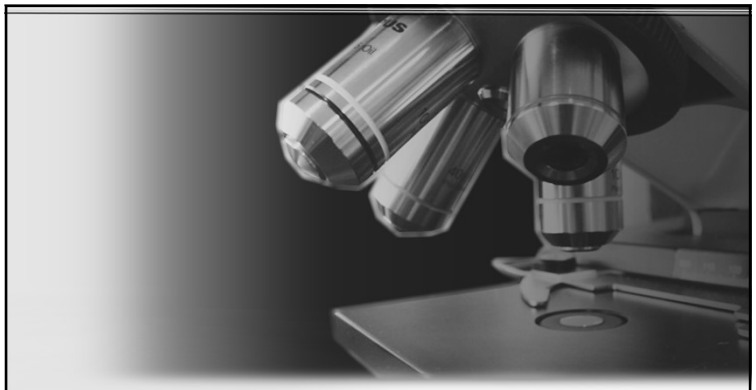


# *General Microbiology*

BIO 3124

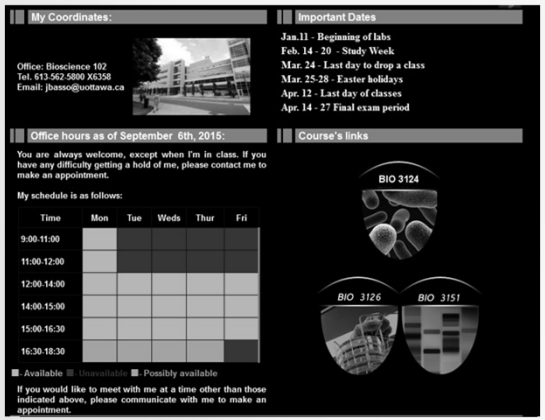
1

## General Information

- Instructor: **John** Basso
- Email: jbasso@uottawa.ca
- Tel. 613-562-5800 Ext. 6358
- Office: BSC102
- My web page :
  http://mysite.science.uottawa.ca/jbasso/home.htm
- Course's web page:
  http://mysite.science.uottawa.ca/jbasso/micro/home.htm

2

## My Web Page




3

## Topics of the Course

- Principals and history of microbiology
- Diversity and classification of microorganisms
  – Anatomy, metabolism, growth
- Control of microbial growth
  – *In vitro* (disinfection & sterilization), *in vivo* (antibiotherapy)
- Virology
  – Anatomy and growth

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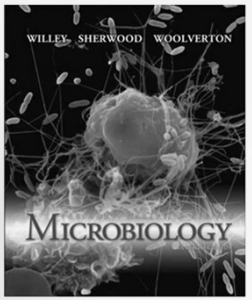
## Topics of the Course *(Cont'd)*

- Principals of immunology
  - Defenses of the human body against infections
- Medical microbiology
  - Disease and diagnostics
- Principals of Epidemiology
  - The spread of infections through populations

5

## Text Book

- There is no textbook required. However, if you would like to obtain a textbook, I would recommend the following which I've made available on the course's web site.

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## Course Evaluation – Midterm Exams

- **3 midterm exams**
  - Each exam will cover the material covered in previous weeks (**non cumulative**) and will contribute towards **15 %** of the final grade
  - These exams will consist of **41 multiple choice questions** given over a 75 minute period
  - Answers are to be submitted on Scantrons
    - 1 point for scantrons which were correctly filled
      - Mandatory information: Name, student number, section
  - Maximum grade: 40/40
    - Ex. 42/40 or 41/40 or 40/40 are all equal to 40/40
  - These exams are closed book

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## Course Evaluation – Final Exam

- This exam is **cumulative**
  - Will contribute towards **50-65%** of the final grade
  - This exam will consist of **91 multiple choice questions** given over a period of 3h
  - Answers are to be submitted on Scantrons
    - 1 point for scantrons which were correctly filled
      - Mandatory information: Name, student number, course code and section
  - Maximum grade: 90/90
  - This exam is open book
    - Any printed material is allowed
    - All electronics, except for calculators, are prohibited

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## Course Evaluation – Problems

- Periodically, 5 series of problems will be made available on the course's web site
- The exact due dates will be announced in class and posted on Blackboard
- You will have one week to complete each series
- Your answers must be submitted on Blackboard
- Total value of **5%** of the final grade

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## Bonus Points on your Final Grade!!

- **8 Quizzes**
  - Quizzes are **optional**
  - Quizzes will be made available on Blackboard every Saturday between 9 am-9 pm
  - You will have 15 minutes to complete the quizzes
  - Each quiz will include 2 questions
  - Persons who obtain **100%** on at least **4** of the quizzes will be attributed **two bonus points**

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## Bonus Points on your Final Grade!!

- **Mystery microorganism**
  - Each week a new clue will be made available on the course's web site
  - Identify the microorganism (genus and species)
  - Indicate the complete name on the first page of your final exam
    - Correct answer = **One bonus point**

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## Calculating the Final Grade

| | Option I | Option II |
|---|---|---|
| **Problems** | **5** | **5** |
| **Midterm exams** | **45[a]** | **30[b]** |
| **Quizzes (≥ 4/8)** | **2** | **2** |
| **Mystery microorganism** | **1** | **1** |
| **Final exam** | **50** | **65** |
| **Maximum final grade** | **103** | **103** |

**a: The grades of the three midterm exams will be used in the calculation of the final grade**

**b: The two best grades out of the three midterm exams will be used in the calculation of the final grade**

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## FAQ

- Do I need to get a textbook?
- I missed an exam, how will my grade be calculated?
- I am one point away from the next letter grade, is there anything I can do?

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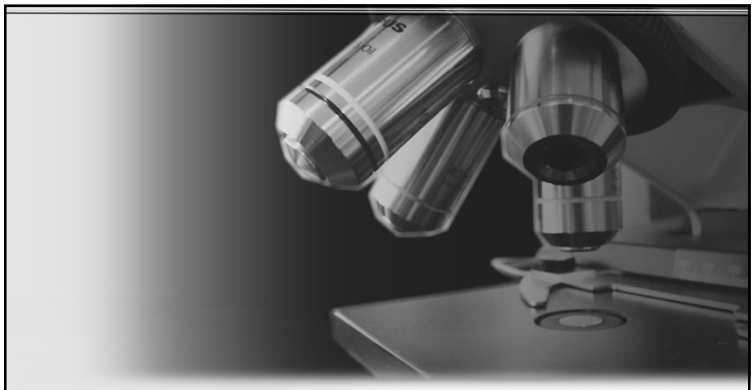

## *Microbiology*

The Study of Microorganisms

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## Definition of a Microorganism

- Derived from the Greek: *Mikros*, «small» and *Organismos*, «organism»
  - Microscopic organism which includes either a single cell (unicellular) or a group of identical cells (non differentiated)
  - Includes bacteria, fungi, *viruses* and protozoans

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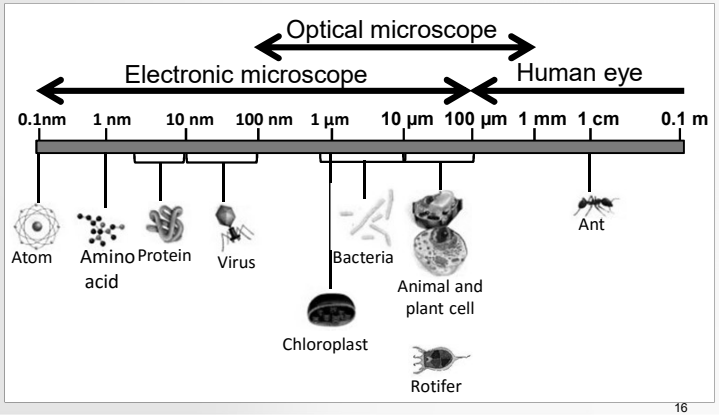
## Micro_?

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## _Organism?

- That is living
  - Has a metabolism
- That can live independently
  - Is not the unit of a multicellular organization
    - Ex. A liver cell is not an organism
    - Exception: Obligate parasite
      - Cannot survive independently, but is an organism!

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## Properties of Life

- Living organisms:
  - Are composed of cells
  - React to their environment
  - Can feed
  - Can obtain and use energy
  - Maintain an internal equilibrium
  - Can grow and reproduce
  - Are subject to evolutionary adaptations

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## Unit of Life – The Cell

- Structural components :
  - Plasma membrane
  - +/- Cell wall
  - Nucleus or nucleoid
  - Cytoplasm
- Chemical components :
  - Proteins
  - Lipids
  - Polysaccharides
  - Nucleic acids

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## Eukaryotic Vs Prokaryotic cells

| **Eukaryote** | **Prokaryote** |
|---|---|
| Nuclear membrane<br>Nucleus | No nuclear membrane<br>Nucleoid |
| More than one DNA molecule | Single DNA molecule |
| Mitotic division | Non-mitotic division<br>Binary fission |
| Organelles | No organelles |

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## React to its Environment

- Organisms interact with their environment and other organisms
- The responses help ensure the survival of the organism and to continue its biological activities
  - Ex. To move from an area lacking nutrients to an area with more nutrients
  - Chemotropism
  - Phototropism

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## Feeding

- Metabolism:
  - Absorption and transformation of chemical compounds
    - Anabolism + Catabolism
      - Anabolism : Macromolecular synthesis
        - » Construction
      - Catabolism : Macromolecular degradation
        - » Destruction
  - Obtain, generate and use energy
  - Elimination of waste products

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## Maintaining an Internal Equilibrium

- The cell attempts to maintain a constant internal environment
  - Ex. pH, solute concentration, osmotic pressure, temperature, *etc*.
- The organism attempts to maintain a constant internal environment
  - Homeostasis

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## Reproduction

- Reproduction:
  - Ability of any organism to generate another organism such as itself
    - Unicellular organisms, such as bacteria, simply divide in two
      - Asexual reproduction: Binary fission
  - Multicellular organisms are often the result of the union of two different cells from different individuals
    - Sexual reproduction; Ex. Sperm + ovum

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## Reproduction – The Genetic Code

- The instructions for the organization and the development of an organism are encoded in the genes
- Genes are composed of DNA
  - The genome
    - Decoding (Transcription + Translation)
      - Generating the cellular machinery
      - Generating new cells
      - Maintain the cell

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## Evolutionary Adaptation

- Organisms acquire changes which are transmitted to future generations allowing them to better respond to their environment
  - Changes at the level of the genome
    - Maintaining these changes depends on selective pressures
      - Ex. Acquiring resistances to antibiotics

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## Organisms and Biological Entities Studied by Microbiologists

- Cellular
  - Fungi
    - Ex Yeast
  - Protists
    - Ex. algae
  - Bacteria
    - Ex. *E. coli*
  - Archaea
    - Ex. Methanogens
- Acellular
  - Virus
    - Composed of nucleic acids and protein
  - Viroids
    - Composed of RNA
  - Prions
    - Composed of protein

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## Fields of Microbiology

- Bacteriology
  - The study of bacteria
- Environmental microbiology
  - The study of microbial processes in the environment
- Food Microbiology
  - The study of pathogenic microorganisms which cause diseases associated with food and the spoilage of food
- Industrial microbiology
  - Use and development of microorganisms in biotechnology
- Medical microbiology
  - The study, diagnosis, and treatment of microbial diseases

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## Fields of Microbiology *(Cont'd)*

- Mycology
  - The study of fungi
- Protozoology
  - The study of protists
- Virology
  - The study of viruses
- Epidemiology
  - The study of the role of microorganism in the health and the diseases of populations
- Immunology
  - The study of the defense mechanisms of the body against viruses, bacteria and fungi

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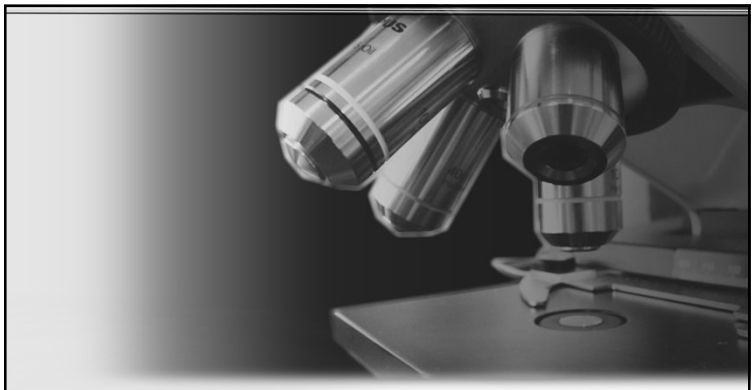

## *History*

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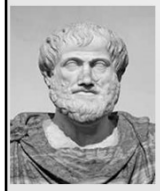
## The origins of Life – The Debate?

- **300 B.C.-** Aristotle
  - Belief that living creatures are created spontaneously from non living matter
  - Life is not required to create life
    - Flooding of fields in the spring results in pools of water that generates frogs
    - Meat left outside rots and generates flies
    - Crops stored under damp conditions rot and generate mice
    - This belief remains unchallenged for over 2000 years



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## Hypothesis on the Origins of Life

- No matter can be created or destroyed
- All that exists is the result of transformations
- Life occurs spontaneously by transforming appropriate ingredients
  - Spontaneous generation

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## 17th Century- Jan Baptista Van Helmont

- Recipe
  - Open container with soiled underwear + wheat
  - After 21 days the smell changes and the ferment from the underwear reacts with the wheat transforming it into adult mice
    - Mice of both sexes are created
    - The adult mice can reproduce themselves

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## Conclusion

- Wheat + ferment from soiled underwear create mice
- Life can be created from inert materials
- Life created spontaneously can also propagate life

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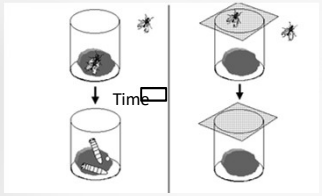
## 17th Century - Francesco Redi

- The first to challenge the theory of spontaneous generation
  - Redi's question: From where do maggots come from?
  - Hypothesis: Maggots come from flies
  - Experiment:

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## 17th Century - A. Van Leeuwenhoek

- The use of a microscope allows him to observe life which is invisible to the naked eye
  - The **animalcules**
- First to observe microorganisms with a microscope
  - First to describe bacteria and protists

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## 18th Century; Controversy: Needham Vs. Spallanzani

- Question: What causes living organisms to appear in a broth?
  - Needham's hypothesis: Spontaneous generation
  - Spallanzani hypothesis : Microbes come from the air. Boiling the broth will kill them

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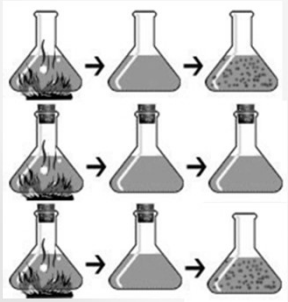
## Controversy : Needham Vs. Spallanzani

- Experiment



Compounds essential for spontaneous generation are destroyed by heat!?

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## 19th Century - Louis Pasteur

- New theory : **Germ theory**
  - Observation
    - Treating milk with heat prevents it from going sour
    - Heat treatments prevents wine fermentation
      - **Pasteurization**
  - Hypothesis
    - Microorganisms in the air fall and grow if they find an appropriate medium such as food

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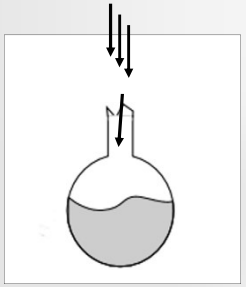
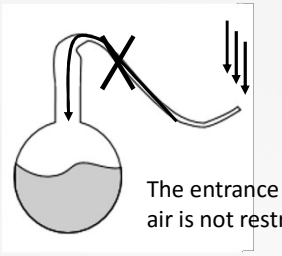
## L. Pasteur - Experimental Method

The microorganisms can reach the medium



The microorganisms cannot reach the medium



The entrance of air is not restricted

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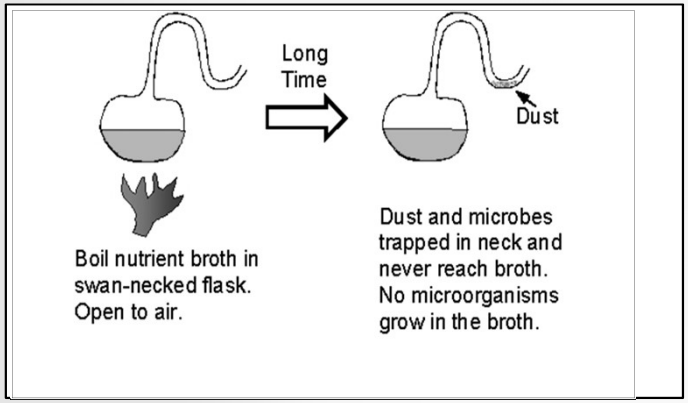
## L. Pasteur - Experimental Method

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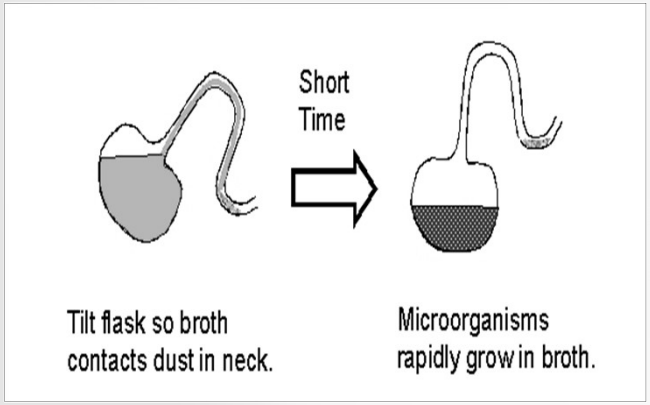
## L. Pasteur –Experimental Method

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## 18 -19th Century - Diseases

- It is generally accepted that there is a link between dirt and disease
- Belief: There are bad seeds in the air called **miasma**
- The miasma that cause disease have a bad smell

## Lister – Father of Antisepsis

- Observation:
  - Notices a high incidence of wound infections following surgeries
    - Proposes that microorganisms in the air are responsible for the infections
- Lister uses carbolic acid, which is used to deodorize sewers, to treat the instruments, wounds, and bandages
  - Observes a large reduction in the incidence of gangrene
    - This eventually leads to sterile surgeries

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## Pasteur – Germ Theory *(Cont'd)*

- If germs can spoil wine and beer, then the same thing can occur in animals and humans
  - Germs are the cause of diseases
- The French silk industry asks Pasteur to find the cause of the high mortality rate of silk worms
  - Pasteur determines that germs are responsible, but never demonstrates that germs cause diseases in humans

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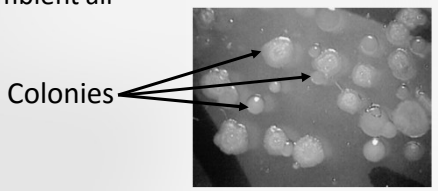
## 19th Century - Robert Koch

- Observation:
  - Heaps of bacteria (**colonies**) of different sizes, colors, and shapes grow on potato slices exposed to ambient air

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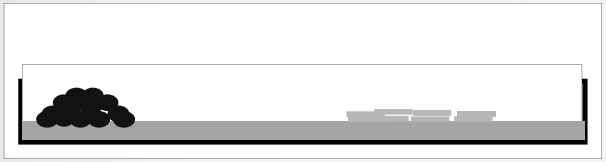

- Conclusion:
  - Colonies are pure cultures arising from single cells of different bacteria since a colony spread repeatedly generates identical colonies

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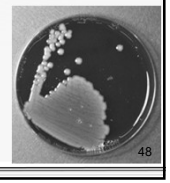
## Koch *(Cont'd)*

- Problem :
  - Several bacteria cannot grow on potatoes!
- Solution :
  - Uses gelatin as a solidifying agent
  - Creates different solid media from liquids such as blood



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## Koch *(Cont'd)*

- Disadvantage of gelatin
  - It is **digested** by several microorganisms
  - It is **liquid** at temperatures above 28°C
- Solution – Agar
  - Polysaccharide derived from an algae
  - Remains solid at temperatures >37°C
  - Melts at 100°C
  - Is not digested by most bacteria

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## 19th Century- Robert Koch

- Studies anthrax which kills livestock
- Grows the bacteria obtained from diseased animals in pure culture
  - *Bacillus anthracis*
- **Observations**:
  - The blood from diseased animals transmits the disease
  - The microorganism can only be found in diseased animals
  - The microorganism grown in the lab transmits the disease to healthy animals

