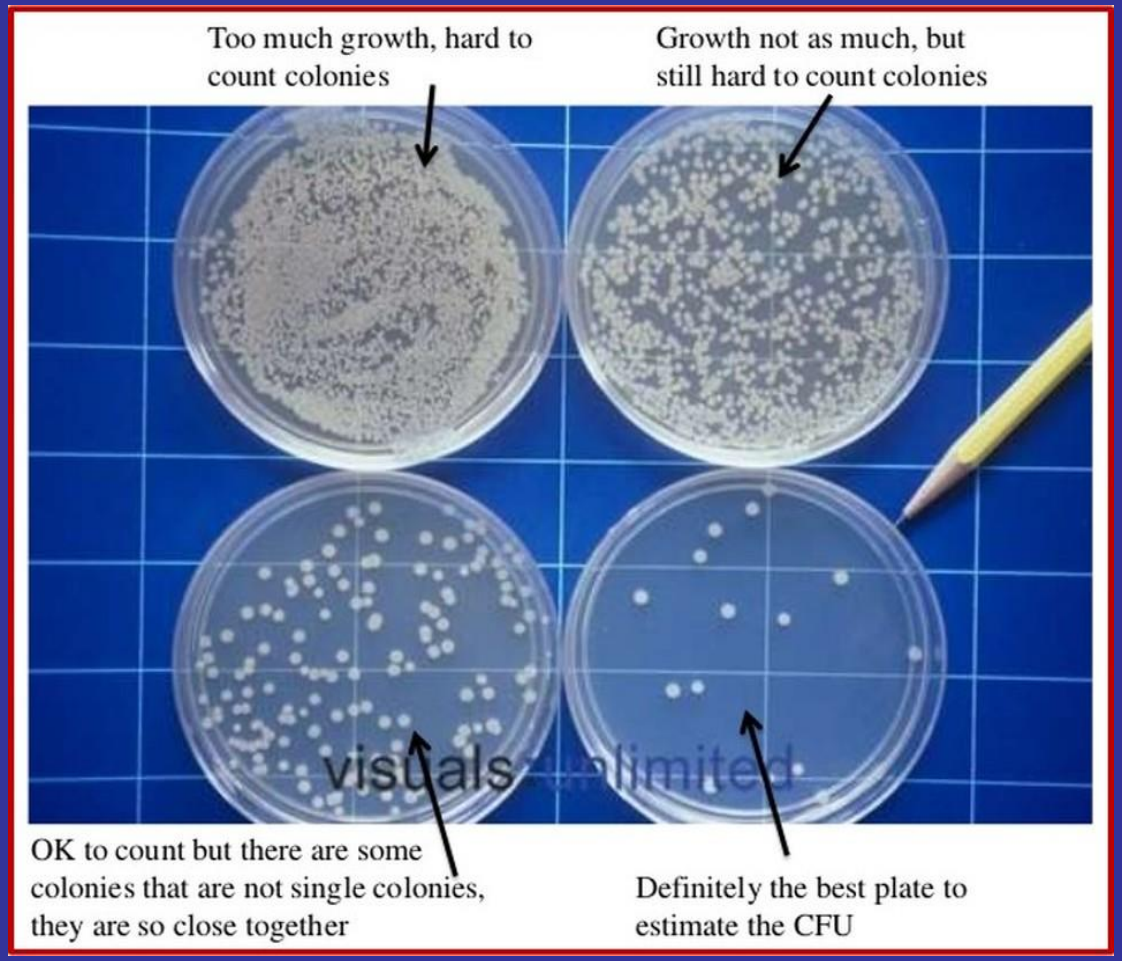


Plate Count

After incubation, count colonies on plates that have 25-250 colonies (CFUs)




Too much growth, hard to count colonies
Growth not as much, but still hard to count colonies
visuals unlimited
OK to count but there are some colonies that are not single colonies, they are so close together
Definitely the best plate to estimate the CFU

Viable count

This is typically carried out by **CFU (colony forming units)**

assay:

1- carry out dilution series

2- plate known volumes on plates

3- count only plates with 30-300 colonies (best statistical accuracy)

4- extrapolate to undiluted cell conc.

- CFU may or may not be same as number of cells --
- Method is accurate, but requires time for incubation.
- Two ways to carry out viable count:

Spread plate: bacteria are spread on the surface of agar using some sterile spreading device.

Advantages: if properly carried out, all colonies should be easily counted.

Disadvantages:

♦takes some time♦ not always reliable in inexperienced hands ♦ cells with low tolerance to oxygen won't grow.

If "spreaders" are present, may overgrow plate surface.

Pour plate: bacteria are mixed with melted agar and cooled; colonies grow throughout the agar.

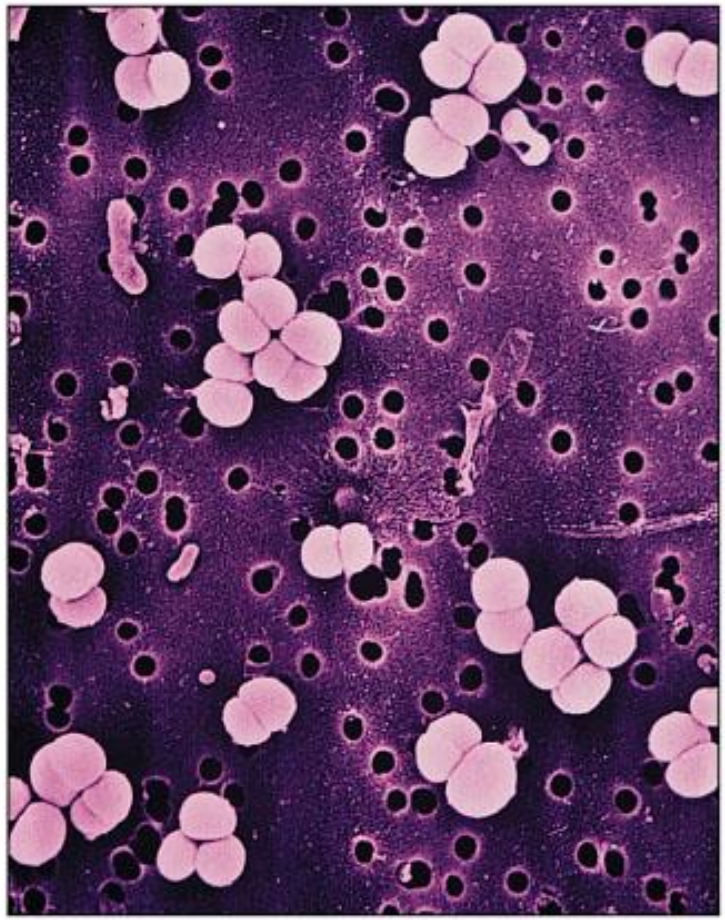
Advantages: colonies well separated.