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## Robert Koch

- Demonstrates a direct link between specific germs and a given disease:
  - 1875 – Identifies the germ responsible for **anthrax**
  - 1882 – Discovers the germ responsible for tuberculosis **(TB)**
  - 1883 – Discovers the germ responsible for **cholera**

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## Robert Koch *(cont'd)*

- Conclusion: Microorganisms are responsible for diseases
  - **Pathogens**
- These results lead Robert Koch to formulate a set of directives for the association of a microorganism with a given disease
  - **Koch's postulates**

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## Koch's Postulates

- The microorganism must be present in **all** cases of disease, but absent from healthy individuals
- The suspected microorganism must be isolated and grown as a pure culture
- The disease must occur when the isolated microorganism is inoculated in a healthy individual
- The same microorganism must be isolated again from the diseased host

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## Limitations of Koch's Postulates

- Can these postulates be applied to all microorganisms which cause diseases?
  - Not all microbes can be grown in pure cultures
    - Ex. Viruses
  - Not all microbes can be grown in a lab
  - The same disease can be caused by different microorganisms
  - Some diseases are caused by a combination of microorganisms

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## Limitations of Koch's Postulates *(Cont'd)*

  - Pathogenic microorganisms can be found in healthy individuals
    - Ex. Carrier state
  - Disease (symptoms) may occur after the disappearance of the microorganism
    - Ex. Immune response – autoimmune diseases
  - May not have a compatible host
    - Ex. HIV
  - Ethical dilemma

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## Postulates Modified for Viruses

- The virus must be isolated from the diseased host
- The virus must be grown in cells from the host
- The pathogenic power of the virus can be eliminated by filtration
- The virus must cause a disease with similar symptoms when inoculated in a compatible host
- An immune response against the virus must be induced following the infection

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## Modern Postulates – Molecular Based

- Fredricks and Relman (1996):
  - A nucleic acid sequence belonging to a putative pathogen should be present in **most** cases of an infectious disease **preferentially** in those organs or sites known to be diseased
  - **Fewer, or no**, copies of pathogen-associated sequences should be found in **hosts or tissues** without disease
  - Number of copies of pathogen-associated sequences should decrease with recovery

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## Modern Postulates – Molecular Based

- Sequence detection predates disease, or copy number correlates with severity of disease
- The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms
- These sequence-based evidence for microbial causation should be reproducible

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## 20th Century- Antimicrobial Agents

- Dr. Gerhard Domagk discovers that a stain, Prontosil, is effective against a broad spectrum of bacteria
  - The sulfanilamide portion of Prontosil is responsible for the antimicrobial action


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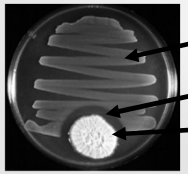
## 20th Century- Antimicrobial Agents *(cont'd)*

- Alexander Fleming discovers a <u>natural</u> product from a fungi that kills bacteria
  - Penicillin





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## Virology – Viruses

- 19th Century
  - Charles Chamberland invents a filter whose pores are smaller than bacteria
  - Demitri Ivanowsky shows that an extract from an infected plant is still infectious after filtration with the Chamberland filter
    - Concludes that the agent is a bacterial toxin
  - Martinus Beijerinck (Father of virology) demonstrates that the Chamberland agent can only replicate in cells
    - Names the agent "contagium vivum fluidum" or living contagion

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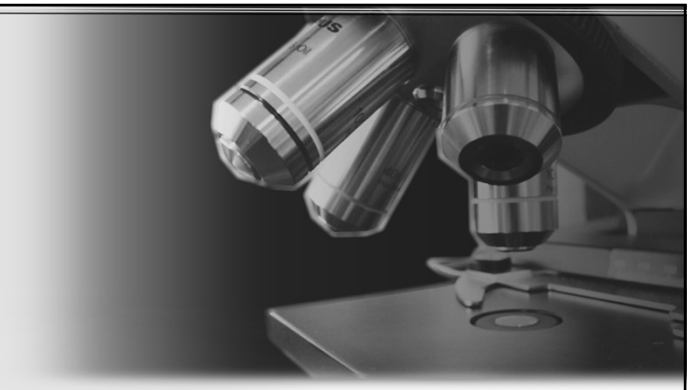
## Immunology

- 1796: A young milk maid informs the physician Edward Jenner that she could not get smallpox because she had already been sick from the cattle disease
- 1796: Edward Jenner inoculates a person with the bovine vaccinia virus
  - The person was protected from smallpox
    - Since the virus is called *Vaccinia,* he names the method - vaccination
      - The protection is referred to as immunization



## *Classification of Organisms*

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## Levels of Classification

- Hierarchical divisions
  - Kingdom (Not used by microbiologists)
    - Microbiologists use the division « **domains** »
  - Phylum
  - Class
  - Order
  - Family
  - Genus
  - Species

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## Domains and Kingdoms

- All organisms originate from a common ancestor the **Progenote**
- Organisms derived from the progenote are grouped in three domains or 6 kingdoms

| Domains | Kingdoms |
|---|---|
| *Archaea* | *Archaeabacteria* |
| *Eubacteria* | *Eubacteria* |
| *Eukarya* | *Animalia* |
| | *Plantae* |
| | *Protista* |
| | *Fungi* |

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## Definition of a Species

- Basic taxonomic unit which represents a specific type of organism
  - In the case of organisms that reproduce sexually the fundamental definition is reproductive compatibility
- This definition cannot be applied to several microbial species (such as bacteria) since they do not reproduce sexually

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## Q. True or False

- All microorganism reproduce asexually?
- All macroorganisms reproduce sexually?

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## Definition of a Species for Microbiologists

- A set of microbial **strains** that share several characteristics and which are significantly different from other sets of strains
- Species are identified by comparisons with known standard reference strains

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## Definition of a Strain

- Population of microbes resulting from a unique individual or a pure culture
  - Different strains represent genetic variations of the **same** species
    - **Biotypes**: Strains with biochemical or physiological differences
    - **Morphotypes**: Strains with morphological differences
    - **Serotypes**: Strains with antigenic differences
    - **Pathotypes:** A disease-causing variant of a microorganism distinguishable from other members of its species by its virulence

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## Nomenclature

- Scientific name – Binomial system
  - Name of genus + name of species
    - The genus name always starts with a capital letter
    - Can be abbreviated
    - The genus name can be used alone
    - The name of the species is never abbreviated
    - The name of the species is never used alone
      - ex: *Bacillus subtilis*
      - *B. subtilis*
      - *Bacillus sp.*
      - *Bacillus*

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## Properties Used for Classification

- Colony morphology
- Cell shape and grouping
- Structure of the cell wall
  - Gram stain
- Specific cell structures
- Biochemical/metabolic characteristics

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## Properties Used for Classification

- Serological testing
  - Uses antiserums specific against a group of microorganisms
    - The antiserum contains proteins (antibodies) which react with antigens on the organism
  - Advantages:
    - Very specific
    - Does not require pure cultures
    - Allows the identification of microorganisms that cannot be grown in the lab

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## Properties Used for Classification

- Molecular properties
  - G + C content
  - Nucleic acid hybridization
  - Nucleic acid sequencing

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## Molecular Properties Used for Classification

- G + C content

$$\text{Mol\%}(G+C) = \frac{G+C}{G+C+A+T} \times 100\%$$

  - Estimate obtained based on the melting point of DNA
  - A higher G + C content results in a higher melting point

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## Molecular Properties Used for Classification

- Nucleic acid hybridization
  - Allows to determine what percentage of single stranded DNA from one species can anneal to the single stranded DNA of a different species to generate double stranded hybrids
  - The higher the percentage the more closely related are the species


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## Molecular Properties Used for Classification

- Nucleic acid sequencing
  - Sequences of genes for specific enzymes
  - Sequences for complete genomes
  - Sequences of 5s and 16s ribosomal RNA genes
    - The comparison of these sequences is commonly used to determine the relationship between different groups of microorganisms

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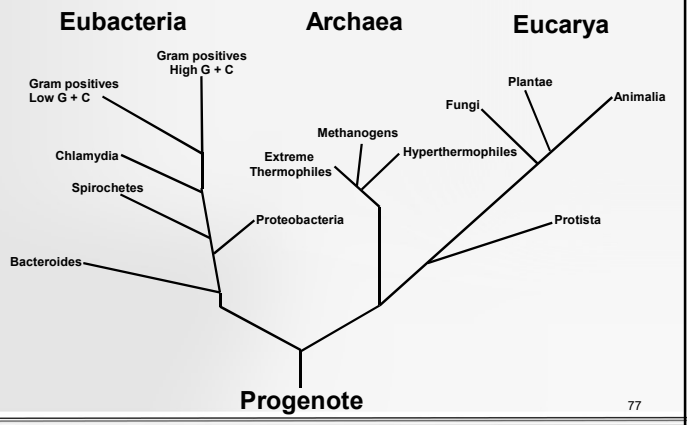
## Three Domain Classification




77

## Domain: *Eubacteria*

- Prokaryote
- The largest group of organisms on Earth
  - Classified according to their…
    - Shape
    - Oxygen requirements
    - Diseases they cause
  - Energy production: photosynthesis or chemosynthesis from organic compounds

78

## The Proteobacteria

- Largest group of bacteria
  - Gram negative
- Have a cell wall made of **peptidoglycan**
- **Have a second membrane that is external to the cell wall**
- Obtain their energy by chemosynthesis from organic compounds or photosynthesis
- Largest group of pathogens

79

## Photosynthetic Bacteria

- Includes Cyanobacteria and some Proteobacteria (green/purple sulfur and green/purple non sulfur bacteria)
- Obtain their energy by photosynthesis
  - Use inorganic electron source
- Use inorganic carbon source

80

## The Bacteroids

- Characteristics similar to Proteobacteria
- Do not tolerate oxygen
- Membrane contains **sphingolipids**
- Mostly **mutualistic**
  - Predominant bacteria in the intestines
- Opportunistic pathogens

81

## Gram Positive Bacteria

- Have a cell wall made of **peptidoglycans**
- **Do not have a membrane external to the cell wall**
- Predominant shapes: Spheres or rods
- Obtain their energy by chemosynthesis from organic compounds
- Several species make spores
- Several pathogenic species

82

## Atypical Bacteria

- Chlamydia
  - **Cell wall is not based on peptidoglycan**
  - **Have second membrane external to the cell wall**
  - Obligate intracellular parasite
  - Cannot generate energy
- Mycoplasms
  - **No cell wall**
  - Undefined shape
  - Obligate intracellular parasite
  - Cannot generate energy

83

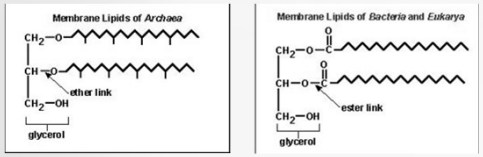
## Atypical Bacteria

- Spirochetes
  - Cork screw shaped
  - Too thin to be observed with a traditional microscope
  - Pathogen of syphilis and lime disease
- Mycobacteria
  - Classified amongst high G+C Gram positives
  - Cell wall with **mycolic acid** which is impermeable to stains
  - Pathogens of Tuberculosis and Leprosy

84

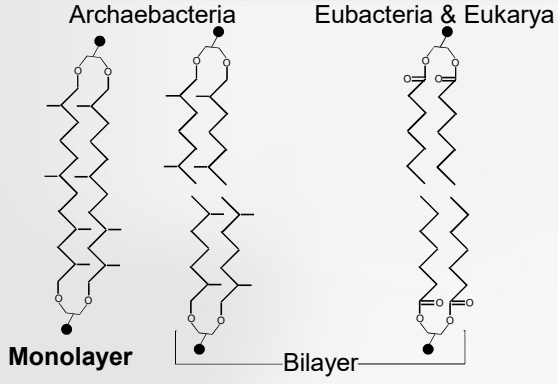
## Domain *Archaea*

- Prokaryotes, but more closely related to eukaryotes
- No peptidoglycan in cell wall
- Cell membrane distinct from both Eubacteria and Eukarya
  - Lipids with **ether** instead of **ester** bonds




85

## Domain *Archaea* - Membranes




86

## Domain *Archaea* - Metabolism

- Mostly extremophiles
  - Live in extreme environments
    - Ex. Acidopihiles, halophiles, thermophiles, psychrophiles
- Most do not require oxygen
- Energy obtained from chemosynthesis using inorganic electron sources
  - No glycolysis

87

## Domain Eukarya: Kingdom Fungi

- Unicellular/multicellular
- Cell wall
- Not organized as tissues
- Energy production: Chemosynthesis from organic compounds
  - Molds, yeast and mushrooms
- Absolute requirement for oxygen

88

## Domain *Eukarya:* Kingdom *Protista*

- Eukaryotic organisms which cannot be classified in any of the other kingdoms
  - Mostly unicellular, some are multicellular
  - Mostly non photosynthetic
  - Mostly motile
  - Absolute oxygen requirement

- Amoeba, green algae, brown algae diatoms, euglena, myxomycetes, ciliated protozoans

89

## Domain *Eukarya:* Kingdom *Plantae*

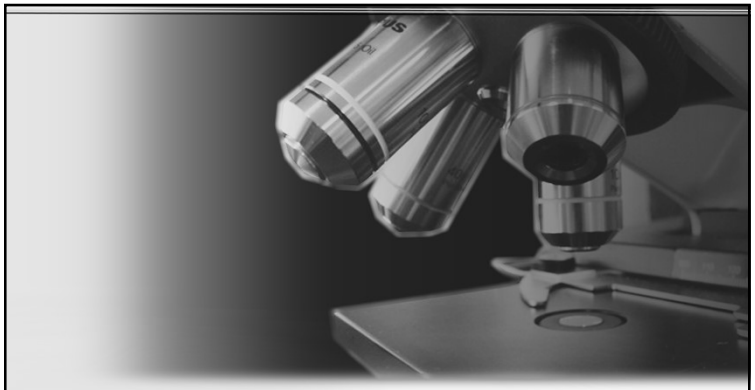
- Multicellular eukaryotic organisms
  - Organized into tissues
  - Perform photosynthesis
  - Cells have cell walls
  - Absolute oxygen requirement
    - Mosses, ferns, conifers, angiosperms, etc.

90

## Domain *Eukarya:* Kingdom *Animalia*

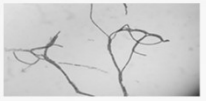
- Multicellular
- **No cell wall**
- Organized into tissues
- Absolute requirement for oxygen
- Energy production: Chemosynthesis
- Obtain their nutrients by **ingestion**
  - Sponges, worms, insects, rotifers, vertebrates

91



## ***Microscopy***

Staining



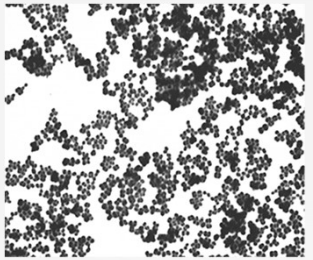
92

## Stains

- Basic : Positively charged
  - Interacts with negative groups
    - Ex. Plasma membrane
- Acid : Negatively charged
  - Interacts with positive groups
    - Ex. Glass

93

## Positive Staining

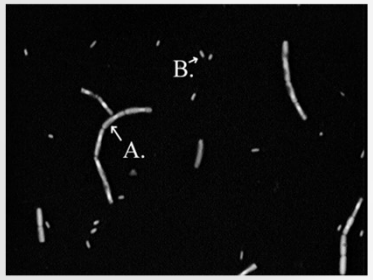


- Staining of the specimen

94

## Negative Staining

- Staining of the background




A. Large rod
B. Small rod

95

## Method

- Simple Staining
  - Single staining agent
  - Basic or acid stain
  - Positive or negative staining
  - Allows to determine the size, shape, and grouping of cells

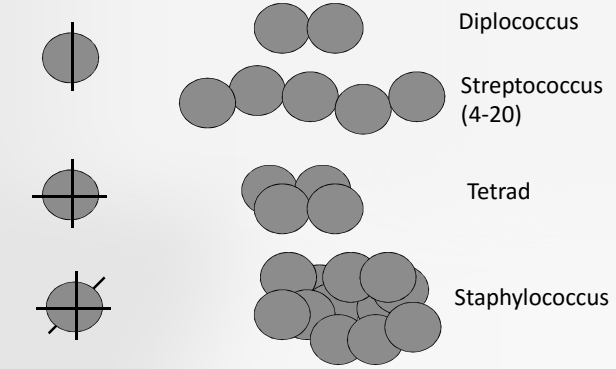
96

## Cell Shapes

- Coccus: 
  - Spheres
  - Division along 1,2 or 3 axes
  - Number of axes along which division occurs gives rise to different groupings
  - Typical groupings according to bacterial genus

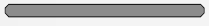
97

## Cocci (Coccus)



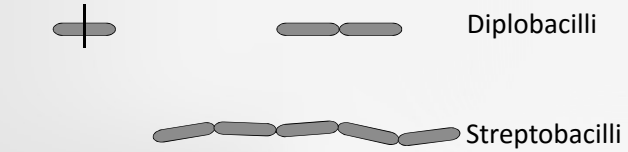

98

## Cell Shapes *(cont'd)*

- Rods : 
  - Division is only along one axis
  - Typical groupings according to bacterial genus

99

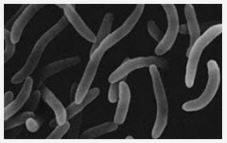
## Rods




100

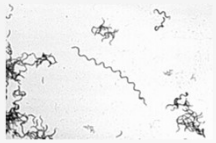
## Other Cell Shapes

Curved rods



Typical of *Vibrio*

Spirals

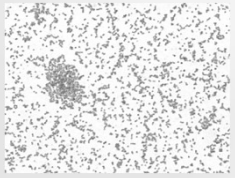


Typical of Spirochetes

101

## Differential Staining - Gram Staining

- Divides bacteria into two groups
- Gram Negatives & Gram Positives



102

## Gram Positive

- Colored purple
  - Low G + C
    - Rod or bacillus
      - Sporulating: Genera *Bacillus* and *Clostridium*
      - Non sporulating: *Lactobacillus and Listeria*
    - Coccus or sphere
      - Genera *Streptococcus*, *Staphylococcus* and *Micrococcus*
  - High G + C
    - Rod or bacillus
      - Genus *Mycobacterium*

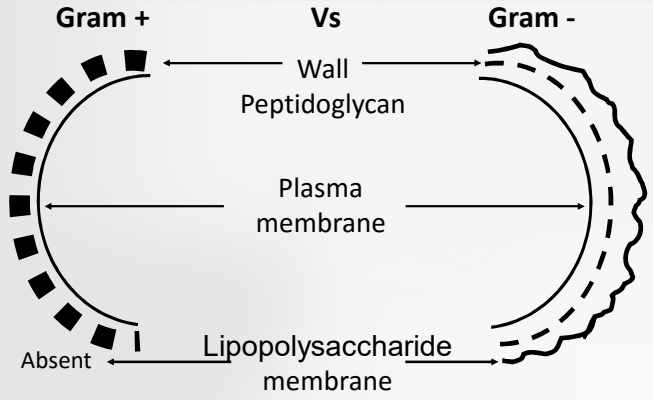
103

## Gram Negative

- Colored red
  - Proteobacteria, Bacteroids, Chlamydia, Spirochetes, Cyanobacteria, green/purple sulfur bacteria, etc.
  - Mostly rods
  - Some genera are cocci:
    - Genera: *Neisseria*, *Moraxella*, & *Acinetobacter*

104

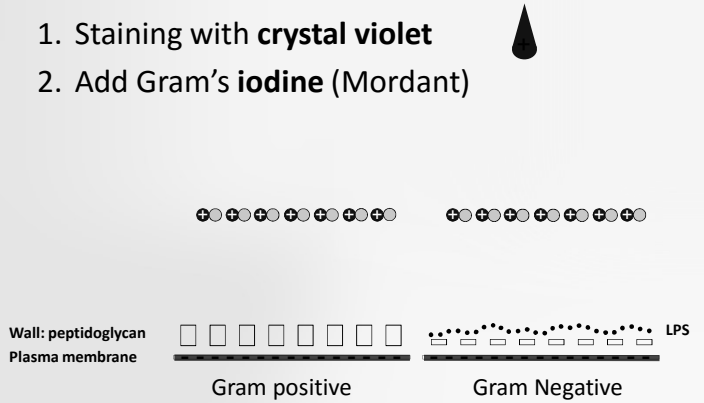
## Cell Wall




105

## Method – Primary Stain

1. Staining with **crystal violet**
2. Add Gram's **iodine** (Mordant)



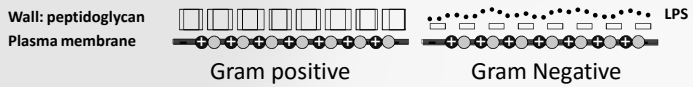

106

## Method – Differential Step

3. Alcohol wash

Wall is dehydrated
– Stain + iodine complex is trapped

Wall is not dehydrated
– Complex is not trapped




107

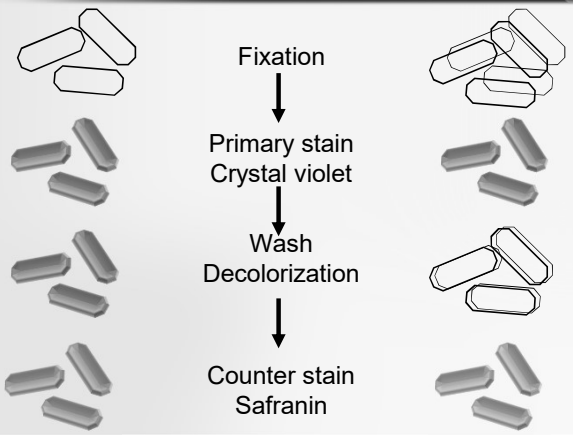
## Method – Counter Stain

4. Staining with **Safranin**




108

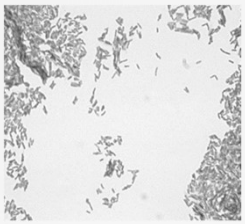
## Summary




109

## Acid Fast Staining

- Diagnostic staining of Mycobacteria
  - Pathogens of Tuberculosis and leprosy
  - Cell wall with mycolic acid



110

## Method

- Principal:
  - High content of compounds similar to waxes, **mycolic acid**, in the cell wall, make these bacteria highly impermeable to stains

111

## Method *(cont'd)*

- Permeabilization of cell wall with heat
- Staining with basic fuschin
  - Cooling of the cell wall returns it to its impermeable state
    - Stain is trapped
- Acid alcohol wash
  - Differential step
    - **Mycobacteria** retain stain
    - Other bacteria lose stain

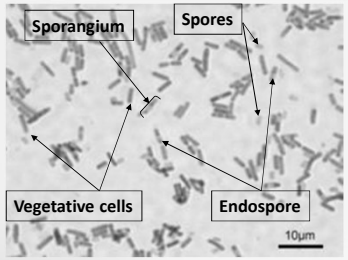
112

## Spore Staining

- Spores:
  - Differentiated bacterial cell
  - Resistant to heat, dehydration, ultraviolet, and different chemical treatments
  - Typical of Gram positive rods
    - Genera ***Bacillus*** and ***Clostridium***
  - Unfavorable conditions induce **sporogenesis**
    - Differentiation of the vegetative cell into an endospore

113

## Malachite Green Staining

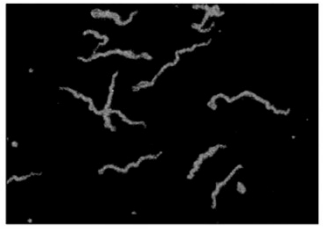



- Permeabilization of spores with heat
- Primary staining with **malachite green**
- Wash
- Counter staining with **safranin**

114

## Fluorescent Staining

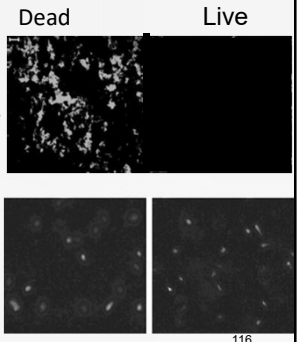
- Uses UV light
- Fluorescent compound absorbs UV light and emits visible light
  - Fluorescent stains
    - Vital stains
    - Metabolic stains
  - Conjugated antibodies
    - Immunofluorescence



115

## Fluorescent Stains– Vital Staining

- Combination of 2 stains
  - SYTOX green
    - Green stain
    - Stains DNA
    - Stains only dead cells
      - Live cells – membrane is impermeable to the stain
      - Dead cells – membranes are damaged and therefore permeable to the stain
  - DAPI
    - Blue stain
    - Stains DNA
    - Stains live and dead cells



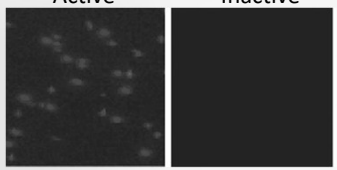

116

## Fluorescent Stains – Metabolic Stainning

- CTC
  - Only stains cells which perform respiration
    - The stain is reduced by succinate dehydrogenase to a red fluorescent product

Metabolism

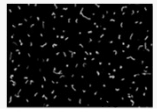
Active Inactive

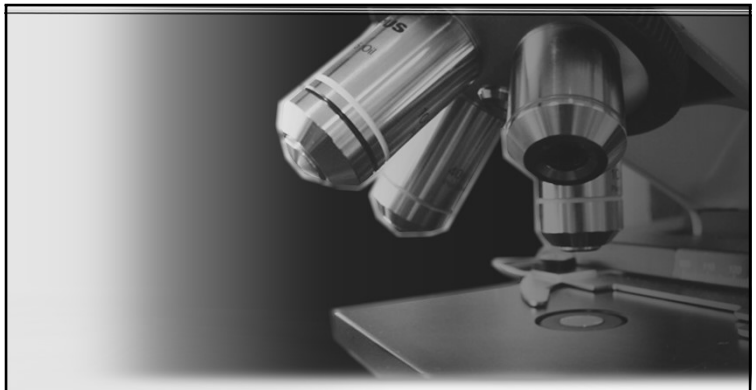


117

## Immunofluorescence

- Makes use of an antibody that recognizes a specific characteristic at the surface of the microorganism
- The antibody is conjugated to a fluorochrome
- The fluorochrome emits visible light when it is excited by UV
- Allows the discrimination of different bacteria





## *Bacterial Anatomy*

119

## Dimensions

- 1-4μm
  - High ratio of surface area relative to volume
    - Favors diffusion and absorption
      - Nutrient uptake and waste elimination
  - Rapid growth
  - High cell density

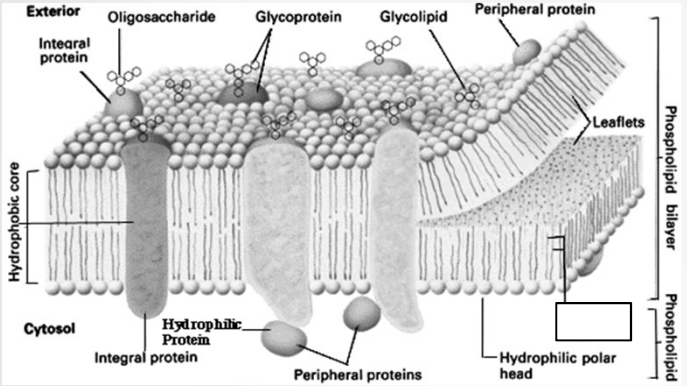
120

## Plasma Membrane

- Properties and functions
  - Surrounds the cell
    - Approx. 8nm thick
  - Separates exterior from interior
  - Selective barrier
  - Allows the concentration of some compounds
  - Allows the excretion of waste products
  - Site of several metabolic processes
    - ex. Respiration
  - Site of ATP generation

121

## The Lipid Bilayer




122

## Plasma Membrane

- Permeability Barrier
  - Maintains the intracellular environment:
    - **Hypertonic**
  - Problem:
    - How does the cell obtain nutrients?

123

## Permeability of the Membrane

| Substance | Rate of uptake |
|---|---|
| • Water | 100 |
| • Glycerol | 0.1 |
| • Tryptophan | 0.001 |
| • Glucose | 0.001 |
| • Chloride ions | 0.000001 |
| • Sodium ions | 0.0000001 |

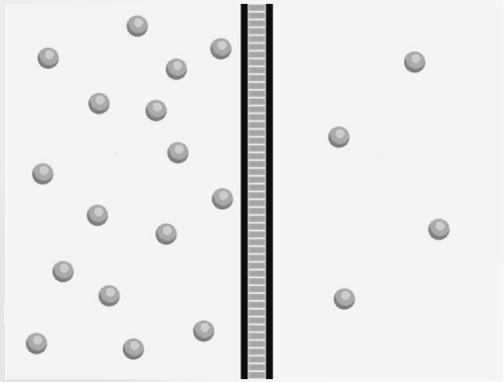
124

## Passive Passage

- Passage of molecules through the membrane without an investment of energy
- Rate of passage is a function of the concentration gradient
- Can not operate against a concentration gradient
- Can not create a concentration gradient
- Two types:
  - Passive diffusion
    - Transporter independent
      - Dependent on membrane permeability
  - Facilitated diffusion
    - Transporter dependent
      - Independent of membrane permeability

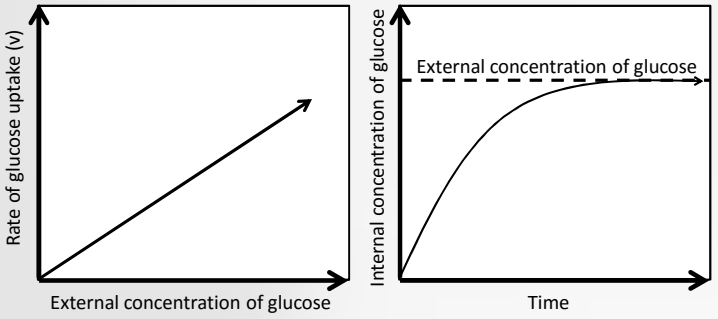
125

## Passive Diffusion



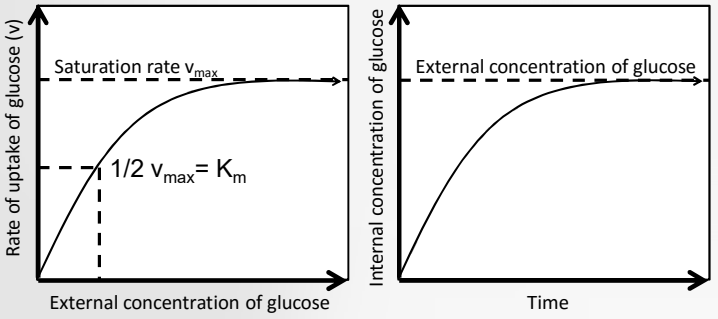
126

## Kinetics of Passive Diffusion




127

## Kinetics of Facilitated Diffusion




$K_m$: Represents the affinity of transporter for the substrate
* The lower the Km the higher the affinity

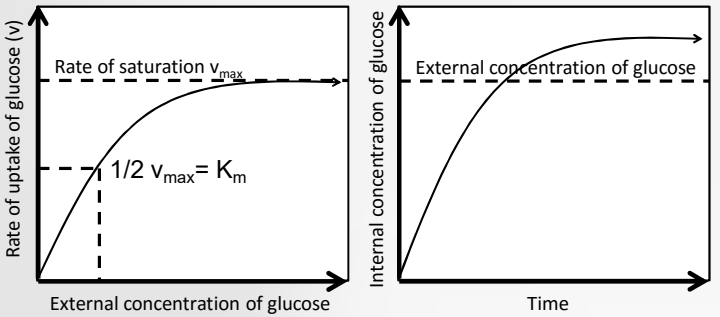
128

## Transport - Active

- Passage of molecules which is dependent on an energy investment
  - ATP or proton gradient
- Passage dependent on a transporter
- Rate of passage is not a function of a concentration gradient
- Can operate against a concentration gradient
- Can create a concentration gradient

129

## Kinetics of Active transport

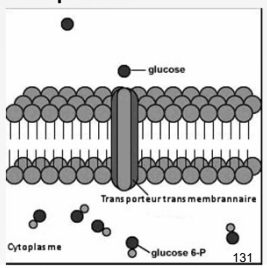



$K_m$: Represents the affinity of transporter for the substrate
* The lower the Km the higher the affinity

130

## Transport – Group Translocation

- Passage <u>and</u> conversion of molecules as they pass through the membrane with an investment of energy
- Passage is dependent on a transporter
- Independent of a gradient

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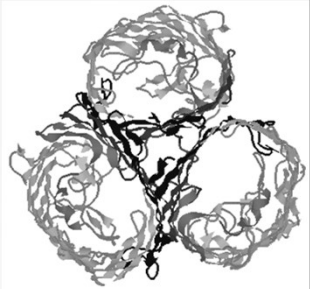
## The Transporters

- Amphipathic proteins
- Transmembrane proteins (Integral)
  - Porins
  - Uniporter
  - Symporter
  - Antiporter
- Used for the transport…
  - Facilitated
  - Active
  - Group translocation

132

## Porins

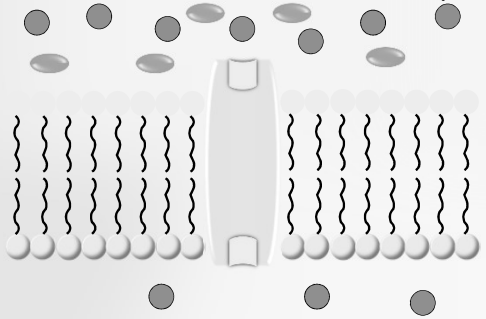
- Structures in the external membrane of Gram-negative bacteria
- Facilitated diffusion of low molecular weight compounds
- Channels for small hydrophilic molecules
- Protein trimers
- Semi-selective
  - Size
  - Ionic properties



133

## UNIporters

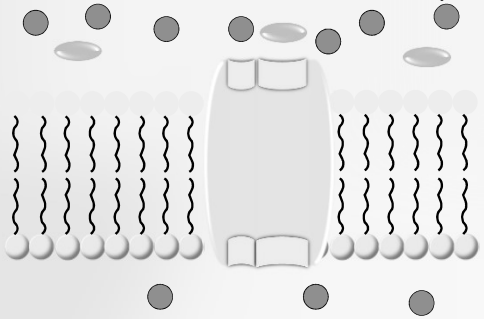
- Selective
- Used for facilitated diffusion or active transport



134

## SYMporters

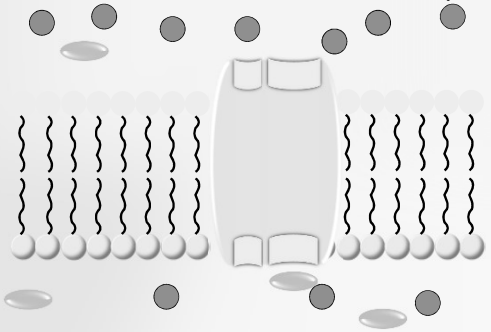
- Selective
- Used for facilitated diffusion or active transport



135

## ANTIporters

- Selective
- Used for facilitated diffusion or active transport



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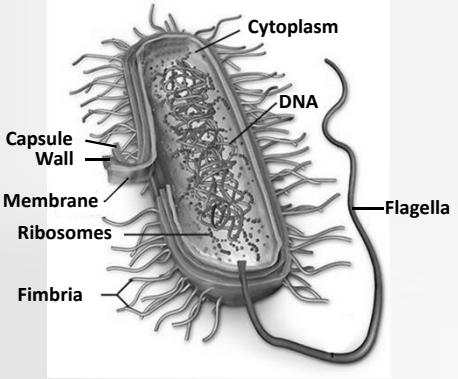
## Summary

| Properties | P. D. | F. D. | Active T. | Transloc. |
|---|---|---|---|---|
| Transporter | - | + | + | + |
| Works against a gradient | No | No | Yes | Not applicable |
| Specificity | No | Yes | Yes | Yes |
| Energy expense | No | No | Yes | Yes |
| As a function of permeability | Yes | No | No | No |
| Transformation | No | No | No | Yes |

137

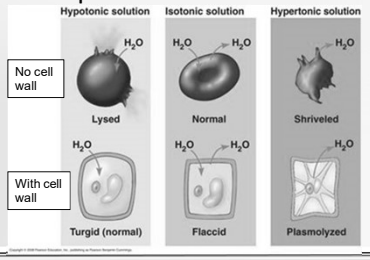
## Structures External to the Membrane




138

## Cell Wall

- Functions:
  - Resist osmotic pressure caused by the entry of water
    - Osmosis (Passive diffusion of water)
  - Defines cell shape

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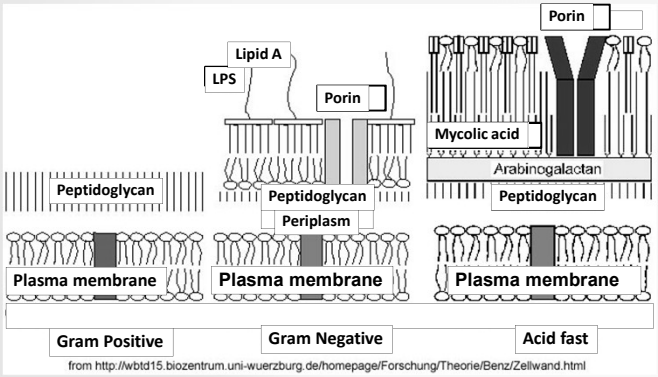
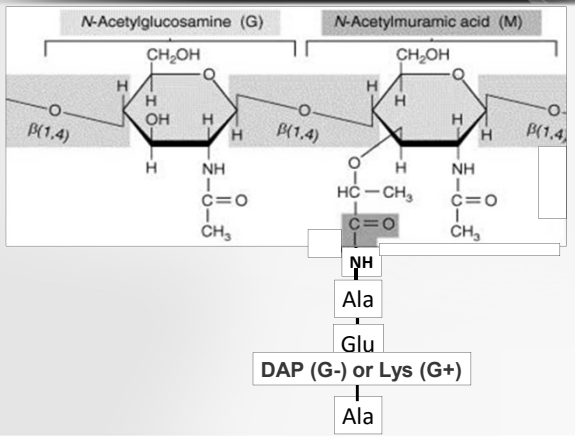
## Summary of Cell Walls




from http://wbtd15.biozentrum.uni-wuerzburg.de/homepage/Forschung/Theorie/Benz/Zellwand.html

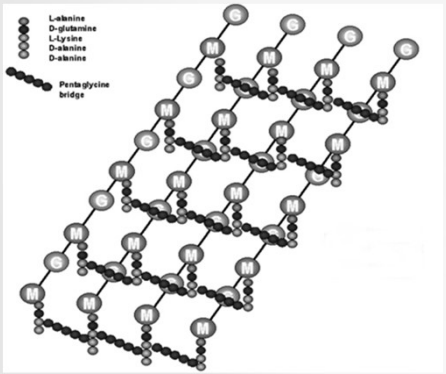
140

## Wall – Units of Peptidoglycan




141

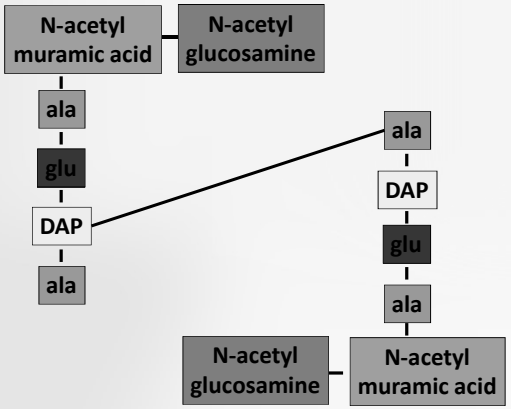
## Wall- Multiple Layers of Peptidoglycan Polymers




- Gram negative
  - 1-2 layers
- Gram positive
  - 10-30 layers

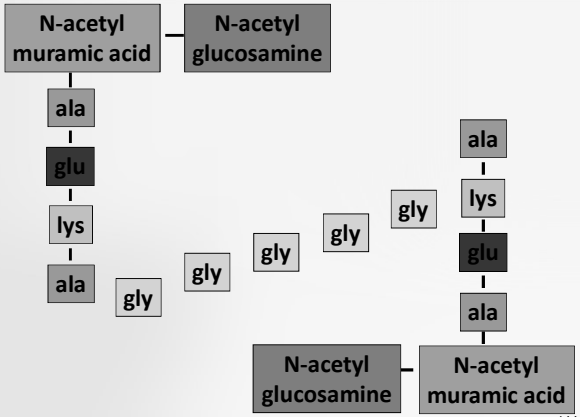
142

## Bridges Between Peptidoglycan Polymers of Gram -




143

## Bridges Between Peptidoglycan Polymers of Gram +

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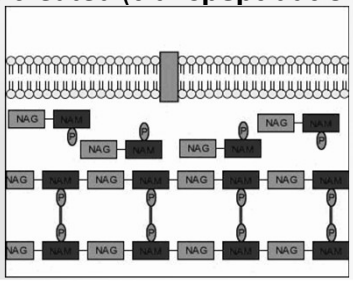
## Assembly of the Cell Wall