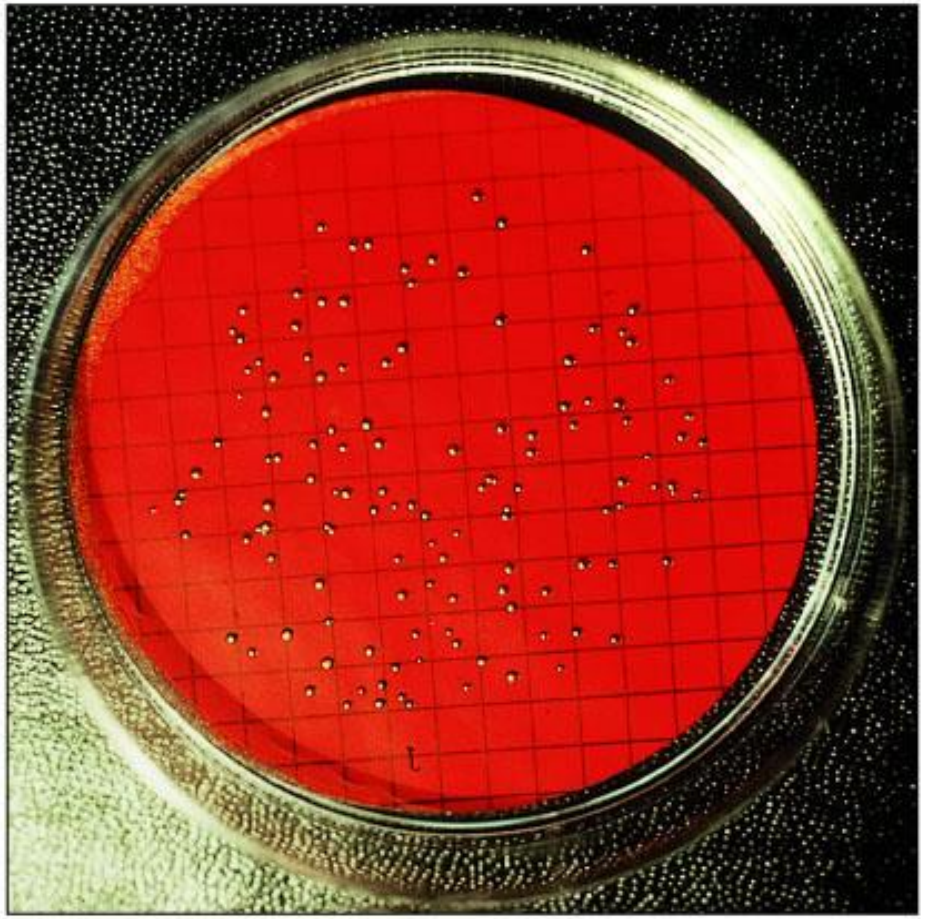
Can allow growth of organisms with lower oxygen tolerance in agar.

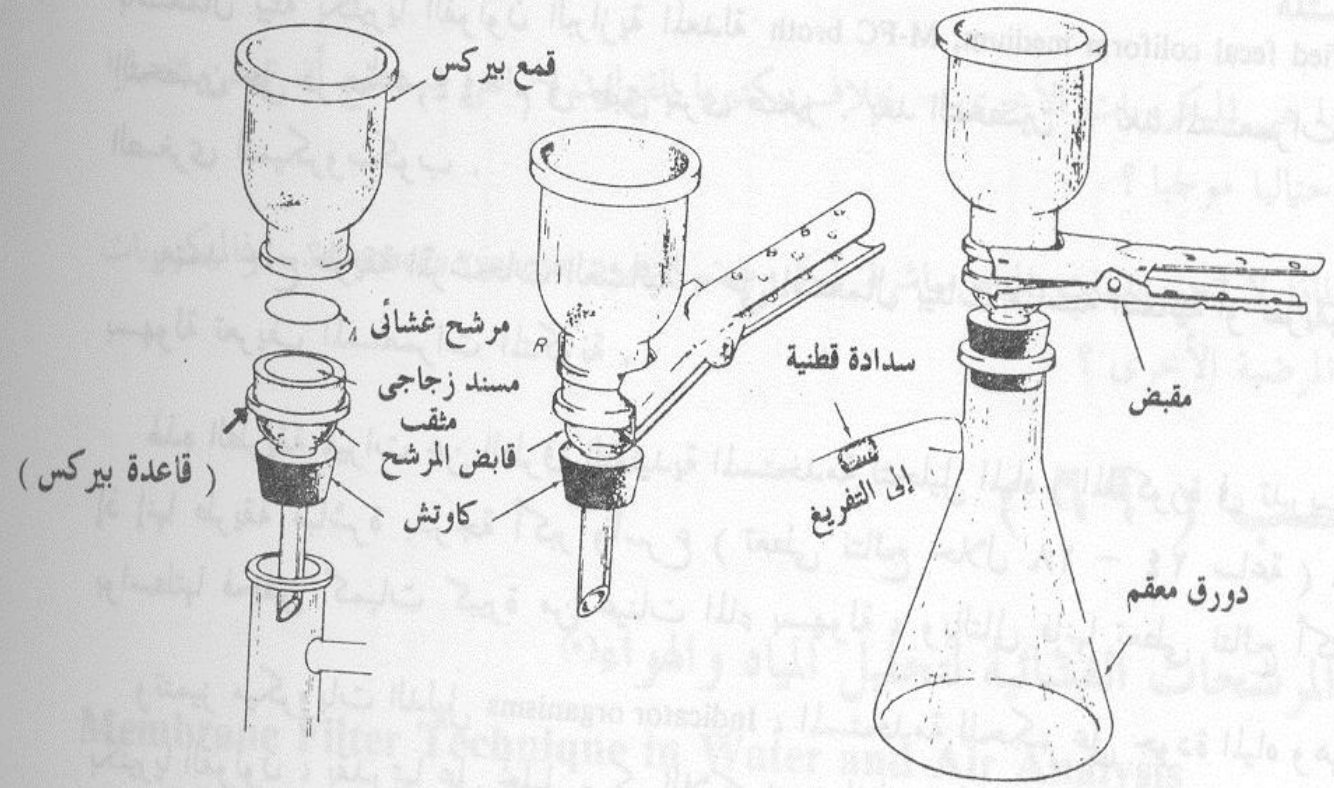
Disadvantages: colonies variable size, harder to see similarity in colony morphology between those on surface and in agar. Counting may be more difficult. Heat may kill some cells before agar cools and gels.

Direct Measurements of Microbial Growth

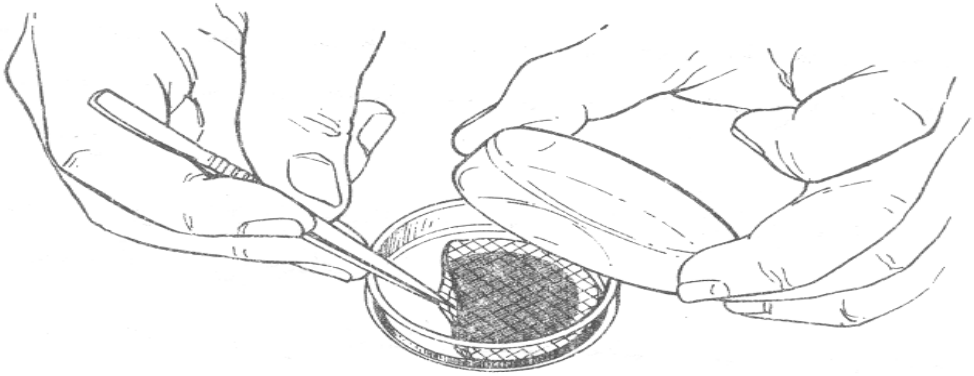
2- Filtration



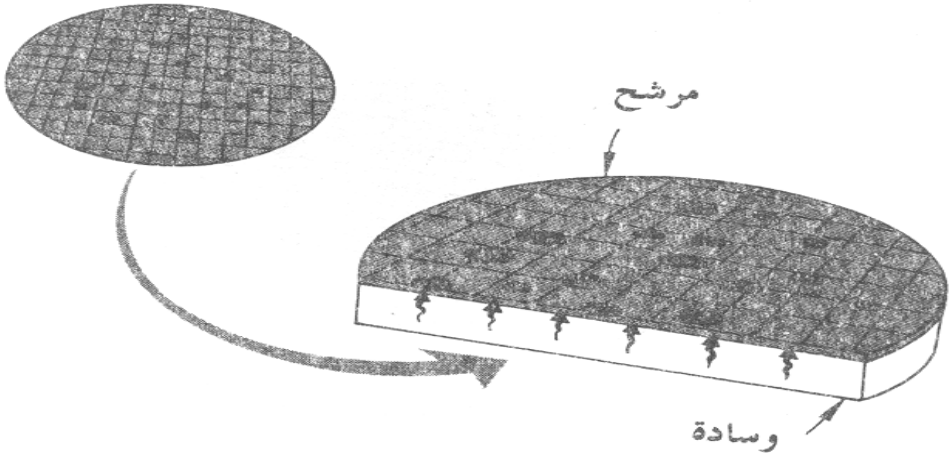




شكل (١) : جهاز المرشح الغشائى .



شكل (٣) : وضع المرشح على الوسادة فى طبق بترى .



شكل (٤) : انتشار سائل المرق من الوسادة إلى المرشح لتدعيم النمو .

Membrane filter technique:

1-Filter a known volume of solution through membrane filter (Bacteria - retaining) with pores diameter of 0.45Mm.

2-Bacteria are retained on its surface.

3-Put the membrane on the surface of thin absorbent pad that is saturated with growth suitable medium.

4-After incubation in Petri dish the colonies are counted under microscope at low power.

Direct Measurements of Microbial Growth

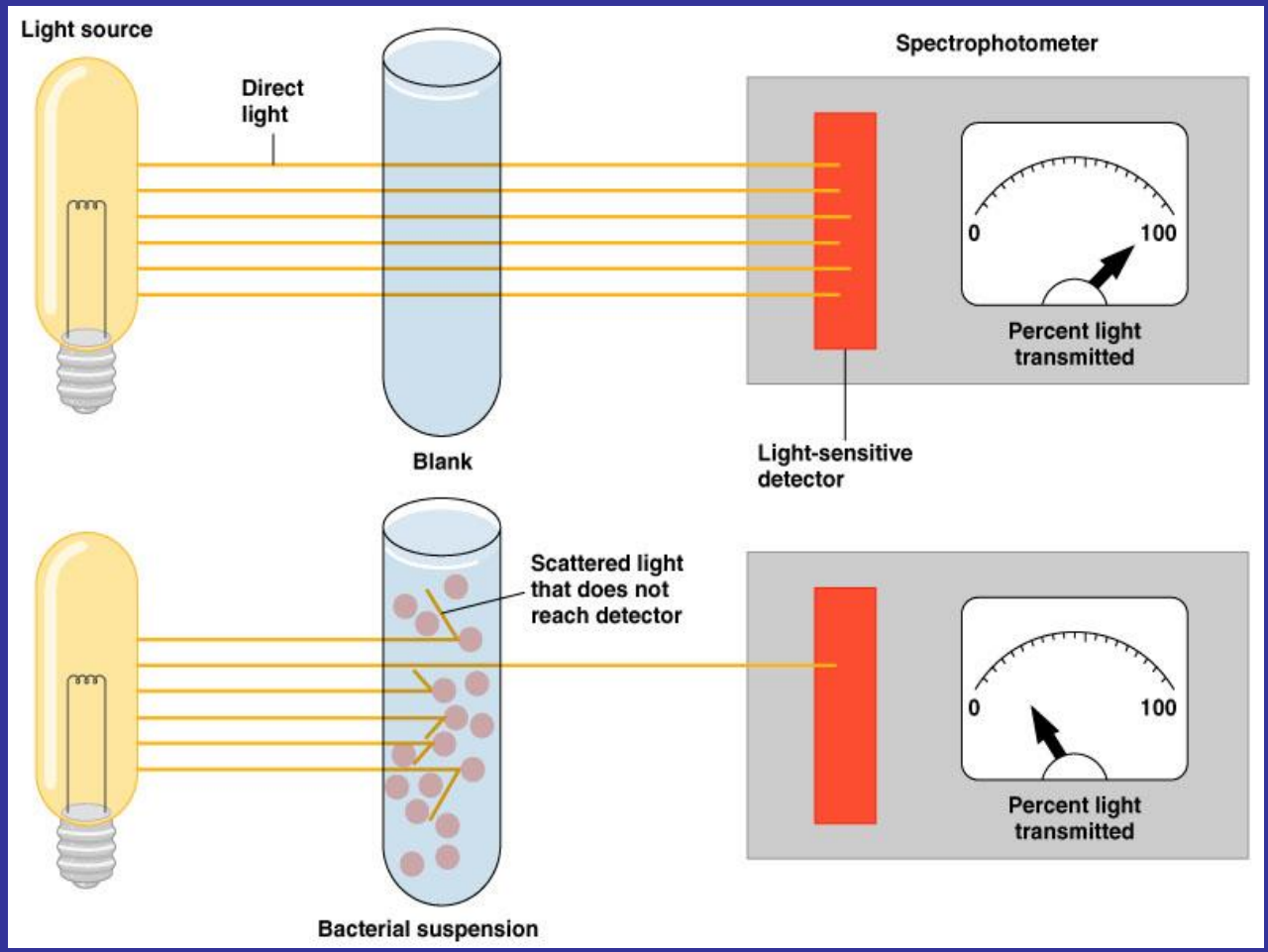
- Multiple tube MPN test
- Count positive tubes and compare to statistical MPN table.