- Cleavage by **autolysin**
- Preformed subunits are added
- Bridges are created (**transpeptidation**)

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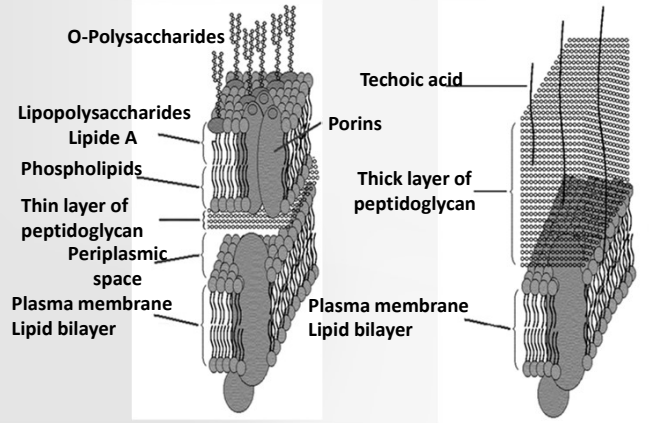
## Walls of Gram – and + Bacteria

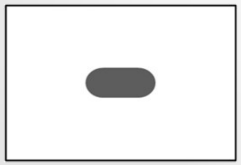



146

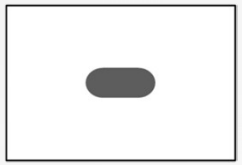
## Compounds which Act on the Wall

Beta-Lactams: Inhibit transpeptidation

Growth without penicillin



Growth with penicillin



ß-lactams only act on actively growing bacteria

147

## Compounds which Act on the Wall

- Lysozyme
  - Cleaves ß-1-4 linkages between N-glucosamine and acetyl-muramic acid
  - Mode of action similar to autolysins
  - Acts on growing or non growing bacteria

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## LPS Layer

- Characteristics :
  - External membrane only in Gram negative bacteria
  - Lipopolysaccharides implicated in the pathogenic potential
    - Lipid A
  - Impermeable to large proteins, polysaccharides and H+

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## Glycocalyx

- Polysaccharide or polypeptide layer surrounding the cell
  - Also called **Extracellular polysaccharide (EPS)**
- Synthesized inside the cell and then excreted
- Two types:
  1. Mucoid layer
     - Poorly organized and attached
  2. Capsule
     - Highly organized and firmly attached
- Functions:
  - Protects against dehydration
  - Protects against phagocytosis
  - Allows adhesion
  - Resistance to the environment

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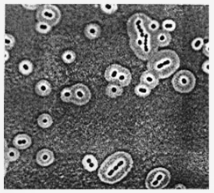
## Glycocalyx - Capsule

- EPS firmly attached to cell wall
- Allows adhesion to surfaces
- Protection against phagocytosis
  - Virulence factor



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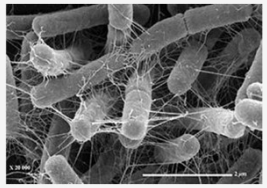
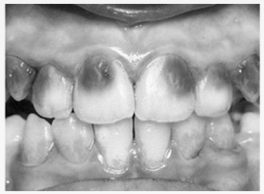
## Glycocalyx – Mucoid Layer

- EPS loosely attached to cell wall
- Scaffold of **biofilms**
- Allows adhesion to surfaces
- Protection against phagocytosis
  - Virulence factor
  - Protection against environmental conditions
    - Dehydration
    - Antibiotics





152

## Fimbriae

- Thin hollow fibers
- Composed of protein subunits
- Allows adhesion
- Associated to pathogenic power

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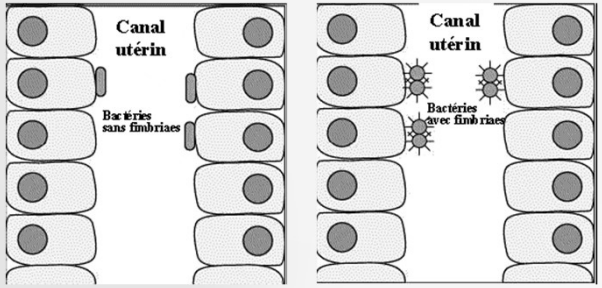
## Pathogenic Power of Fimbriae

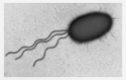



154

## Bacterial Motility

- Sliding
  - Small rotating protein particles (ball bearings) or secretion of surfactants

- Swimming
  - Flagella
    - Long rigid appendages composed of a single polymer of a single protein; la flagellin



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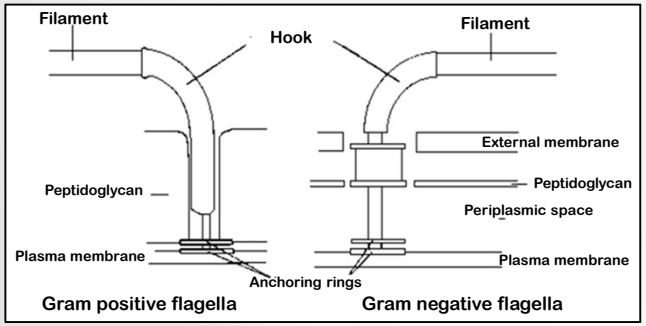
## Structure of Flagella

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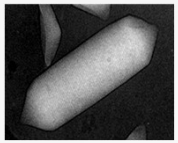
## Bacterial Motility *(cont'd)*

- Gas vesicles – Floatation apparatus
  - Small hollow rigid cylinders composed of two proteins
  - Impermeable to water
  - Permeable to atmospheric gases
  - Found in aquatic bacteria
  - ex. Cyanobacteria



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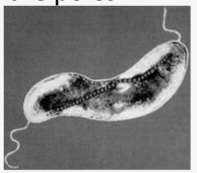
## Bacterial Motility *(cont'd)*

- Magnetosomes - magnetotactic bacteria
  - Chains of magnetite particles
    - $Fe_3O_4$
  - Each particle represents a miniature magnet
  - Allows to orient themselves towards the poles



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## *Anatomy of Eukaryotic Cells*

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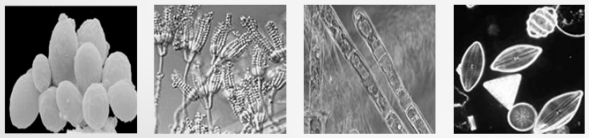
## Cell Wall

- Present in several eukaryotic microorganisms

Yeast Fungi Algae Diatoms



- Present in some macroorganisms
- Composed of various polysaccharides, such as cellulose, chitin and glucans

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## Plasma Membrane

- Same architecture as that of prokaryotes
  - Lipid bilayer
- Problem - smaller surface area to volume ratio
  - Less efficient passage
  - Solution - Contains **sterols**
    - **Cholesterol**
    - Increases fluidity and permeability to non polar compounds

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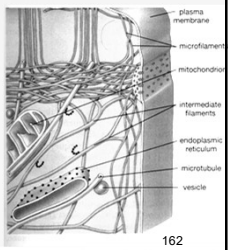
## Cytoskeleton

- Internal protein network of microfilaments, intermediate filaments and microtubules
  - Confers cell shape
  - Allows the creation of compartment
  - Used for internal transport
  - Allows motility

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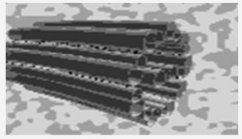
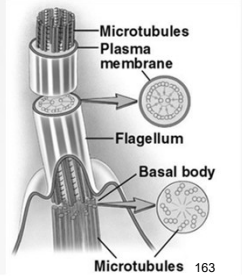
## Eucaryotic Motility

- Flagella and cilia
  - Flexible cylinders
  - Composed of tubulin
- Extension of the cytoskeleton
- Covered by the plasma membrane



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## Eucaryotic Organelles

- Architecture:
  - Compartments enveloped by lipid bilayers

| | |
|---|---|
| Mitochondria/Chloroplast | ATP synthesis |
| Nucleus | Genome |
| Golgi | Transport-Export |
| Endoplasmic Reticulum | Protein synthesis |
| Lysosome | Digestion |

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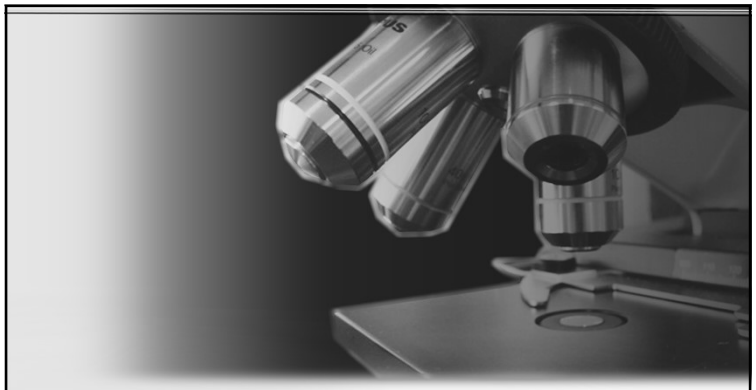

## *Nutrition*

Macronutrients required for biosynthesis:
C,H,N,O,P,S

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## Carbon

- Required for the synthesis of all organics
  - Carbohydrates
  - Lipids
  - Proteins
  - Nucleic acids
- Sources
  - Organic
    - Monosaccharides, disaccharides, polysaccharides, proteins, lipids, nucleic acids, phenols, etc.
  - Inorganic
    - $CO_2$ and CO

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## Phosphorous

- Required for the synthesis of:
  - Nucleic acids
  - Phospholipids
  - ATP
- Sources:
  - Organic and inorganic
    - The inorganic form is the most used

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## Nitrogen

- Required for the synthesis of:
  - Amino acids
  - Nucleic acids
  - Peptidoglycan
- Sources:
  - Organic: amino acids
  - Inorganic: $NH_3$, $NO_3$ & $N_2$

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## Sulfur

- Required for the synthesis of:
  - Amino acids (Cysteine/Methionine)
  - Vitamins (thiamine and biotin)
- Sources:
  - Organic: amino acids
    - Cysteine and methionine
  - Inorganic:
    - S, $SO_4$

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## Hydrogen and Oxygen

- Required for the synthesis of all organics!
  - Carbohydrates
  - Lipids
  - Proteins
  - Nucleic acids
- Sources:
  - Organic:
    - Any organic compound
  - Inorganic:
    - $H_2$ (Methanogens only)
    - $H_2O$ (Mainly autotrophs)

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## Nutritional Classification

- Carbon source
  - Heterotrophs :
    - Preformed organic molecules
  - Autotrophs:
    - Inorganic molecules
      - $CO_2$ and CO

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## Nutritional Classification *(cont'd)*

- Source of energy
  - Phototrophs:
    - Light
  - Chemotrophs:
    - Oxidation of either organic or inorganic compounds
- Source of e-
  - Organotrophs:
    - Reduced organic molecules
  - Lithotrophs:
    - Reduced inorganic molecules

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## Nutritional Types

- Nomenclature:
  - Source of **C**arbon-**E**nergy-**E**lectrons
    - Ex. Autotroph photolithotroph
    - Heterotroph photoorganotroph
    - Autotroph chemolithotroph
    - Heterotroph chemoorganotroph

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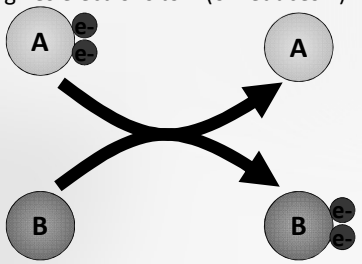
## Nutrient Requirements

- Prototrophs vs. Auxotrophs
  - Prototroph
    - A species or a strain of a microbe capable of growing on a minimal medium consisting of an organic or inorganic carbon source, with inorganic sources of all other nutrient requirements
  - Auxotroph
    - A species or genetic strain requiring one or more organic nutrients (such as amino acids, nucleotide bases, vitamins, etc.) for growth

## Producing Energy

- Obtaining energy depends on oxidoreduction reactions

A gives electrons to B (or reduces B)




B accepts electrons from A (or oxidizes A)

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## Reduction Potential or Redox Potential

- Measure of the tendency (willingness) of a chemical species to accept electrons and thus be reduced.
  - Unit of measure : $E^o$ (volts)
    - The more negative $E^o$ is the lower the redox potential
      - Greater tendency to give rather than accept electrons
    - The more positive $E^o$ is the greater the redox potential
      - Greater tendency to accept rather than give electrons

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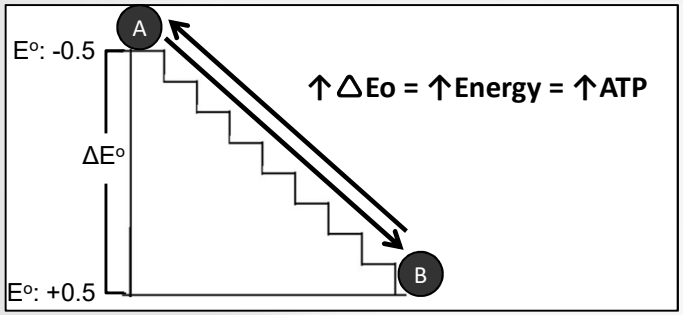
## Redox Vs Energy




**AH + B → A + BH : ΔEº= -1.0 ; Energy output**

**A + BH → AH + B : ΔEº= +1.0 ; Energy input**

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## Producing Energy *(cont'd)*

- Oxidative-Respiration
  - Aerobic
    - $O_2$ used as a final e- acceptor
  - Anaerobic
    - Inorganic final e- acceptor other than $O_2$ is used
- Fermentation
  - Organic final e- acceptor is used

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## Microbial Energy Metabolism

- Glycolytic pathways
- Respiration
- Fermentation
- Chemolithotrophy
- Photosynthesis

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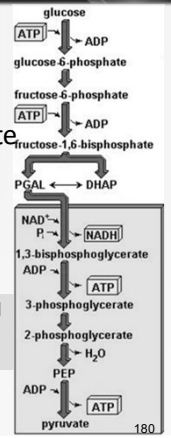
## Glycolytic Pathways

- Glycolysis:
  - Most common glycolytic pathway
  - Partial oxidation of glucose to pyruvate
  - Net production of 2 ATP
  - 2 NAD are reduced to NADH




Each of these steps is carried out **twice** for each glucose molecule

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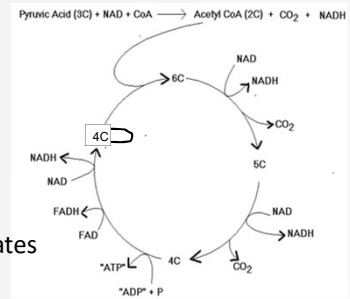
## Respiration

- Features
  - Pyruvate is completely oxidized to $CO_2$
  - NADH is oxidized to NAD
    - Essential for continued operation of glycolytic pathways
  - Uses an inorganic electron acceptor
    - Aerobic respiration: $O_2$ is the final e- acceptor
    - Anaerobic respiration: An inorganic substance other than $O_2$ is the final e- acceptor
      - Ex. nitrate, nitrite, sulfate
  - Additional ATP are made

## Respiration - Krebs Cycle

- Summary/1 pyruvate molecule:
  - Energy equivalents
    - 1 ATP
  - Reducing equivalents
    - 4 NADH
    - 1 FADH
  - One carbon compounds
    - 3 $CO_2$
  - 4C Biosynthetic intermediates
    - α-ketogluterate
    - Succinate
    - Oxaloacetate

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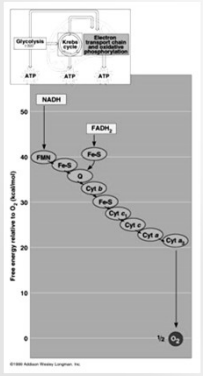
## Respiration – Electron Transport




- Aerobic respiration :
  - Final e- acceptor: $O_2$
  - 3 ATP/NADH
  - 2 ATP/FADH
- Anaerobic respiration :
  - Final e- acceptor other than $O_2$:
    - $NO_3$, $NO_2$, $SO_4$, etc.

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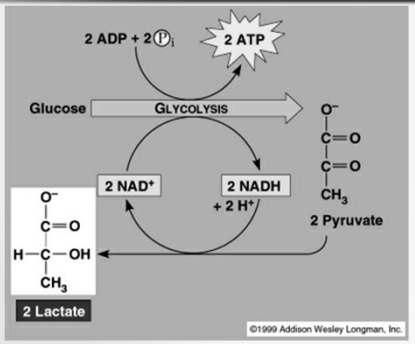
## Fermentation

- Features
  - Pyruvate is reduced to organic acids or alcohols
  - Final e- acceptor is organic
  - NADH is oxidized to NAD:
    - Essential for continued operation of glycolytic pathways
  - $O_2$ is not required
  - No additional ATP made
  - Gasses ($CO_2$ and/or $H_2$) may be released

## Fermentation - Lactic




- Organic electron acceptor - **Pyruvate**
- Regeneration of NAD $^{+}$

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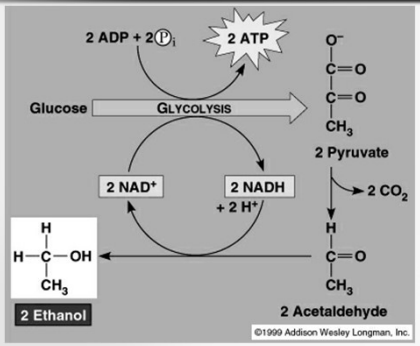
## Fermentation - Ethanolic




- Organic electron acceptor- **Acetaldehyde**
- Regeneration of NAD $^{+}$

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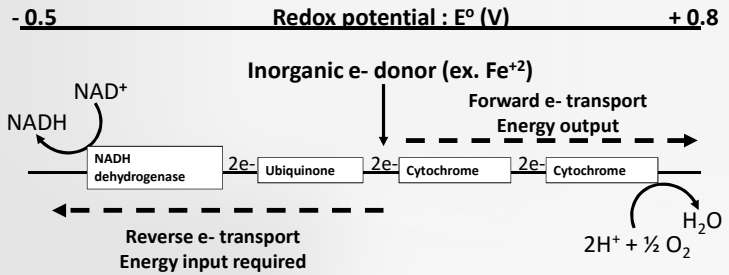
## Chemolithotrophy

- Features
  - Uses a reduced inorganic e- donor
    - Ex. Nitrite, sulfur, hydrogen
  - e- go through an electron transport pathway
    - Coupled to the synthesis of ATP and NADH
  - e- are used to reduce a final e- acceptor
    - $O_2 \rightarrow H_2O$ or $CO_2 \rightarrow$ Methane
  - ATP and NADH are used to convert $CO_2$ to sugars
    - Calvin cycle - autotrophs

## Chemolithotrophy – ETC

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## Photosynthesis

- Features:
  - An e- donor reduces a photosynthetic pigment
  - Light energy is used to make the redox potential of reduced photosynthetic pigment more negative
  - e- go through an electron transport pathway
    - Coupled to ATP synthesis
  - e- are used to reduce a final e- acceptor
  - ATP is used to convert $CO_2$ to sugars
    - Calvin cycle - autotrophs

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## Photosynthesis – 2 Types

- Anoxygenic - Cyclic
  - Electron donor: Organic or inorganic ($H_2S$, S or $H_2$)
  - Final e- acceptor : Oxidized photosynthetic pigment
- Oxygenic- Non-cyclic
  - Electron donor: $H_2O$
    - $H_2O + Pig_{ox} \rightarrow ½\ O_2 + Pig_{red}$
  - Final e- acceptor: NAD or NADP

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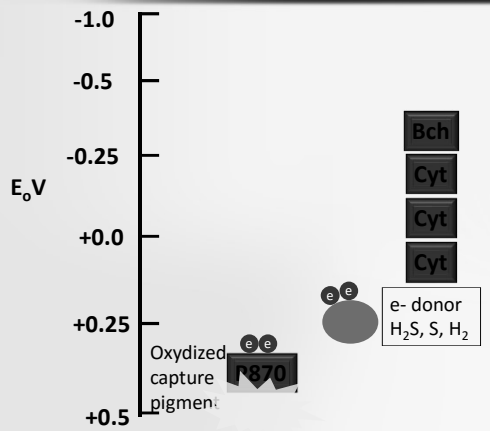
## Anoxygenic Photosynthesis