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	9	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360

Estimating Bacterial Numbers by Indirect Methods

Turbidity

Often, can estimate cell numbers accurately by measuring visible turbidity.
Light scattered is proportional to number of cells.
This **only** works above cell densities of 107 in pure cultures.
With less than 107 cells/ml, cannot detect bacteria.



Optical technique:

The bacterial culture is considered as colloidal suspension prevents and reflects light, so the absorbed or reflected light is proportional to cells concentration.

Turbidimetre measures absorbed light.

spectrophotometer is used to measure the reflected or transmitted light.

Turbidity is expressed as absorbance.

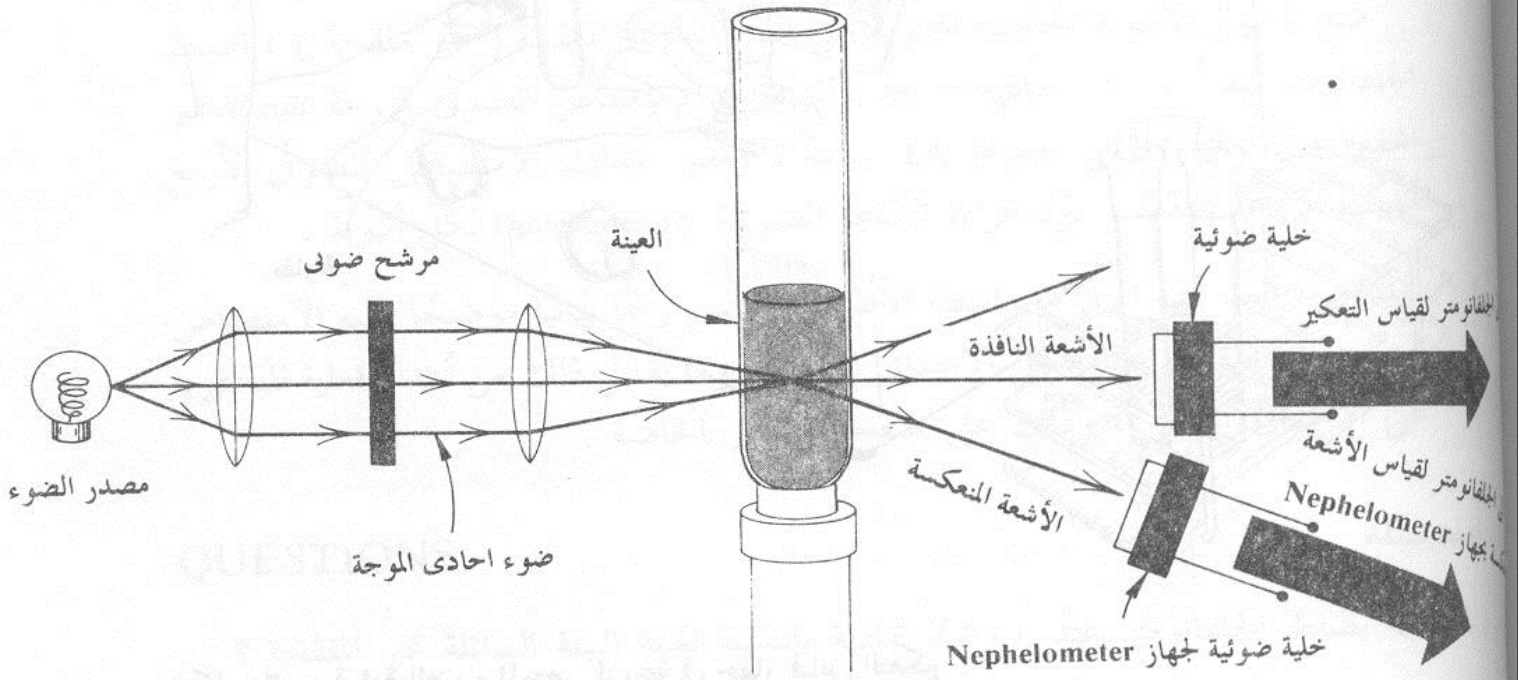
Absorbance method

Use a spectrophotometer to accurately measure

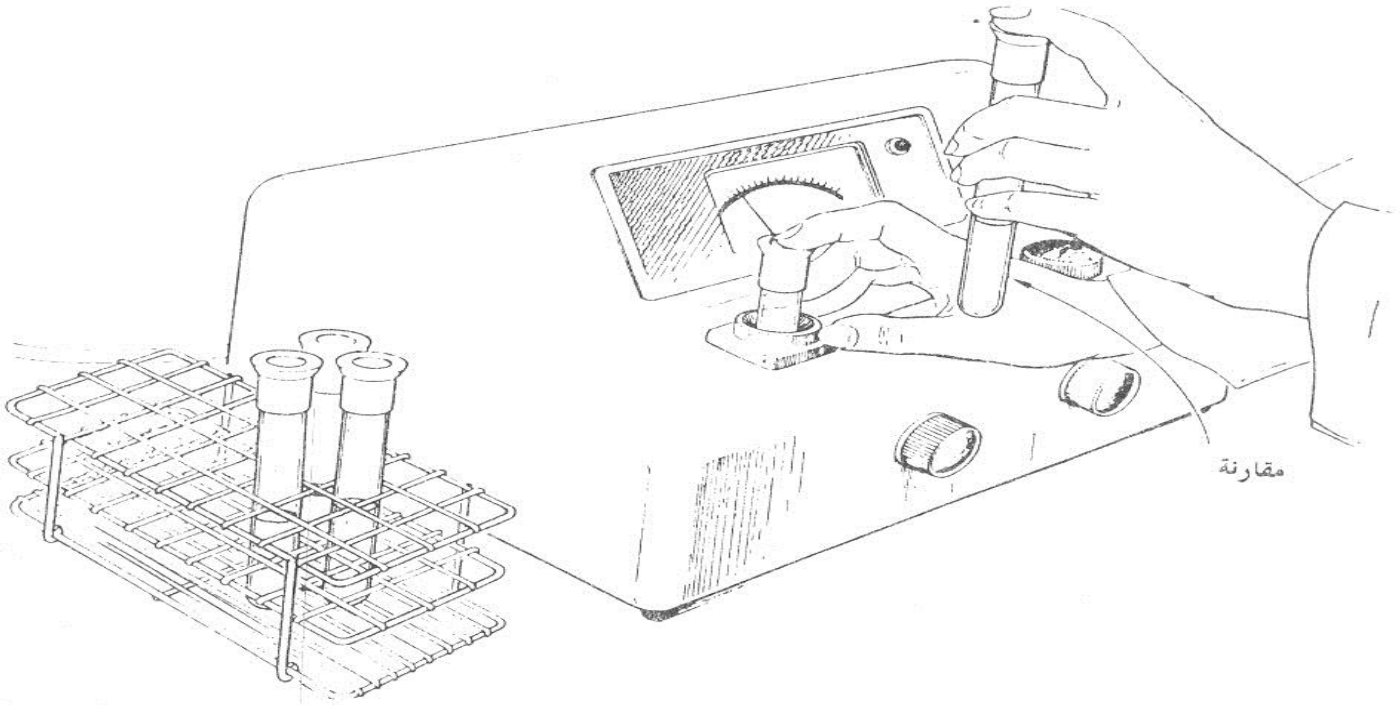
absorbance, usually at wavelengths around 400-600 nm.

Accurate measure of cells when concentration not too high.

Easy and quick to measure (can sample in less than a minute)



شكل (١) : رسم تخطيطى لجهاز قياس الألوان الضوئى الذى يمكن أن يستعمل كجهاز لقياس التعكير أو كجهاز لقياس الأشعة المنعكسة .



شكل (٢) : قراءة الضوء الممتص لمزرعة فى جهاز قياس التعكير .

Measuring the volume

- Special graduated centrifuge tubes are used.