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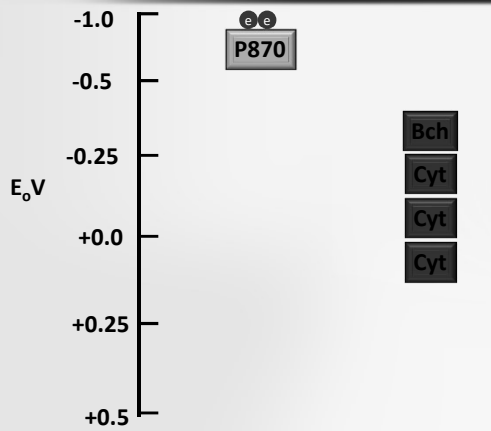
## Anoxygenic Photosynthesis

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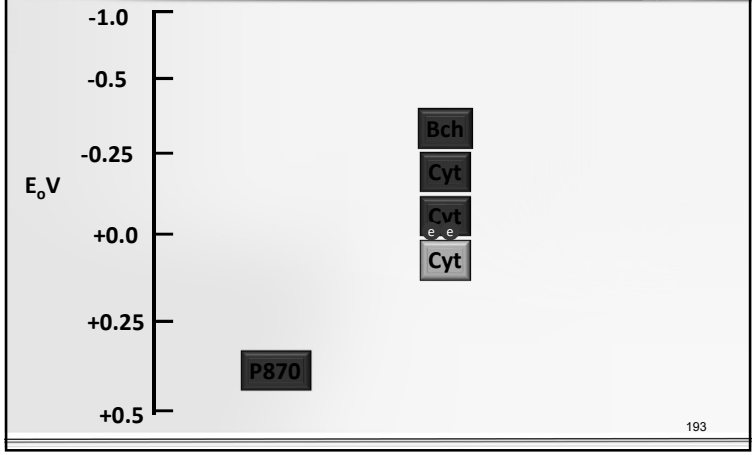
## Anoxygenic Photosynthesis

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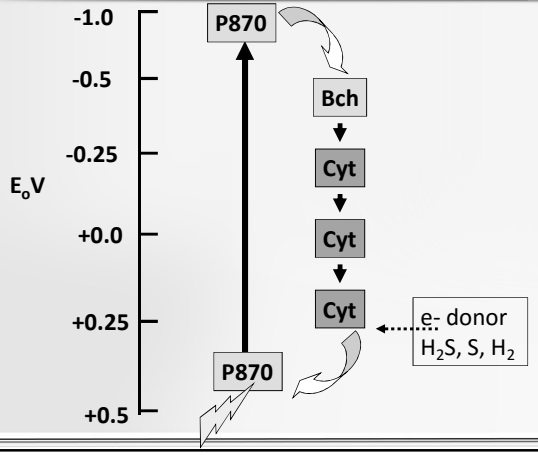
## Anoxygenic Photosynthesis

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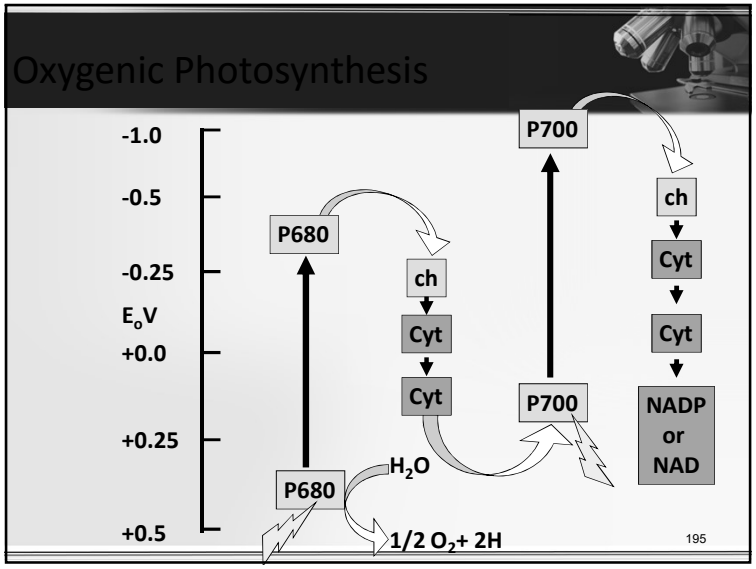
## Oxygenic Photosynthesis

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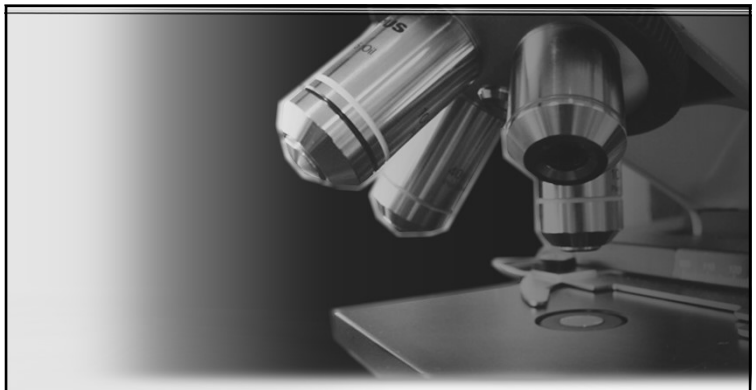
## Comparison of Photosyntheses

| Characteristics | Non cyclic | Cyclic | |
|---|---|---|---|
| | Cyanobacteria | Green/purple sulfur bacteria | Green/purple nonsulfur bacteria |
| e- donor | $H_2O$ | Sulfur compounds | Organic or $H_2$ |
| Production of $O_2$ | Yes | No | No |
| e- acceptor | NAD or NADP | Photosynthetic pigment | Photosynthetic pigment |
| Environnement | Aerobic | Anaerobic | Anaerobic |

196



## *Culture Media*

197

## Nutritional Complexity

- Nutritional complexity is a function of the biosynthetic capacity
- The greater the biosynthetic capacity, the lower the nutritional requirements

198

## Complex Media

- Composed of rich and complex ingredients
  - Ex. Soya protein extracts
  - Milk protein extracts
  - Blood products
  - Tomato juice, etc.
- Exact chemical composition is unknown
- Can be **selective** and/or **differential**

199

## Defined Media

- Known chemical composition
  - Can contain up to 80 different ingredients
  - Can be very simple
  - Allows the growth of a restricted number of microorganisms
  - Composition is highly variable according to the microorganism
- Can be **selective** and/or **differential**

200

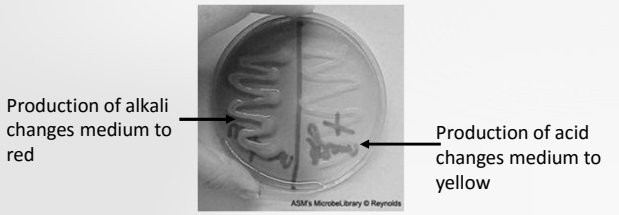
## Selective Media

- Contain compounds which **inhibit** or **kills** the undesired organisms
  - Ex. Medium containing penicillin only allows the growth of penicillin resistant microorganisms

201

## Differential Media

- Allows to discriminate different species
- Often contain pH indicators
  - Allows to discriminate different metabolisms




202

## Environmental Parameters

- Oxygen requirements
- pH
- Temperature
- Solute concentration/Water availability

203

## Oxygen Requirements

- Aerobic:
  - Absolute requirement of oxygen for survival
  - Oxygen is used as a final electron acceptor
  - Oxygen is used by bacteria which have an oxidative metabolism or perform aerobic respiration
- Microaerophilic:
  - Absolute requirement for low oxygen concentrations
  - High concentrations are deadly

204

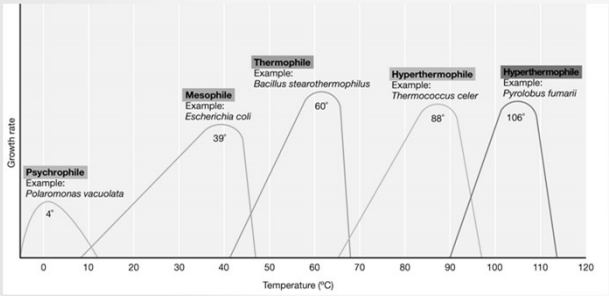
## Oxygen Requirements *(cont'd)*

- Anaerobic/Aerotolerant:
  - Oxygen is tolerated but not required
- Facultative anaerobes:
  - Facultative oxygen requirement
  - Can choose to use oxygen or not
  - Have an oxygen dependent and an oxygen independent metabolism
- Strict or obligate anaerobes:
  - Oxygen is not used nor tolerated; cannot survive in the presence of oxygen

205

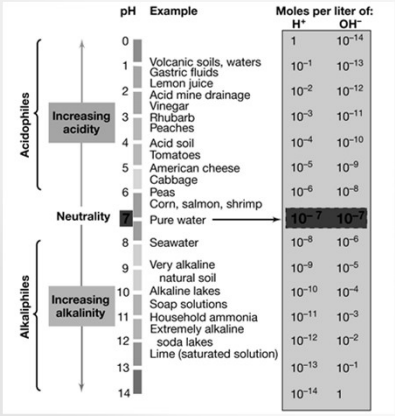
## Temperature

- Microorganisms are **poikilothermic**
  - Do not control their temperature
- Different species have different optimums




206

## pH




207

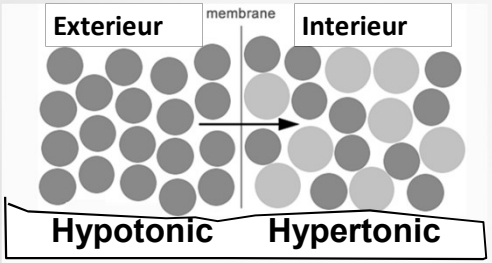
## Controlling pH

- Selective permeability of the membrane
- Antiporters $K^+/H^+$ or $Na^+/H^+$
- Protein buffers
- Chaperones
- Excretion of acid or alkaline waste products

208

## Water Activity ($a_w$)

- Measure of water availability
  - $a_w$ : 1% of relative humidity




$a_w$ (External) > $a_w$ (Internal)

Why?

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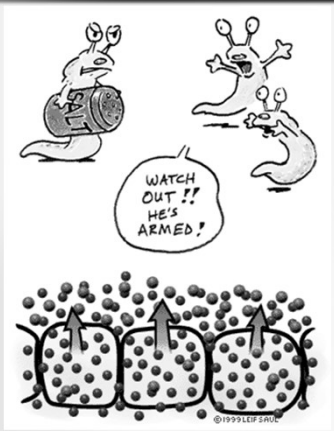
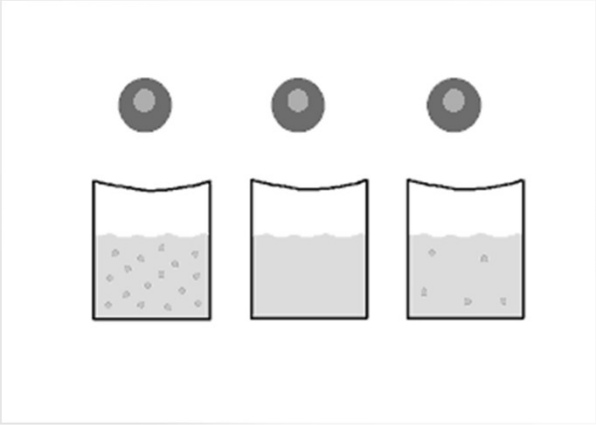



210

## Osmosis – Diffusion of water



211

## Control of the $a_w$

- Control the internal concentration of **compatibles solutes** :
  - Solute that allows metabolism to occurs even when its concentration is high
    - Amino acids, carbohydrates

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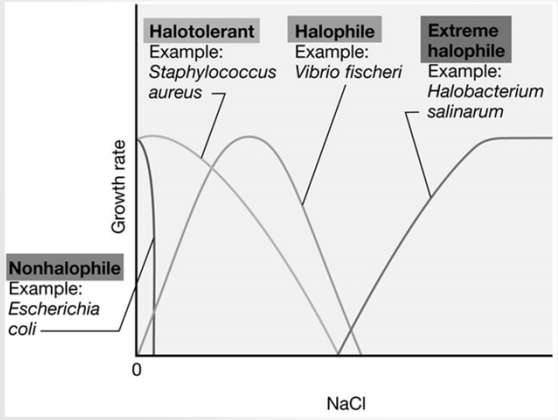
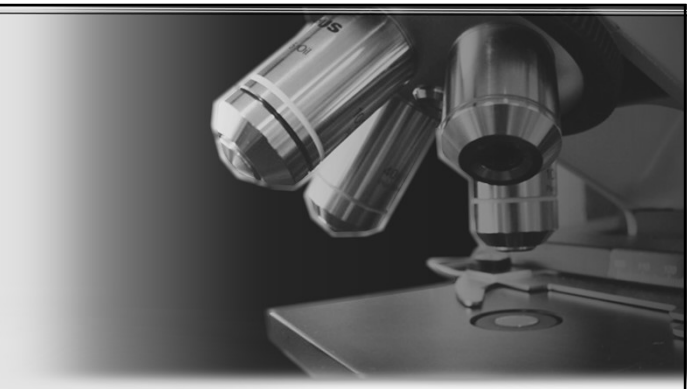



213

## The Suffix "phil" Vs "tolerant"

- -phil
  - The suffix "phil" describes optimal conditions where the microbe can grow at maximum speed
    - Ex. Thermophile: Growth of the microbe occurs at maximum speed at a high temperature rather than at a low one
- -tolerant
  - The suffix "tolerant" describes a non-optimal condition where the microbe can survive
    - There is no growth or growth occurs at a reduced speed
      - » Ex. Thermotolerant: the microbe survives elevated temperatures, but prefers lower temperatures

214



## *Bacterial Growth*

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## Bacterial Growth

- Increase in the number of cells
- The bacterium reproduces by binary fission
  - ($1 \rightarrow 2$, $2 \rightarrow 4$….$2n$)
- Growth measurements monitor changes in the total number of cells or the mass of cells

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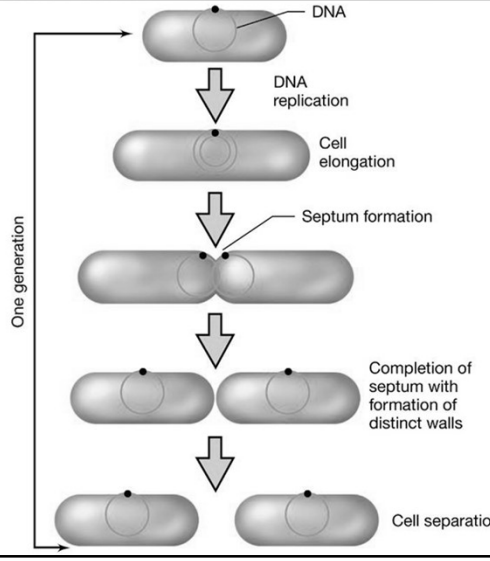
## Binary Fission

- Asexual reproduction
  - DNA replication → cellular elongation→ septum formation→ septum completed and cell wall formation→ cellular separation and creation of daughter cells
    - The quantity of all molecules doubles : proteins, DNA, RNA, lipids for membranes, cell wall materials, etc.
    - Everything is distributed almost equally

217




218

## Growth in Batch Cultures

- CLOSED system
  - No addition of new nutrients
  - No elimination of waste products
  - Cells are not withdrawn
    - Ex. Production of yogurt, beer fermentation, blood infection
- Cell density increases until something becomes limiting

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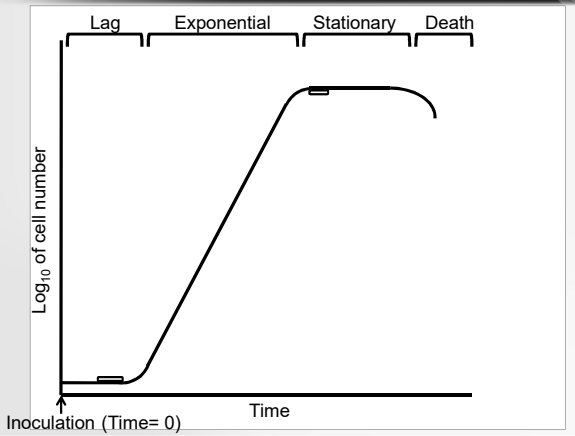
## Growth Profile of Batch Cultures




220

## Lag or Adaptation Phase

- No increase in the number or the mass of cells
- Active synthesis of components required for growth in the given medium
  - Metabolic adaptation

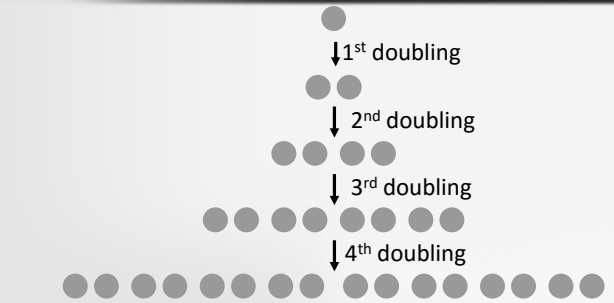
221

## Exponential or Logarithmic Phase

- Development and cellular division occurs at maximum speed
- The number and mass of cells doubles at regular intervals
- The population is in physiological and biochemical equilibrium
- Cell number and mass increase by an **exponential factor ($2^n$)**
  - n = number of divisions or generations

222

## Exponential Division




Final number of cells (N) = Initial number of cells ($N_0$) X ($2^n$)

n = number of generations

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## Exponential Division

| Time (h) | Number of generations (n) | Number of cells (N) | Time (h) | Number of generations (n) | Number of cells (N) |
|---|---|---|---|---|---|
| 0 | 0 | 1 ($2^0$) | 4.5 | 9 | 512 ($2^9$) |
| 0.5 | 1 | 2 ($2^1$) | 5 | 10 | 1024 ($2^{10}$) |
| 1 | 2 | 4 ($2^2$) | 5.5 | 11 | 2048 ($2^{11}$) |
| 1.5 | 3 | 8 ($2^3$) | 6 | 12 | 4096 ($2^{12}$) |
| 2 | 4 | 16 ($2^4$) | 6.5 | 13 | 8192 ($2^{13}$) |
| 2.5 | 5 | 32 ($2^5$) | 7 | 14 | 16384 ($2^{14}$) |
| 3 | 6 | 64 ($2^6$) | 7.5 | 15 | 32768 ($2^{15}$) |
| 3.5 | 7 | 128 ($2^7$) | 8 | 16 | 65536 ($2^{16}$) |
| 4 | 8 | 256 ($2^8$) | 8.5 | 17 | 131072 ($2^{17}$) |

224

## Growth Parameters of Log Phase

- Generation time: **g**
  - Time required for the number of cells to double
    - **$g = \Delta t/n$**
- Number of divisions : **n**
  - Number of times the cell number doubles
    - **$N = N^o (2^n)$**
- Growth rate: **μ**
  - Rate at which cell number changes over time
    - **$\mu = \ln 2/g$**

225

## Sample Calculations

- After 4 h of growth, an *E.coli* culture goes from 100 cells to 6.6 X $10^6$ cells
  - What was n for the 4h period?
  - What is the generation time?
  - What is the growth rate?

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## Sample Calculations

- If you start with one cell, how many will you have after 4 generations?
  - $N_o$= Initial number of cells
  - N = Number of cells after n generations
  - n = number of generation
    - Formula : **$N = N_o(2^n)$**
    - N = 1 ($2^4$) = 16 cell
- How many would you have if you started with 100 cells?
- How many would you after 5 generations if you started with 100 cells?

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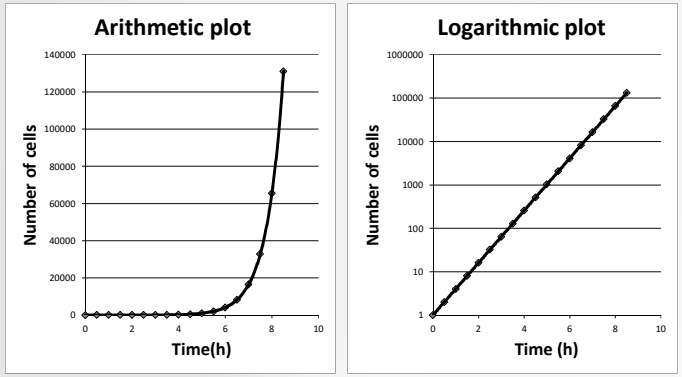
## Sample Calculations

- *E. coli* has a generation time of 20 minutes. If you start with 1 cell, how many will you have after 2 hours?
- After 5 hours?

228

## Growth Parameters from Graphs

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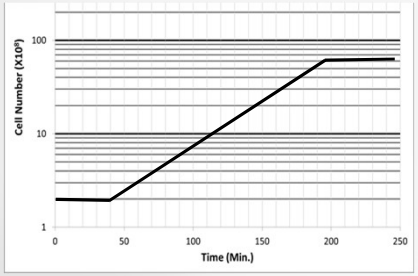
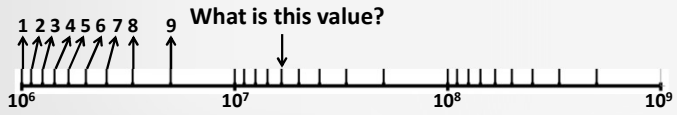
## Growth Parameters from a Graph




All growth parameters must be determined from the logarithmic phase!
In this case, between 40-190 min.

230

## Reading a Log Scale

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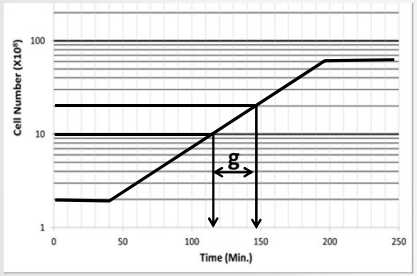
## Determining Generation Time




Method 1:
- Choose two points that represent 1 doubling of cell number
  - Ex. 10 and 20
- Determine time span

Method 2:
- Choose any two points and determine coordinates (cell number and time)
- Calculate n for time span
- Calculate g: Δt/n

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## Stationary Phase

- Arrest in cell growth
- The population is no longer in equilibrium
- Arrest due to a lack of nutrients, oxygen, or an excessive accumulation of waste products, etc.
- Represents the maximum yield under the given conditions
  - Yg : Mass of microorganisms formed/mass (g) of consumed substrate
  - Ym: Mass of microorganisms formed/mole of consumed substrate

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## Death Phase

- Exponential loss of viability due to a prolonged lack of nutrients or a prolonged exposure to waste products
- Not necessarily a loss in mass

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## Measurements of Growth

- Counting microorganisms
  - Relative abundance
    - Turbidity measurements
  - Direct counts
    - Absolute counts
  - Viable counts
    - Absolute number of growing bacteria

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## Turbidity Measurements

- Measures the quantity of light that can go through a sample
- The less light that passes the more dense is the population
- Measurements of optical density or percent transmission

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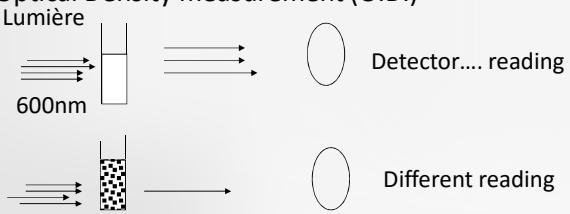
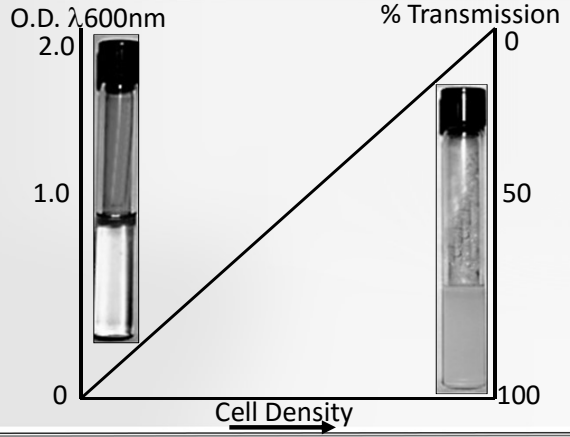
## Turbidity Measurements

- Spectrophotometer (A600):
  - Optical Density measurement (O.D.)




237




238

•Advantages:
  - Quick

- Disadvantages:
  - Relative measurement
  - Does not discriminate between dead and living
  - Does not discriminate between bacteria and detritus
  - Does not discriminate between different microbes

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## Direct Counts

- The sample to be counted is applied to a **hemacytometer** slide which holds a **fixed volume** in a counting chamber
  - The number of cells is counted
  - The number of cells for a given volume is determined

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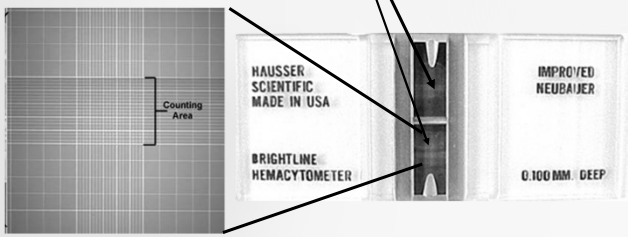
## Hemacytometer

- This slide has **2** independent counting chambers

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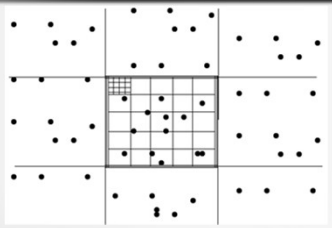
## Determining the Direct Count



- Count the number of cells in 3 independent squares
  - 8, 8 and 5
- Calculate the average
  - (8 + 8 + 5)/3 =7
  - Therefore 7 cells/square

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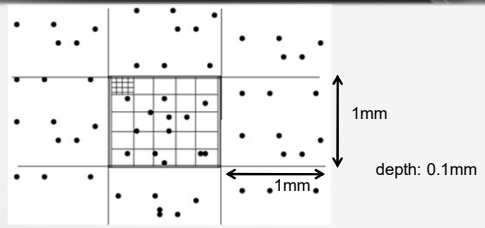
## Determining the Direct Count *(cont'd)*




- Calculate the volume of a square:
  = 0.1cm X 0.1cm X 0.01cm= 1 X $10^{-4}$$cm^3$ or ml
- Divide the average number of cells per square by the volume of one square
  - Therefore 7/ 1 X $10^{-4}$ ml = 7 X $10^4$ cells/ml

243

- Advantages:
  - Quick
  - Growth is not required
  - No information about organism required
- Limits:
  - Does not discriminate between live and dead
  - May be difficult to distinguish bacteria from detritus

244

## Sample Problem

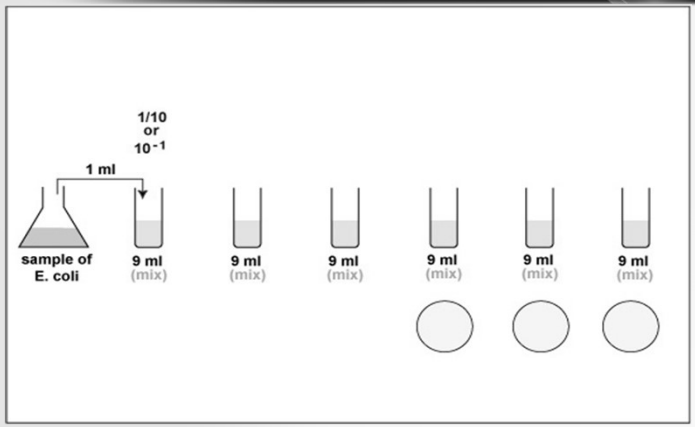
- A sample is applied to a hemacytometer slide whose counting chamber has the following dimensions: 0.1mm X 0.1mm X 0.02mm and a total of 100 squares. Counts of 6, 4 and 2 cells were recorded in three independent squares.
  - What is the volume of the counting chamber?
  - What is the volume of one square?
  - What is the number of cells per milliliter in the sample?

245

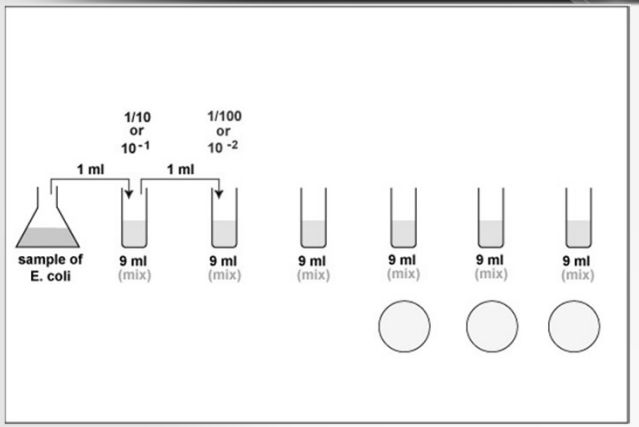
## Viable Counts

- **A viable cell**: a cell which is able to divide and form a population (or colony)
  1. A viable cell count is done by **diluting** the original sample
  2. Plating aliquots of the dilutions onto an **appropriate** culture medium
  3. Incubating the plates under appropriate conditions to allow growth
     - Colonies are formed
  4. Colonies are counted and original number of viable cells is calculated according to the dilution used

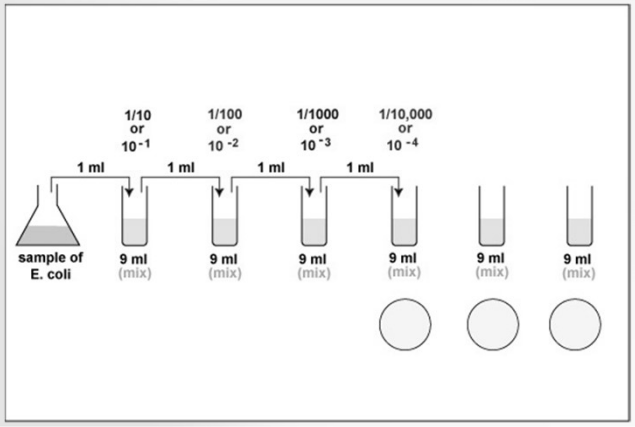
## Dilution of Bacterial Sample



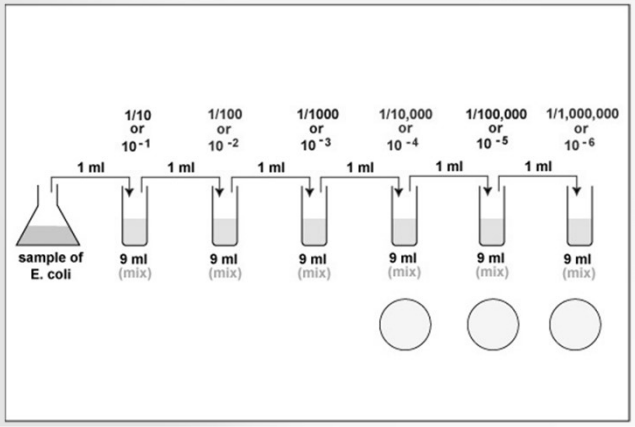

## Dilution of Bacterial Sample



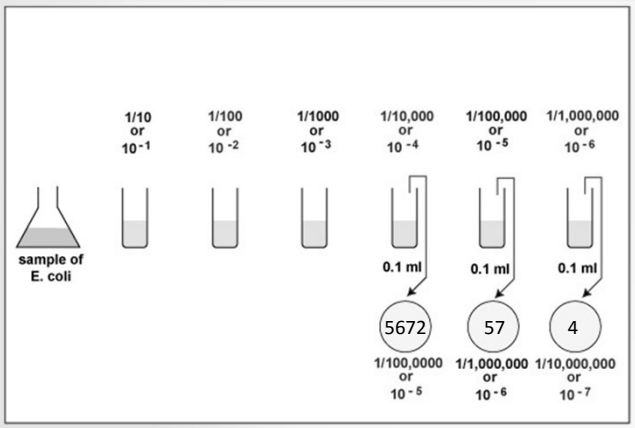
## Dilution of Bacterial Sample




## Dilution of Bacterial Sample




## Plating of Diluted Samples




## Viable Counts

- The total number of viable cells is reported as Colony-Forming Units (CFUs) rather than cell numbers
  - Each single colony originates from a colony forming unit (CFU)
  - A plate having 30-300 colonies is chosen
  - **Calculation**:
    - Number of colonies on plate X reciprocal of dilution (dilution factor) = Number of CFU/mL
      - Ex. 57 CFU X $10^6$ = 5.7 X $10^7$ CFU/mL

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## Viable Counts

- Advantages:
  - Determines the number of live organisms
  - Can discriminate between different microorganisms
- Disadvantage:
  - No universal medium
  - Requires the growth of the microorganism
  - Only living cells develop colonies
  - Clumps or chains of cells develop into a single colony

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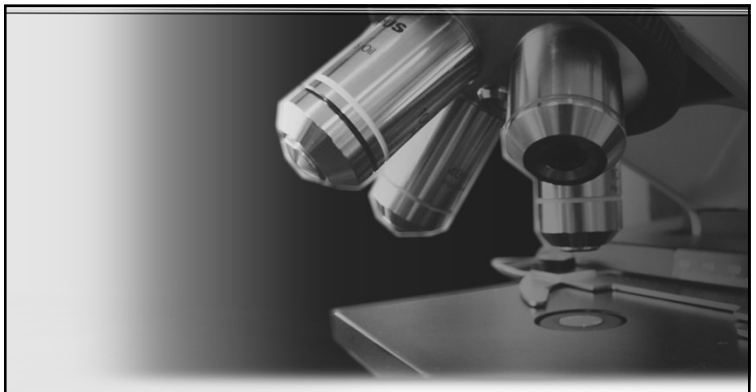

## *Control of Microbial Growth*

Disinfectants and Antiseptics

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## Method

- Three approaches for the control of microbial growth
  - Chemical
    - Disinfectants and antiseptics
  - Physical
    - Heat
    - Ultraviolet
    - Irradiations
  - Mechanical elimination
    - Cleaning
    - Filtration

255

## Terminology

- Cleaning
  - The elimination of visible adherent dirt (blood, proteins and debris), dust or other foreign matter by manual or chemical processes
    - Does not infer the presence or absence of microorganisms
      - Cleanliness ≠ Sterility

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## Disinfection

- The use of chemical or physical agents to kill or inhibit the growth of microorganisms
  - Disinfectants
    - Chemical products used on inanimate objects
  - Germicides
    - Chemical products which can be used on either animate (living) or inanimate things
  - Antiseptics
    - Chemical products used on living tissues

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## Other Definitions

- Contamination
  - Contaminant:
    - Non-intentional presence of a microorganism
  - Decontamination:
    - Operation used to reduce or eliminate a contaminant
  - Sanitation: Reducing the level of microbial contamination to prevent transmission in public establishments
    - Restaurants, bathrooms, etc.

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## Factors which Influence the Efficacy

- Microbial load
  - Number of microbes
- Environment
  - Presence of organic matter
  - Concentration of the agent
  - Temperature
  - pH
- Length of exposure

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## Factors which Influence the Efficacy

- Microbial characteristics
  - Glycocalyx
  - Biofilms
  - Cell wall
  - Resistances
  - Spores

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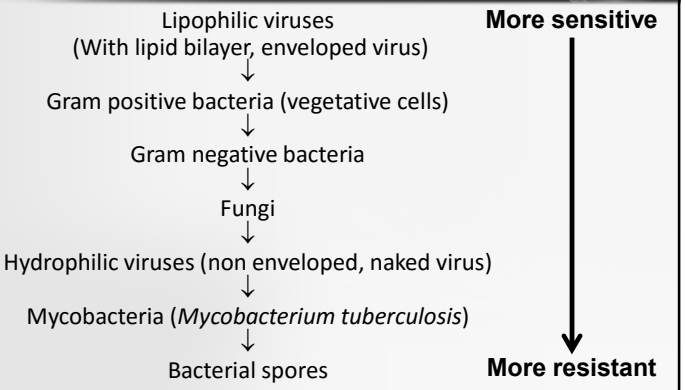
## Order of Sensitivity

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## Disinfectants and Antiseptics

- Ideal characteristics
  - Broad action spectrum
  - Powerful
    - Small amount required for a high efficiency
  - Low toxicity in humans
  - Non corrosive
  - Stable
  - Hydrophilic and hydrophobic
  - Low surface tension
  - Odorless or with a pleasant smell

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## Modes of Action of Chemical Agents

- Denaturation of proteins or DNA
- Mutagenesis of DNA
- Modification of proteins or of DNA
- Interference with the plasma membrane
- Oxidation of functional groups

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## Types of Chemical Agents

- Seven major categories:
  - Phenol and phenolic compounds
  - Alcohols
  - Halogens
  - Oxidative agents
  - Heavy metals
  - Aldehydes
  - Surfactants

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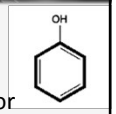
## Phenol and Phenolics




- Phenol (carbolic acid)
  - Used for the first time by Lister
  - Rarely used, since it's an irritant and has a strong odor
- Phenolics: Chemical derivatives of phenol
  - Cresols: Lysol
  - Bisphenols
    - Used in hospital centers
- Denatures proteins and destroys membranes
- Bactericide, fungicide, sporicidal
- Very toxic
- Caustic
- Antiseptic/disinfectant

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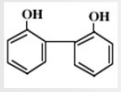
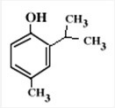
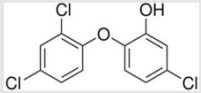
## Examples of Phenolic



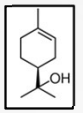

O-phenylphenol


Thymol


Triclosan

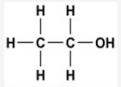




Turpineol


266

## Alcohols

- Ethanol (60-95%) and isopropanol
  - Effective against bacteria, fungi, and enveloped viruses
    - Inefficient against spores and naked viruses
  - Denatures proteins and dissolves lipids





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## Halogens

- Four members :
  - Iodine
  - Chloride
  - Bromide
  - Fluoride
- Bactericide, fungicide and viricide
- Iodine inactivates proteins by interacting with disulfur linkages
- Chlorine, bromine and fluoride are strong oxidizing agents

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## Oxidative Agents

- Release hydroxyl free radicals which inhibit bacterial metabolism
  - Very effective against anaerobic organisms
    - Very effective against deep tissue infections

269

## Oxidative Agents

- The three most commonly used are:
  - Hydrogen peroxide
    - Common household antiseptic
  - Ozone
    - Very reactive form of oxygen used for the treatment of water
  - Peracetic acid
    - Peroxide of acetic acid
      - Very effective sporicidal
        Used to sterilize surfaces and medical materials

270

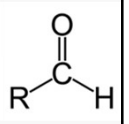
## Heavy Metals - Hg, Ag, Zn, Cu

- Interact with proteins causing their denaturation and inactivation
  - Mercury
    - Mercury and silver used to be used in clinical situations
      - Mercury is not used anymore
      - Silver is still used for surgical bandages
  - Zinc
    - Used in mouth washes
    - Used as antifungal in paints
  - Copper
    - Algaecide; used in pools

271

## Aldehydes

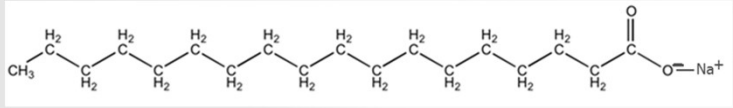
- Compounds with a terminal –CHO group
  - Denatures proteins and inactivates nucleic acids
- Two very reactive aldehydes are used as antimicrobials
  - Glutaraldehyde and fomaldehyde
- Bactericide, sporicidal, fungicide and viricide




272

## Surfactants

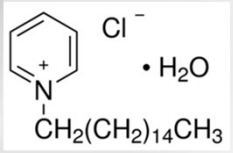
- Soaps
  - Sodium or potassium salts of fatty acids
  - Effective for the mechanical elimination of microbes from surfaces
  - Ineffective as an antimicrobial

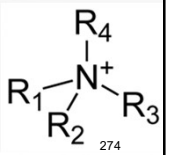


273

## Surfactants

- Detergents
  - Positively charged organic compounds
  - Ex. Quaternary ammonium compounds
  - Dissolves lipid membranes
  - Bactericide, fungicide, and viricide against enveloped viruses
  - Ineffective against spores and naked viruses





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## Evaluation of Disinfectant Efficacy

- **Quantitative suspension tests**
  - Viable counts are performed on a test microorganism exposed to the chemical agent
  - The number of surviving organisms (B) is counted and compared to the original inoculum size (A)
    - **Microbicidal effect (ME) = Log (A) - Log (B)**
    - ME = 1 → killing of 90% of the initial number
    - ME = 2 → 99% killed
  - A generally accepted requirement is:
    - ME ≥ 5 →99.999% of the germs are killed