- A known volume of the culture is put into the tube and after centrifugation the volume of deposited cells is measured.

Dry weight

- The cells are collected dried and weighed in crucible.

Growth Measurement of Filamentous fungi

Dry weight:

Weighing the dried fungus is the most widely used and often the most convenient method of measuring growth. Tissue is placed in a tarred pan, heated for 24 hr at 80^{o}C, allowed to cool, and then weighed.

Advantages:

Accurate (not neglected growth density)-
Metabolites can be measured

Limitations of this method are

that bulk quantities of tissue are required,

and it is mostly c.w materials that is measured.

In addition when solid media are used, it is often a tedious, if not impossible, process to separate the tissue from the medium.

Obviously, continuous change in the growth of a specimen cannot be observed.

Linear extension

This method involves assessment of the linear increase of either individual hyphae or colony diameter per unit time.

Advantages

Linear extension is nondestructive measure of growth, and thus continued observation can be made.

It is the most rapid method when solid media are used.

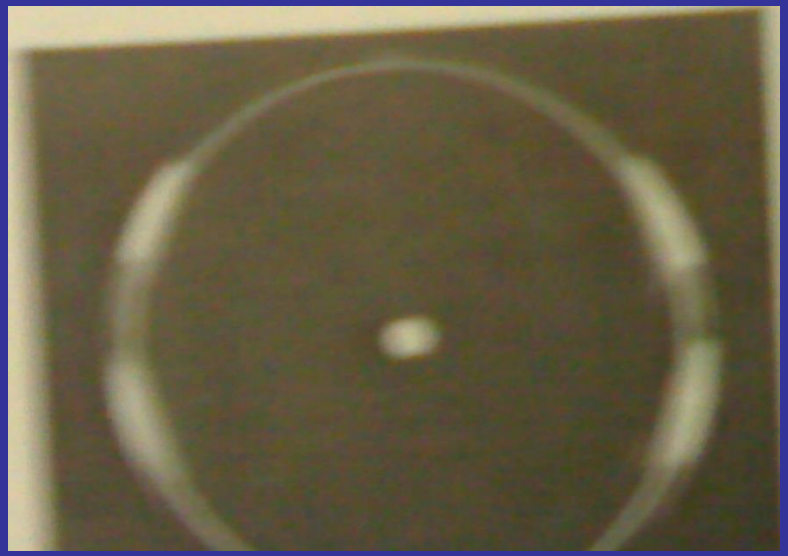
Easy to transport.

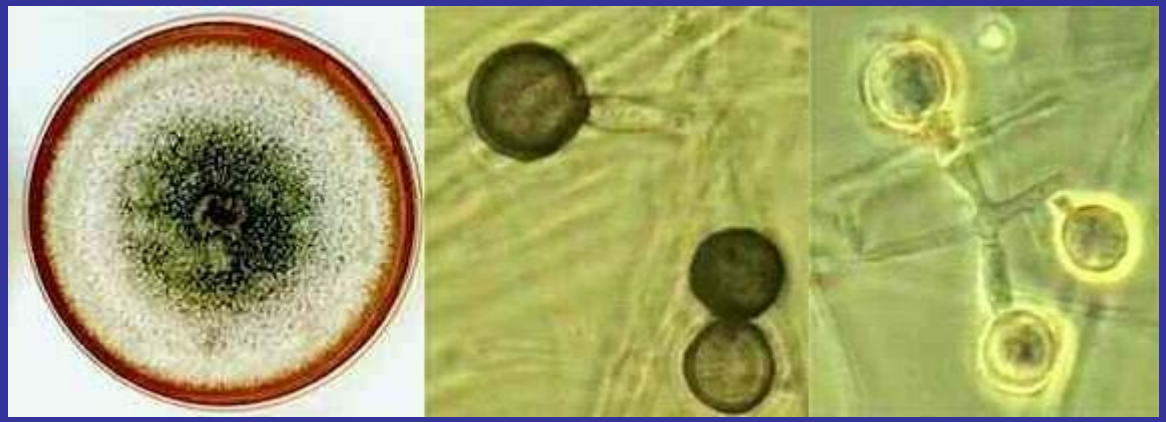
Useful to study morphology

Disadvantages

It neglects the vertical growth of hyphae.

Metabolites cannot be measured.





Cell component:

- The changes in concentration of certain cellular constituents such as chitin and glucosamine have been used as a measure of fungal growth.
- Acid hydrolysis releases glucosamine which can be separated chromatographically, and the amount recovered correlates well with fungal dry weight.
- Sterols such as ergosterols and cholesterol are also good indicators of fungal growth.

Metabolism

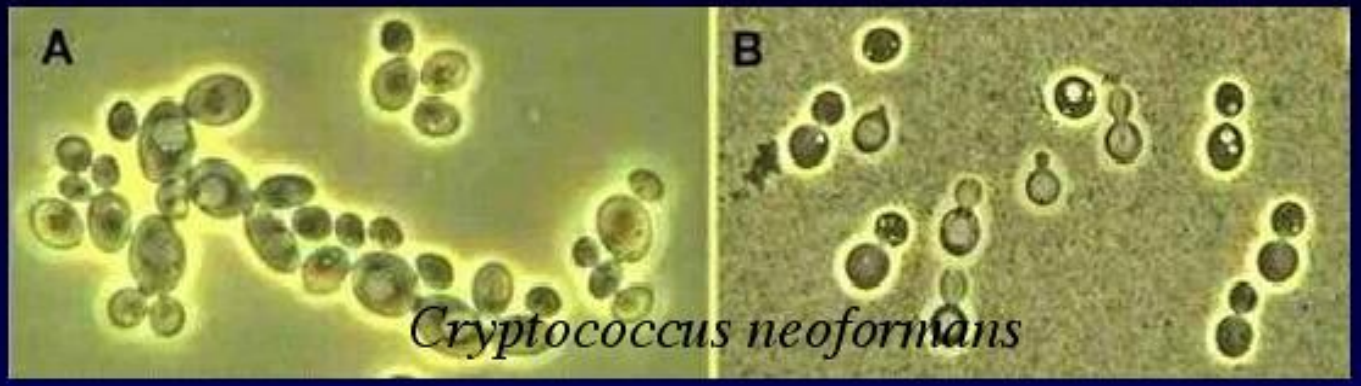
- Estimates of metabolic activity are often used to measure fungal growth.
- CO2 production can be used to determine the growth kinetics.
- C^{14}-glucose incorporation into cell constituents can be used for measuring growth.

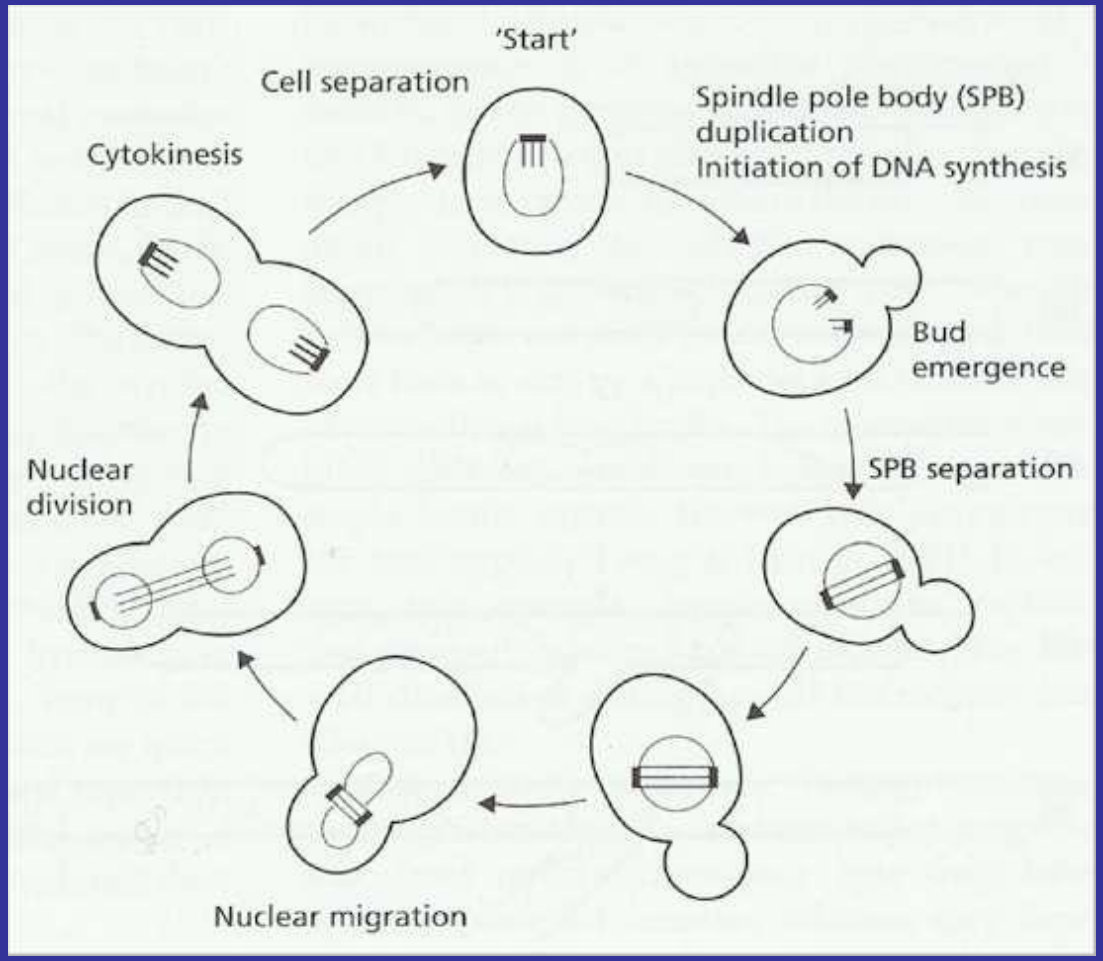
Types of growth in fungi

1- Yeast type growth:

Recorded in yeast and is characterized by budding. The growing protoplast leads to the formation of a bud, which after increase in size separates to form a new cell.