275

## Evaluation of Disinfectant Efficacy

- Phenol coefficient test
  - Potency of a disinfectant is compared with that of phenol
  - Highest dilution that kills bacteria after a 10 minute exposure is used to calculate phenol coefficient
  - The higher the phenol coefficient value, the more effective the disinfectant is

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## Evaluation of Disinfectant Efficacy

- Phenol coefficient test *(Cont'd)*
  - The reciprocal of the appropriate dilution of test disinfectant is divided by that for phenol to obtain the coefficient
    - Ex.: Phenol dilution = 1/90 and the maximum effective dilution for disinfectant X = 1/450
      - Phenol coefficient = (1/450) ÷ (1/90) = 5
      - Agent X is therefore 5X more effective than phenol

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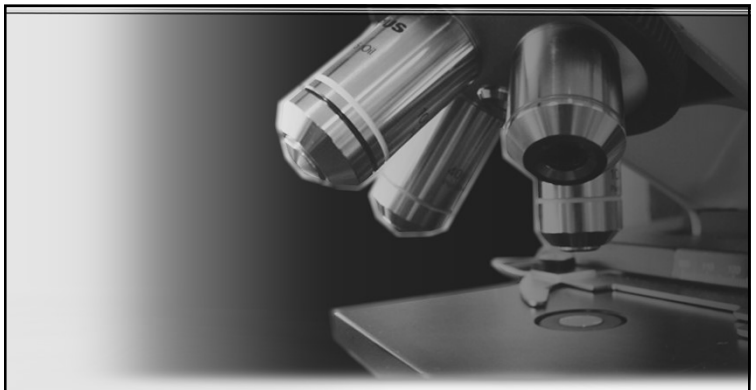

## *Control of Microbial Growth*

Physical Methods: Sterilization

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## Definitions

- Sterilization
  - Killing or removing all forms of microbial life (including endospores)
    - The method most commonly used for sterilization is the use of heat
- Commercial sterilization
  - Thermal treatment which kills *Clostridium botulinum* spores, the causative agent of botulism, in canned foods
    - Does not kill spores of thermophiles which are not pathogenic

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## Modes of Heat Sterilization

- Humid heat
  - Kills microbes by denaturing proteins
    - Boiling (100°C)
    - Pasteurization (65 - 140°C)
    - Autoclave (121°C)
- Dry heat
  - Kills by oxidation
    - Pasteur oven (121 - 250°C)
    - Incineration (870 - 1200°C)

280

## Boiling

- 10 minutes at 100°C at sea level
  - Kills vegetative form of bacterial pathogens
  - Kills most viruses and fungal spores
  - Endospores and some viruses are not destroyed
    - Hepatitis virus: Can survive up to 30 minutes
    - Endospores: Can survive for periods of 20 hours or more

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## Pasteurization

- Used for drinks
- Kills pathogenic agents without affecting the taste of the food
  - Does not sterilize
  - Classical pasteurization
    - 63°C for 30 seconds
  - HTST Pasteurization
    - 72°C for 15 seconds
  - UHT Pasteurization
    - 140°C for 3 sec. under vacuum

282

## Autoclave

- Makes use of humid heat under pressure
- Temperature of 121°C at twice the atmospheric pressure
- All organisms and endospores are killed in 15 minutes

283

## Dry Heat Sterilization

- Pasteur oven
  - Temperature between 121 and 250°C
  - Long exposure times (2-12 hours)
  - Not recommended for chemical products
  - Can be used for some metals and glass
- Incineration
  - 870 to 1200°C
  - Burns and physically destroys
  - Used for needles, glass, corpses, etc.

284

## Parameters of Death by Heat

- Thermal death point (TDP)
  - Lowest temperature at which all bacteria are killed within 10 minutes
- Thermal death time (TDT)
  - Length of time required to kill all the bacteria at a given temperature
- Decimal reduction time (DRT– D value)
  - Time required to kill 90% of a microbial population

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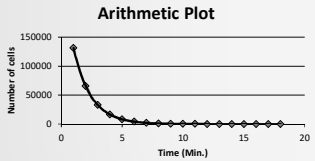
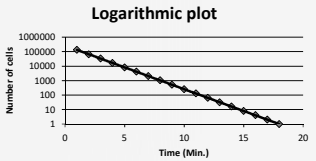
## Profile of Thermal Mortality



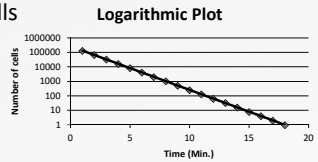




- Death is exponential
  - It's therefore impossible to reach zero
    - Established standards:
      - TDP and TDT: < 1 cell
      - Sterility in the lab: $10^{-6}$ cells or spores
      - Sterility for food: $10^{-12}$ cells or spores

286

## Parameters of Mortality

- Decimal reduction time (D)
  - **Time** required to achieve a reduction of one log or an inactivation factor of 10
  - Formula: **$\text{Log } (N/N_0) = -t/D$**
    - t: Length of time
    - N: Number of surviving cells
    - $N_o$: Initial number of cells
      - $N_o/N$: Inactivation factor

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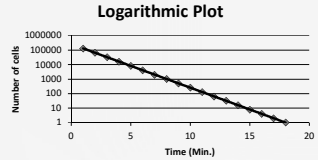
## Parameters of Mortality

- Mortality constant (k)
  - **Rate** of mortality
    - Negative slope
  - Formula: **$-kt = \ln (N_o/N)$**
    - T: Length of time
    - N: Number of surviving cells
    - $N_o$: Initial number of cells
      - $N_o/N$: Inactivation factor



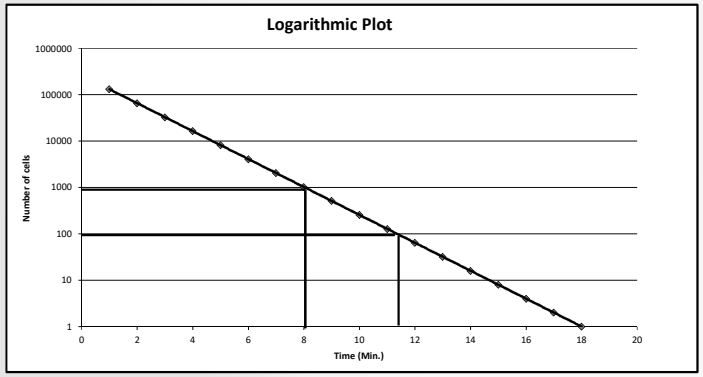

288

## Sample Problem

- A treatment at 100°C for 1h reduced a bacterial population from $10^8$ to $10^2$ cells
  - What is the inactivation factor achieved?
  - What is $D_{100}$?
  - What is the mortality rate?
  - How much time would be required to reduce the population to $10^2$?
  - What would be the TDT?

289

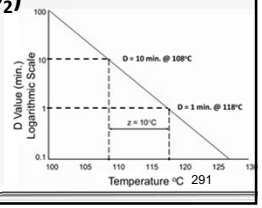
## Determining D from a Graph




290

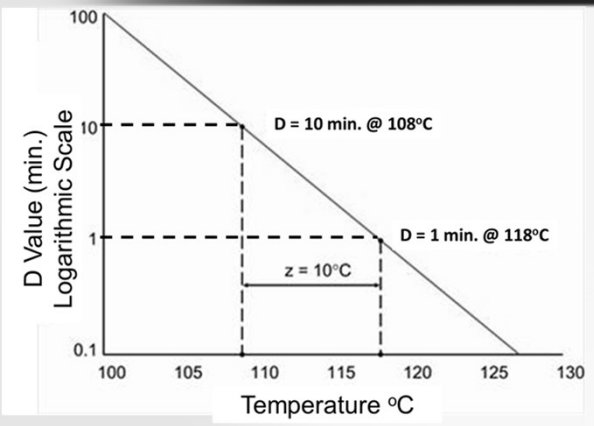
## Relative Resistance of Microorganisms

- Z value
  - **Temperature** change required to change the D value by 1 log
  - **Temperature** change required to change the D value by a factor 10
  - Formula: $-\mathbf{Z = (T_1-T_2)/(\log D_1-\log D_2)}$
    - T: Temperature
    - D: Decimal reduction time




291

## Determining Z from a Graph




292

## Relationship Between D and Z

- If Z =10°C
  - Each 10°C increment change of temperature would change the D value by one log
  - Thus if D at 110°C = 10 minutes
    - At 120°C it would be = to 1 minutes
    - At 130°C it would be = to 0.1 minutes
    - At 140°C it would be = to 0.01 minutes
  - What would be the D value at 80°C?

293

## Sample Problem

- The Z value of an organism is 2°C. If 18 min are required at 75°C to reduce the population from $10^9$ to $10^6$; at what temperature should this organism be subjected to achieve the same result in 10.8 seconds?
- 18 minutes = 1080 seconds
  - Thus to go from 1080 to 10.8 sec. = 2 log
    - 2 log = 2Z = 4°C
    - Since we want to **reduce** the amount of time, we must **increase** the temperature by 4°C
      - Thus 75 + 4 =79°C

294

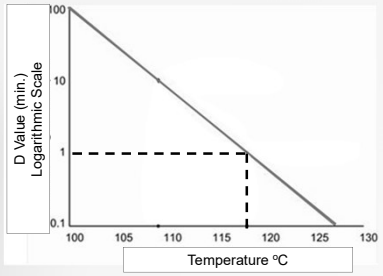
## Thermal Death Point (TDP)

- Minimum temperature required to reduce a population to less than one cell in 10 minutes
  - Ex. What is the TDP of a culture which has $10^8$ cells?
    - Calculate the inactivation factor wanted : $10^8/0.99 = 10^8$
    - Calculate the number of decimal reductions wanted : 8D
      - » Thus 8D = 10 minutes or D = 1.25 minutes
    - Determine form the graph illustrating the level of temperature sensitivity the temperature which corresponds to the D value you want

295

## Determining the TDP

- Want a D value equal to 1.25 minutes
  - According to the graph this corresponds to a minimum temperature of…
    - ~ 117°C

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## Radiation Sterilization

- 3 types of radiations kill microbes
  1. Ionizing radiations
     - Ionizing radiation : Gamma rays and X rays
     - Cause mutations in DNA
       - Used for the sterilization of pharmaceutical products and disposable medical supplies
  2. Ultra violet light
     - Damages DNA and causes mutations
     - Used to sterilize surfaces
       - Ex. Operating room

297

## Radiation Sterilization

3. Microwave radiations
   - Causes water molecules to be heated
     - Can kill vegetative cells in humid food products
     - Endospores, which do not contain water, are not damaged
     - Ineffective on solid foods
       - » Unequal penetration

298

## Filtration

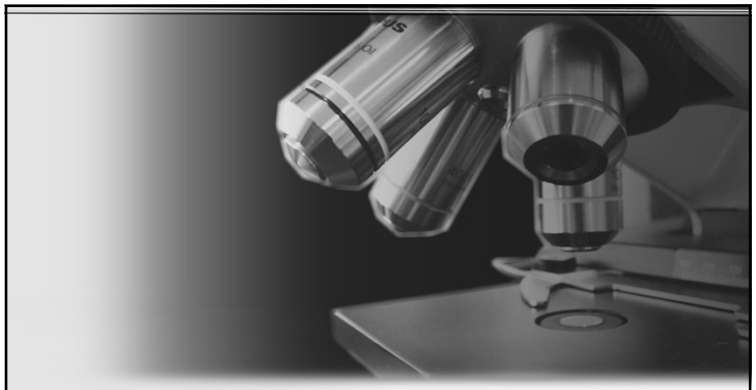
- Exclusion principle using filters
  - Elimination of microbes by the passage of a liquid or gas through a filter whose pore size prevents the passage of…
    - Bacteria: pores of 0.22 and 0.45µm
      - Does not retain mycoplasms and viruses
    - Virus : pores of 0.01µm

299

## Filtration

- Used for the sterilization of thermosensitive materials
  - Vaccines, enzymes, antibiotic, and some culture media
  - High efficiency filters for particles in the air (HEPA; High Efficiency Particulate Air)
    - Used in operating rooms to eliminate bacteria in the air

300



## *Control of Microbial Growth*

*In Vivo*: Antibiotherapy

301

## Antimicrobial Drugs

- Antibiotic or Antibacterial
  - Against bacteria
- Antifungal
  - Against fungi
- Antiviral
  - Against viruses

302

## The Drugs: Antibiotics

- Definitions:
  - Literal: Anti (against) biotic (life)
  - Old def.: Any compound made by a microorganism which inhibits or kills bacteria
  - New def.: Any compound which inhibits or kills bacteria

303

## Desired Characteristics

- **High** selective toxicity
  - Must kill or inhibit the targeted organism with minimal deleterious effects on the host
    - **Penicillin**: (High selective toxicity)
      - Targets the cell wall
    - **Cyanide**: (Low selective toxicity)
      - Targets electron transport of eukaryotes/prokaryotes

304

## Desired Characteristics *(cont'd)*

- **High** toxic or lethal dose (LD50)
  - Concentration of the agent qui that is toxic for the **host**
    - Penicillin: (3 000 mg/Kg)
    - Arsenic: (15 mg/Kg)
- **Low** therapeutic dose
  - Concentration of the agent required for the clinical treatment of an infection
    - Penicillin : 12.5 mg/Kg
    - Garlic: 300 mg/Kg

305

## Therapeutic index

- Toxic dose/Therapeutic Dose
  - Want a therapeutic dose which is?
    - ❖**High**

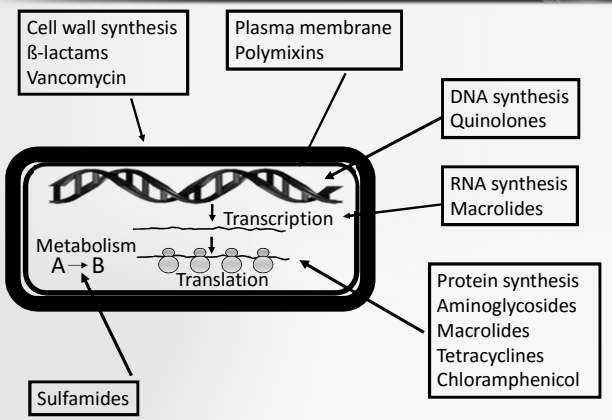
306

## Action Spectrums

- Narrow:
  - Restricted efficacy against some types of microorganisms
    - Ex. Only acts against Gram -
- Broad:
  - Effective against a wide diversity of microorganisms
    - Ex. Acts on Gram + and -

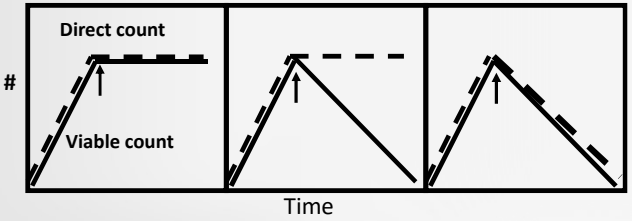
307

## Antibacterial Targets




308

## Modes of Action

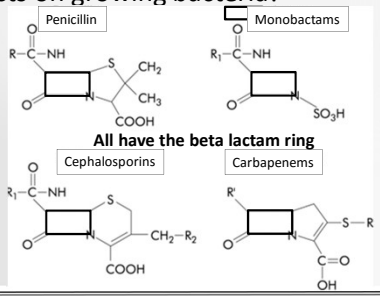



- Bacteriostatic
  - Inhibits growth
  - Non lethal
  - Reversible
- Bactericide
  - Kills
  - Irreversible
- Bacteriolytic
  - Kills
  - Cell lysis
  - Irreversible

309

## Beta-Lactams

- Bacteriolytic
- Inhibit synthesis of the cell wall
  - Only acts on growing bacteria!




310

## Penicillins & Cephalosporins

- Natural penicillin – penicillin G
  - Narrow spectrum; only acts on Gram positives
- Aminopenicillin – ampicillin and amoxicillin
  - Broad spectrum; acts on Gram positives and negatives
- Cephalosporins – Ex. Cefepime & Ceftazidime
  - Developed to have a broader action spectrum as compared to penicillins

311

## Monobactams & Carbapenems

- Monobactams
  - Very narrow action spectrum; useless against Gram positives and anaerobes
- Carbapenems – Last generation of beta lactams
  - Very broad action spectrum
  - Acts against Gram positives, negatives, anaerobes and aerobes

312

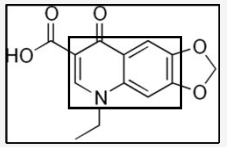
## Adverse Effects of Beta Lactams

- Severe allergies amongst 10% of the population
- Gastro-intestinal problems
  - Vomiting, nausea, diarrhea
- Immune effects
  - Immunodepression
- Neurological problems (carbapenem)
  - Irritability, confusion, seizures

313

## Quinolones

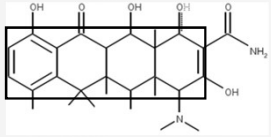
- Bactericides
  - Inhibit DNA synthesis
  - Broad spectrum
  - Side effects:
    - Severe gastrointestinal problems
  - Ex. Ciprofloxacin




314

## Tetracyclines

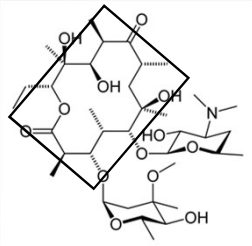
- Bacteriostatic
  - Inhibits protein synthesis
  - Broad spectrum
  - Side effects:
    - Hepatic toxicity
    - Renal toxicity
    - Vitamin deficiency




315

## Macrolides

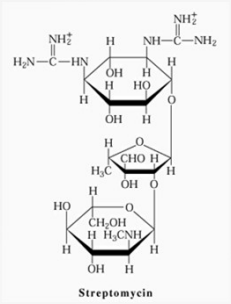
- Bacteriostatic
  - Inhibits protein synthesis
  - Narrow spectrum
  - Side effects
    - Diarrhea
    - Hepatic damage
  - Ex. Erythromycin & Clarithromycin




316

## Aminoglycosides

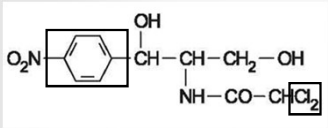
- Bactericides
  - Narrow spectrum
  - Inhibit protein synthesis
  - High level of toxicity
  - Side effects:
    - Allergies
    - Renal damages
    - Deafness
  - Ex. Gentamycin, streptomycin




317

## Chloramphenicol

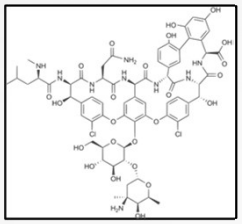
- Bactericides
  - Narrow spectrum
  - Inhibit protein synthesis
  - Side effects:
    - Only used in extreme cases
    - Hematological toxicity



318

## Glycopeptide Antibiotics

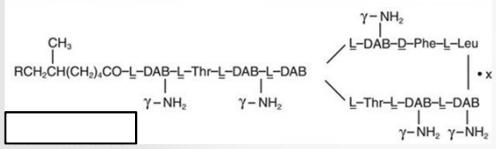
- Composed of polycyclic amino acids
- Inhibits cell wall synthesis
- Acts mostly against Gram Positives
- Used as a last recourse
  - Ex. Vancomycin



319

## Peptide Antibiotic –Polymixin B

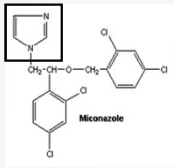
- Disrupts the plasma membrane
  - Binds lipid A and phospholipids
  - Only acts against gram negatives
- Topical use only
  - Can cause lysis of eukaryotic cells



320

## Topical Antifungals

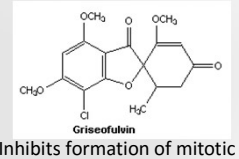
- Imidalzole derivatives:
  - Miconazole, Clotrimazole, Cetoconazole
- Targets and modes of action:
  - Extraction of sterols from plasma membrane
  - Inhibition of plasma membrane synthesis
  - Low therapeutic index

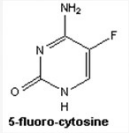

321

## Systemic Antifungals

- Targets and modes of action:
  - DNA replication
  - Cell division
  - Very low therapeutic index
  - Only used in extreme cases




- Inhibits formation of mitotic microtubule fibers




- Nucleoside analog: Inhibits DNA synthesis

322

## Antimicrobial Therapies

- Empirical
  - The infectious organism is unknown
  - Broad spectrum antibiotic recommended
- Definitive
  - The infectious agent was identified
  - A specific therapy is chosen
  - Narrow spectrum antibiotic recommended
- Prophylactic or preventive
  - Prevent an initial infection or a reinfection