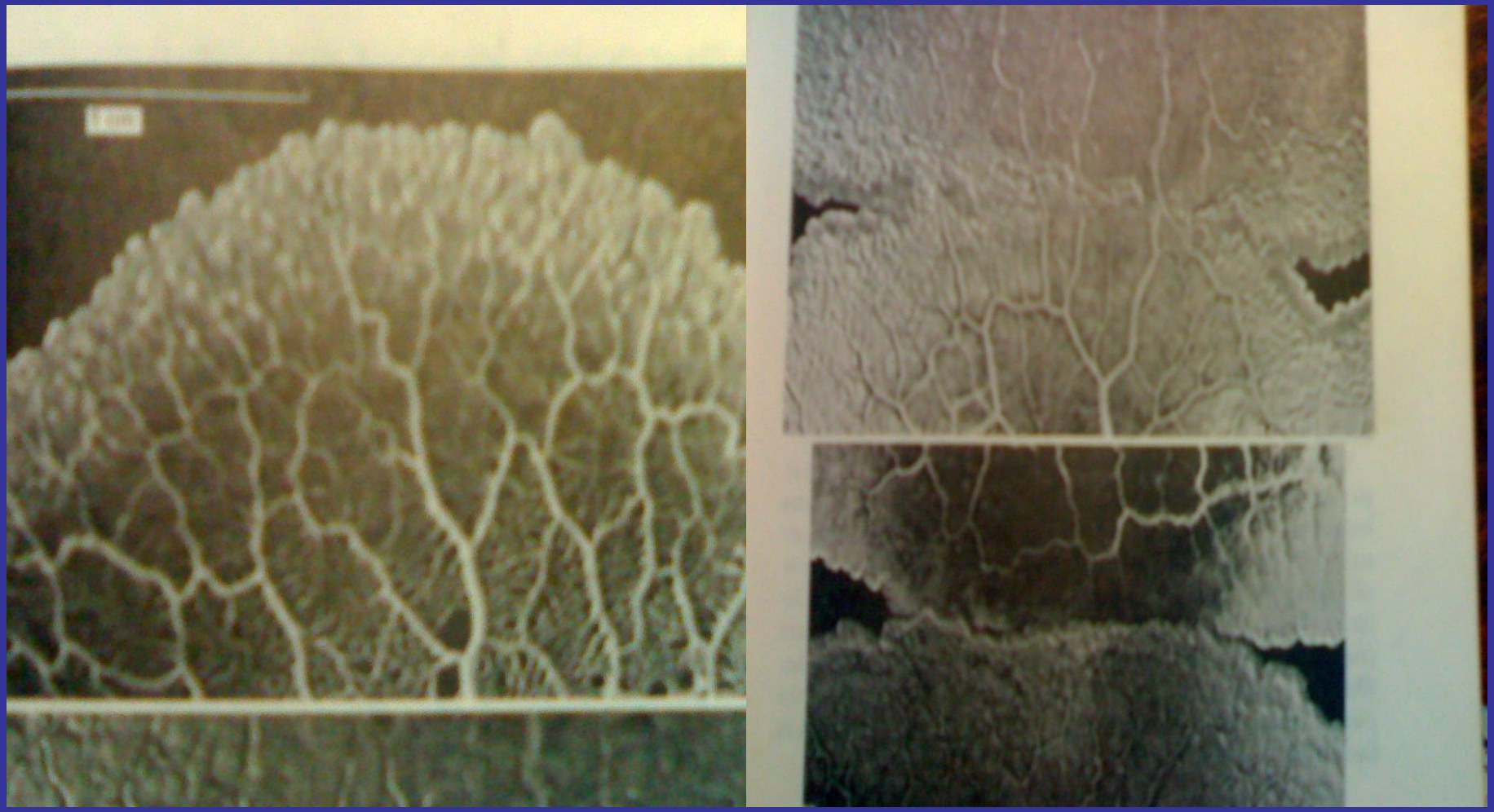
Yeasts and Yeast-like fungi




'Start'
Cell separation
Spindle pole body (SPB) duplication
Initiation of DNA synthesis
Cytokinesis
Bud emergence
SPB separation
Nuclear division
Nuclear migration

2- Plasmodial growth:

The protoplast replicates anywhere in the plasmodium. Different portions show different density and inclusions and thus the replication capacity may remain confined to one or the other part of the plasmodium.



Apical growth :

In filamentous fungi growth is affected at the tip of the hyphae into which new protoplast continuously streams.

Site of cellular extension:

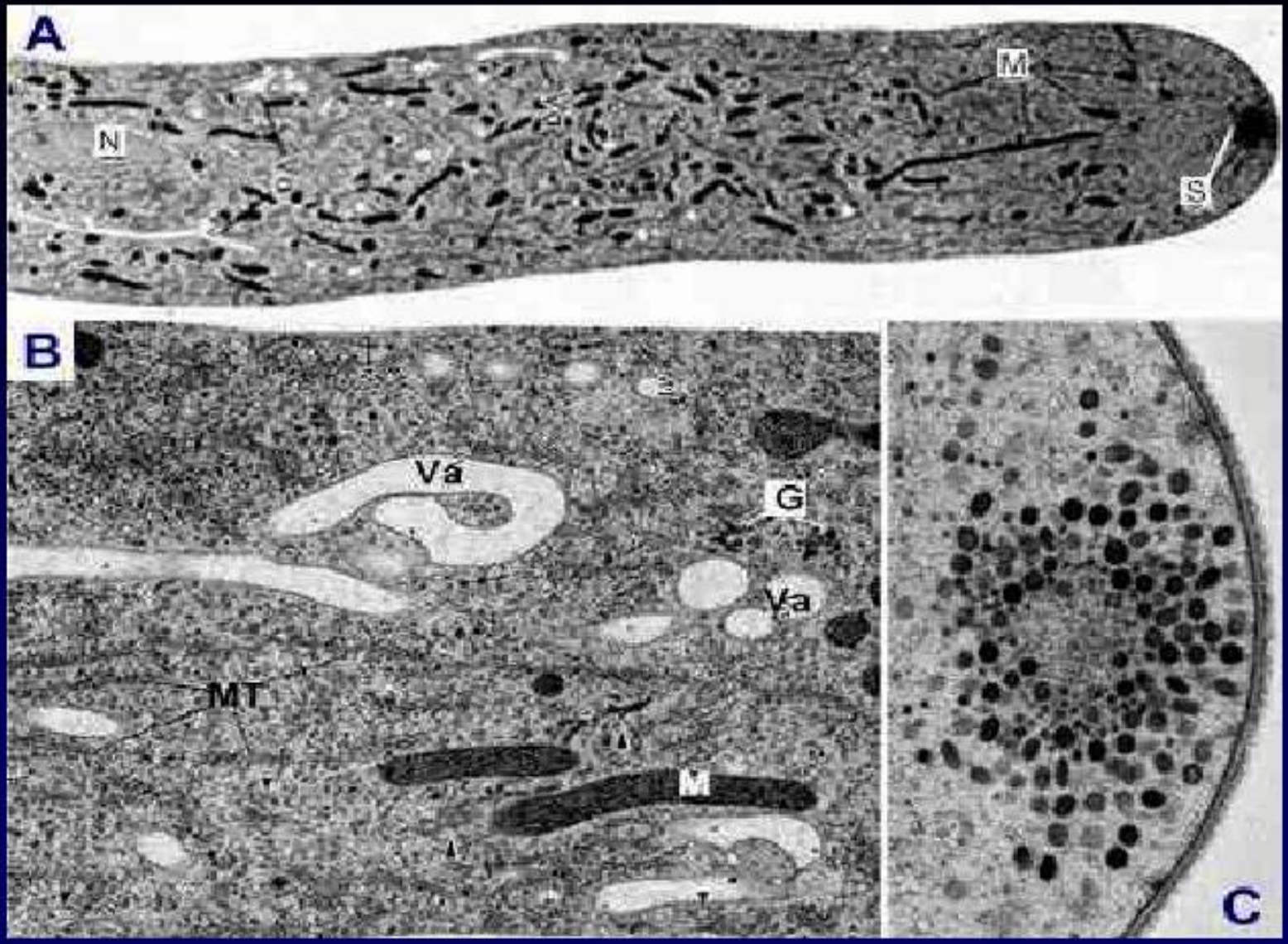
It is restricted to the hyphal tip, comprising 50-100 microns of apical portion

The apical portion has been distinguished into three zones:

i-The extreme tip or apical zone, which is concerned with cell extension and wall synthesis due to incorporation of new materials.

ii- A sub-apical zone, rich in cytoplasm and inclusions, perform other activities concerned with growth, besides translocation of requisite materials to the tip.

iii- The distal portion, which in between sub-apical and the rest of the hypha and it is very much vacuolated to supply


A
N
M
S
B
Va
G
Va
MT
M
C

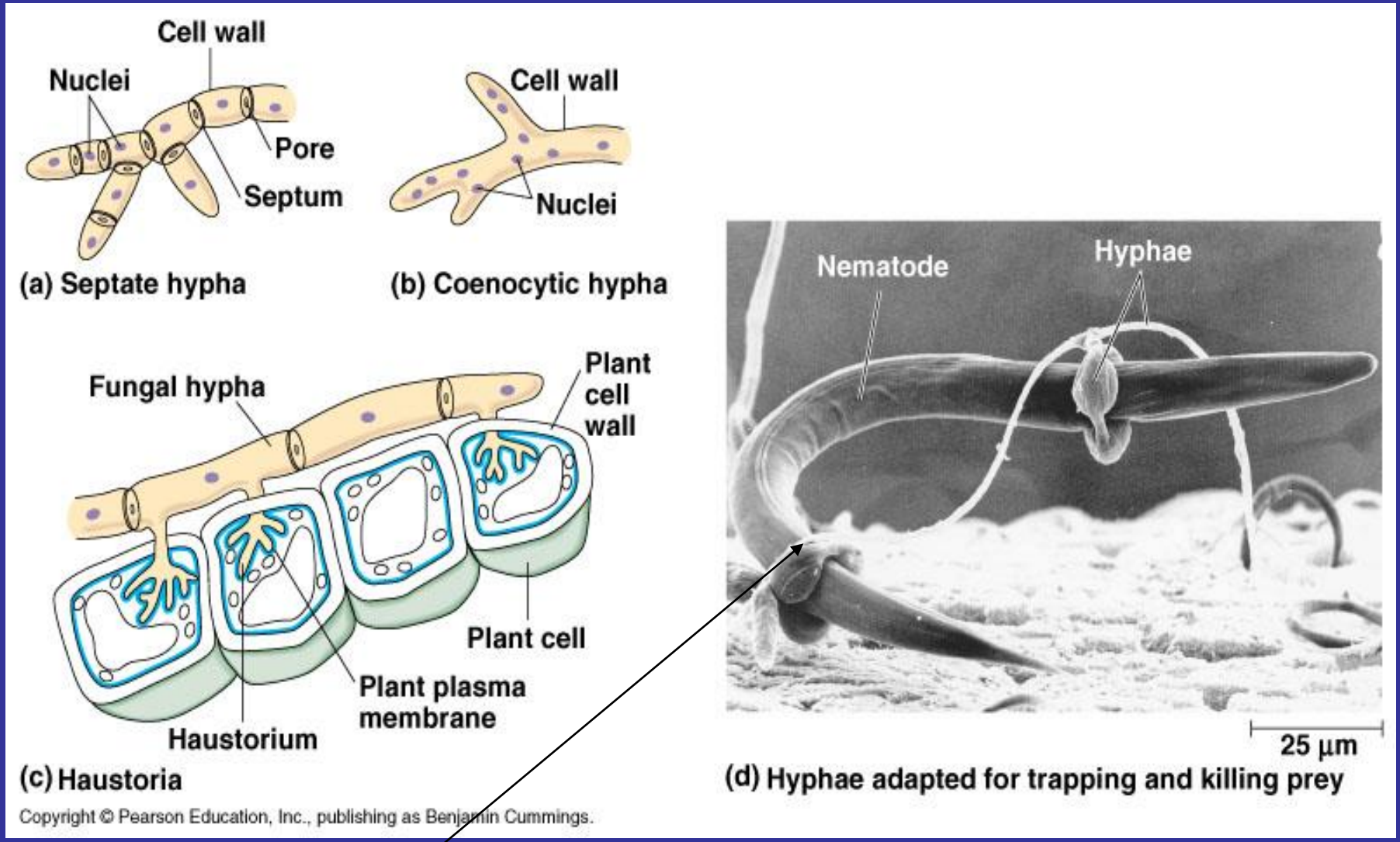
Fungal Growth
Rhizomorphs

- Rhizomorphs = mycelial cords
- These are important components of the disease caused by *Armillaria mellea*!

Fungal Growth

Stromata and Sclerotia

- Stromata (plural), stroma (singular)
  - a mass or matrix of vegetative hyphae
  - a compact, somatic "cushion" on which fruiting bodies are formed
- Sclerotia (plural), sclerotium (singular)
  - are hard resting bodies resistant to unfavorable conditions
  - LONG dormancy periods
  - germinate when favorable conditions return



Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.

Constricting rings