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## Choice of Appropriate Antibiotherapy

- Determine the site of the infection
- Sample and isolate the pathogen
- Determine sensitivity
- Choose the administration route
  - Oral
  - Intravenous
  - Topical

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## The Infection Site

- Most important criteria for choosing the appropriate antimicrobial!
  - Allows to determine the presumptive identity of the infectious organism
    - Particularly useful for empirical therapies
  - Allows the determination of the dose and administration route
    - The effectiveness of the therapy depends on the concentration of the antibiotic at the site of the infection and its relationship to the MIC
      - **The concentration at the site must be higher than the MIC**

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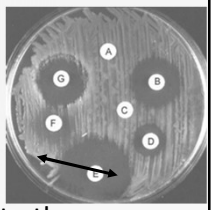
## Sensitivity: Kirby Bauer Assay

- Medium is inoculated with bacteria to be tested
- Discs containing antibiotics are deposited on the medium
- A concentration gradient is established due to the diffusion of the antibiotic in the medium
- Following the incubation, the inhibition zones are measured
- The sizes of the zones are compared to those established to determine whether the organism is sensitive or resistant

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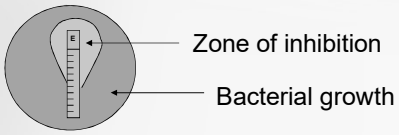
## E-Test

- Same principal as the Kirby Bauer assay
- Makes use of a plastic strip with a predefined gradient of antibiotic concentrations
- The results are read directly on the strip
  - The intersection point of the zone of inhibition and the strip

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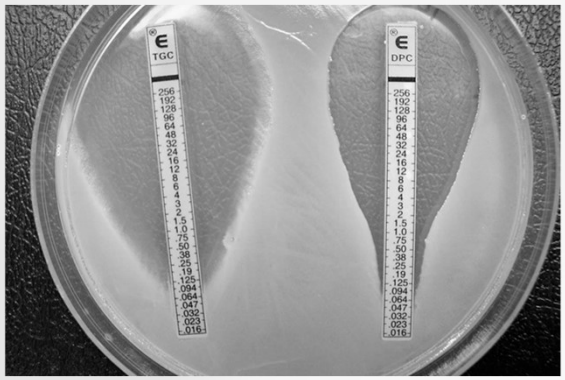
## E-Test

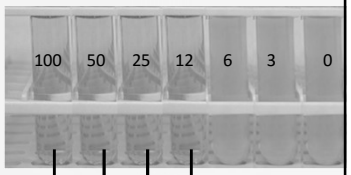



328

## Determining Efficacy

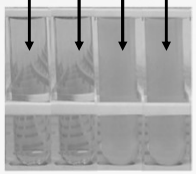
- Minimal Inhibitory Concentration

Cultures with different concentrations of antibiotique




**MIC=12µg/ml**

Subculture without antibiotics



**MBC=50µg/ml**

- Minimal Bactericide Concentration

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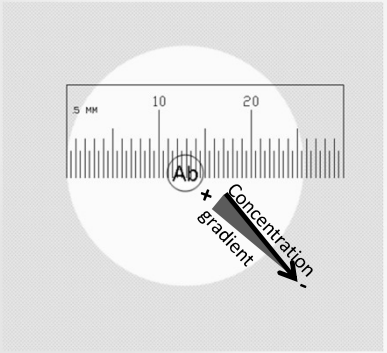
## Diameters of Inhibition Vs Concentration




27mm = to MIC
< 27mm = Conc. > MIC
> 27mm = Conc. < MIC

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## Pharmacodynamics of Antibiotics

- Behavior of antibiotics *in vivo*
  - Interaction of antibiotics with the bacteria
    - The antibiotic must reach the site where the microbe resides
    - The concentration of the antibiotic at the infection site must be above the MIC
    - The antibiotic must occupy a sufficient number of sites on the target
    - The antibiotic must remain in contact with the target for a sufficient amount of time

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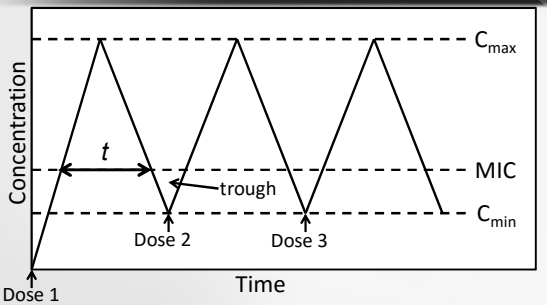
## Concentration of Antibiotics *In Vivo*




- $C_{max}$: Maximum concentration attained for a given dose
- $C_{min}$: Minimal concentration attained between doses
- *t*: Time during which the concentration is maintained above the MIC

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## *In Vivo* Sensitivity

- **Sensitive** pathogen
  - MIC is lower than $C_{min}$
- **Resistant** pathogen
  - MIC is higher than $C_{max}$
- **Intermediate** sensitivity pathogen
  - MIC is between $C_{min}$ and $C_{max}$
    - A combination of antibiotics is recommended

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## Example

- *In vivo conc.* of antibiotic "A"
  - $C_{min}$: 5 µg/ml
  - $C_{max}$: 40 µg/ml
    - Therefore:
      - MIC < 5 µg/ml = **Sensitive** microorganism
      - MIC > 40µg/ml = **Resistant** microorganism
      - MIC between 5 -40 µg/ml = **Intermediate** sensitivity microorganism

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## *In Vivo* Modes of Action of Antibiotics

- Concentration independent activity
  - Time dependent activity
  - The effect is a function of the length of time that the target is in contact with the antibiotic at a concentration above the MIC
    - Efficacy is evaluated by t > MIC
  - Efficacy remains the same at all concentrations above the MIC
    - Typical of ß-lactams, vancomycin, macrolides, and tetracyclines

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## *In Vivo* Modes of Action of Antibiotics

- Concentration dependent activity
  - Time independent activity
  - The effect is a function of the quantity of antibiotic that is in contact with the target
    - Efficacy is evaluated by the ratio of : $C_{max}$/MIC
  - The efficiency is not affected by the amount of time of the exposure
    - Typical of quinolones and aminoglycosides

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## Drawbacks of Antibiotherapy

- Kills the natural flora
- Does not act on bacterial toxins
  - Ex. cholera, diphtheria, botulism, tetanus
- Creates a selective pressure for antibiotic resistant strains
- Favors opportunistic infections
  - Ex. *Clostridium difficile* Associated Diarrhea (DACD)

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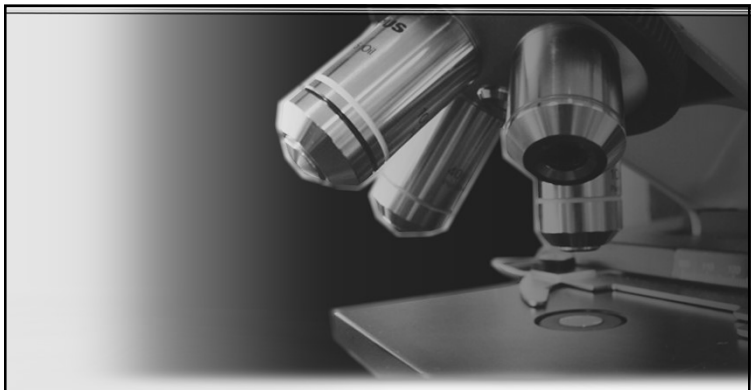

## *Antibiotic Resistance*

•338
338

## Consequences of an Antibiotherapy

- Treatment of an invading organism
  - A life is saved
- Establishment of a selective pressure
  - If the invading microorganism does not develop a resistance it will die
  - 1945- A. Fleming warns that the inappropriate use of penicillin will lead to the selection of resistant bacteria
    - A few years later the first resistant strains appear

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## The Number of Resistant Bacteria is increasing at an Alarming Rate

- To what is this increase attributable?
  - 1990: 300 metric tons of antibiotics are used in humans
  - Approx. 30X more in agriculture
  - 70% are not used appropriately
    - Doses which are too low
    - Prescribed for too short durations
    - Prescribed for viral infections
    - Prescribed for bacterial infections which would have resolved themselves

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## Reasons for Such a High Consumption

- The patient
  - Wants a rapid treatment
  - Publicity
- The physician
  - Wants to satisfy the patient
  - Avoid legal proceedings
  - Cost
    - Antibiotherapy is less expensive than other tests and treatments
- Industry
  - Publicity and pressure from pharmaceutical companies

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## Natural or Innate Resistances

- Resistance is not acquired; **Natural trait**
  - Streptomycetes are resistant to the antibiotics they produce
  - Gram negative bacteria: LPS layer acts as a permeability barrier
  - Some bacteria do not possess the antibiotic's target
    - Mycoplasms – No cell wall; therefore resistant to penicillins and cephalosporins
    - Mycobacteria – No peptidoglycan; therefore resistant to penicillins and cephalosporins

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## Acquired resistance

- The microorganism **acquires** a resistance to one or more antibiotics
  - Following the administration of a given antibiotic or of an antibiotic with similar properties
    - Occurs mostly in the **trough** below the MIC
  - After an encounter of a microorganism that acquired a resistance
    - Transfer of resistance

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## How are Resistance Acquired?

- Microorganisms normally and randomly acquire spontaneous mutations
- The presence of antibiotics exerts a selective pressure
  - The presence of the antibiotic creates a selective environment for microorganisms that acquired by chance a favorable mutation

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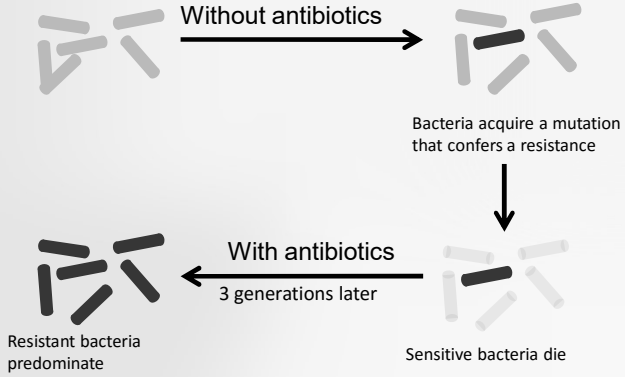
## Spontaneously Acquired Resistance

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## Mechanisms of Resistance

- Impermeability:
  - Reduced number of porins
  - Biofilms
- Inactivation:
  - Proteins that bind and inactivate the antibiotic
- Transport:
  - Pumps the antibiotic outside the cell
    - The internal concentration is below the MIC
- Degradation of the antibiotic
- Modification of the target
  - The antibiotic cannot bind its target

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## Transfer of Resistances

- Exchange of genes/mutations between different strains or species
  - No selective pressure required

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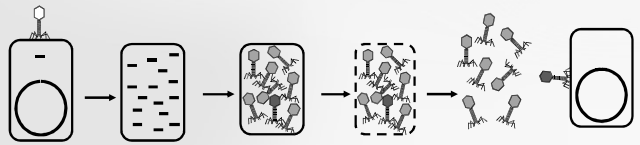
## Transfer Mechanisms - Transduction

- Viral infection of donor bacteria
- Viral DNA replication and fragmentation of bacterial DNA
- Assembly and packaging of DNA
- Release of phage
- Infection of recipient bacteria
- Integration into the genome



•Any gene can be transferred

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## Transfer Mechanisms - Transformation

- Death and lysis of donor bacteria
- DNA fragmentation
- Uptake of DNA by recipient bacteria
- Recombination
- DNA exchange
- Multiplication

•Any gene can be transferred

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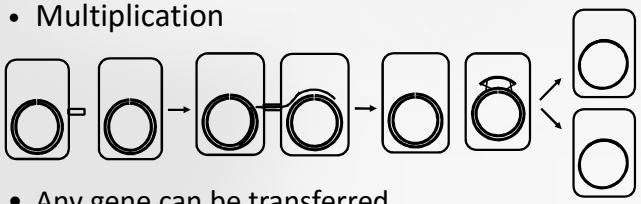
## Transfer Mechanisms - Conjugation

- Donor bacteria with pilus
- Mating with recipient bacteria
- DNA transfer
- Recombination
- Multiplication



- Any gene can be transferred

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## Preventing Acquired Resistances

- Education
- More effectively controlled usage
- Eliminate the prophylactic usage of antibiotics in livestock
- Reduce usage in household products
- Reduce prophylactic usage
- Discovery and synthesis of new antibiotics
- Use a combination of antibiotics

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## Combination Antibiotherapy

- Simultaneous administration of two antibiotics
  - Reduce the probability of acquiring a resistance
  - Use antibiotics for which the microorganism has an intermediate sensitivity
  - Treatment of an infection involving multiple microorganisms

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## Results of Combination Antibiotherapy

- Additive effect (indifferent) :
  - The activity of a combination of two antibiotics is equal to the sum of the individual activities
- Synergistic effect:
  - The activity of a combination of two antibiotics is higher than the sum of the individual activities
- Antagonistic effect:
  - The activity of a combination of two antibiotics is less than the sum of the individual activities

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## Determining Effect of the Combination

- Determine fractional inhibitory concentration (FIC)
  - [(MIC of drug A in combination) ÷ (MIC of drug A alone)] + [(MIC of drug B in combination) ÷ (MIC of drug B alone)]
- Synergism, FIC ≤0.5
- Indifference, FIC >0.5 and ≤4
- Antagonism, FIC >4

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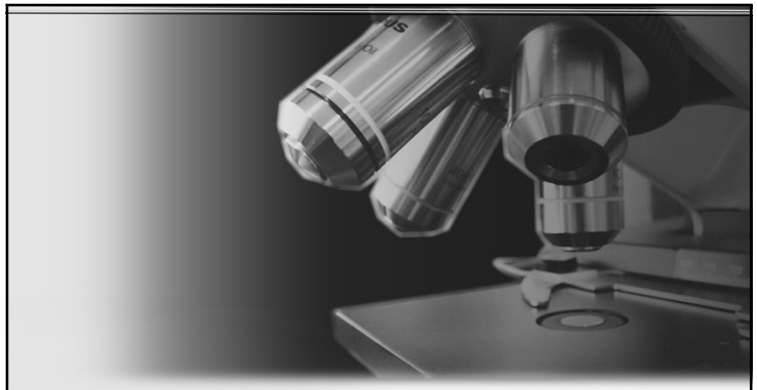

## *Probiotics*

An alternative or complement to antibiotherapy

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## Definition

- For (Pro) life (biotic)
- Live microbial feed supplements that have beneficial effects on the host by improving its intestinal microbial balance

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## Newborn Microbiota

- Mother's microbiota
  - Maternal vaginal and intestinal flora constitutes the source of bacteria, which colonizes the intestine of new born
- Mode of delivery
- Birth environment

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## Factors Affecting Intestinal Microbiota

- Antibiotics and other drug intake
- Microbial infections
- Diet (highly processed, low fiber foods)
- Chronic diarrhea
- Stress
- Radiation and chemotherapy

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## Characteristics of Effective Probiotics

- Able to survive passage through digestive tract
- Able to attach to the intestinal epithelia and colonize
- Able to utilize the nutrients in a normal diet
- Non pathogenic and non toxic
- Capable of exerting a beneficial effect on host
- Anti-inflammatory, antimutagenic, immunostimulatory

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## Probiotics: Modes of Action

- Adherence and stimulation of immune system
  - Enhance secretory antibodies
- Competition for adhesion sites and essential nutrients
- Production of antimicrobial factors
  - Acidophilin, bacteriocin, etc.
- Provide favorable environment for growth of other beneficial bacteria

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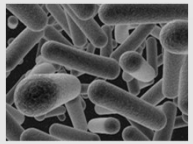
## Common Probiotic Strains

- *Lactobacillus* species
  - *L. acidophilus*
  - *L. plantarum*
  - *L. casei*
  - *L. brevis*
  - *L. delbreuckii*
- *Bifidobacterium* species
  - *B. adolescentis*
  - *B. bifidum*
  - *B. longum*
  - *B. infantis*
  - *B. breve*



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## Probiotics: Proposed Uses

- Infectious diarrhea
- Antibiotic-associated diarrhea
- Irritable bowel Syndrome (IBS)
- Bacterial vaginosis
- Recurrent UTI's
- *H. pylori* infections
- Radiation induced diarrhea
- Constipation

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## Probiotics: Prescribing

- Which organism to use?
  - *Lactobacillus sp.* best studied
- What dose?
  - 10 billion organisms/day
- For How long?
  - Probiotics do not permanently colonize the intestine, thus, daily consumption required

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## Probiotics: Prescribing (*cont'd*)

- Any side effects?
  - 2% risk bloating/gas
  - May cause health problems in immunocompromised individuals
    - Skin rash, fever, bloody stools etc.

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## Probiotic Foods





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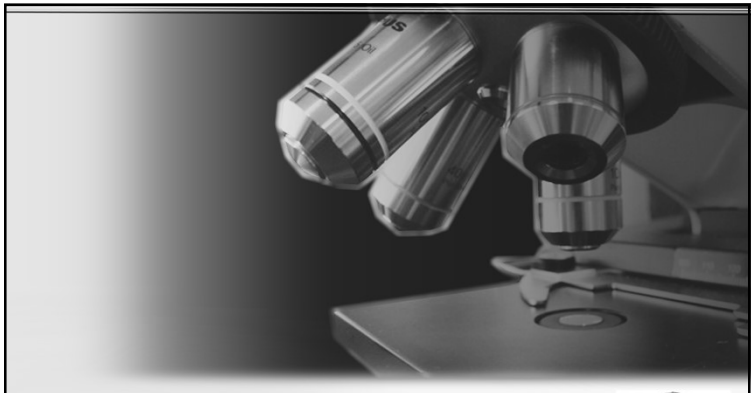

## *Virology*

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## Characteristics of Viruses

- Obligate parasite
- Incapable of multiplying independently
- DNA **or** RNA genome
- Absence of DNA and RNA together in the same virion
- Genomes packaged in a protein capsid

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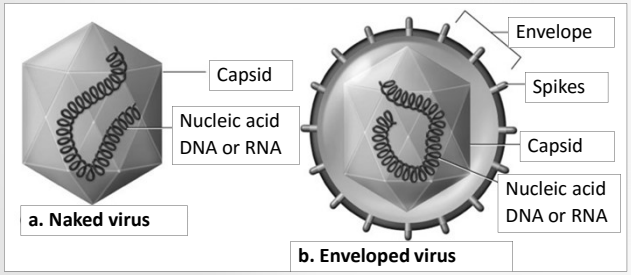
## Virion Anatomy

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## Capsid

- Protein shell that encloses the genome
- Protects the genome from physical, chemical and enzymatic conditions
- Has receptor proteins (spikes) that enable the recognition and attachment to the host cell
- For some viruses, the capsid is surrounded by a lipid bilayer
  - Has enzymes or receptors (spikes) required for attachment and penetration

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## Classification

- 3 primary means of classification:
  - Morphology of protein coat (capsid)
  - Presence or absence of envelope
  - Genome: Nucleic acid

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## Capsid Shapes

- Helical
  - Ex. Ebola virus
- Isometric or Polyhedral
  - Ex. Influenza
- Pleomorphic
  - Ex. Bacteriophages

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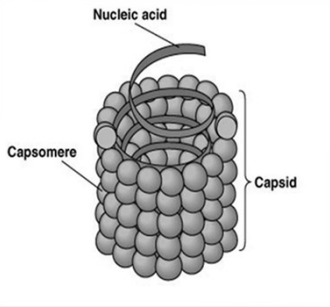
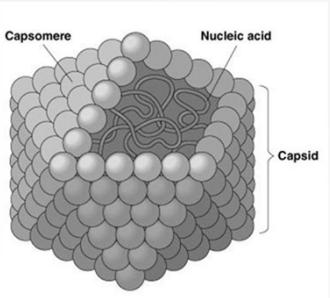
## Capsid Shapes *(cont'd)*

Helical




Polyhedral

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## Genomes

- Single stranded DNA
- Double stranded DNA
- Single stranded RNA
- Double stranded RNA
- One or more molecules (segmented)
- Circular or linear

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## Classification of Viruses

- Groups
  - I. (ds DNA)
  - II. (ss DNA)
  - III. (ds RNA)
  - IV. (+ ss RNA)
  - V. ( - ss RNA)
  - VI. (RNA rev. trans.)

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## Viral Taxonomy

- Family names end in -viridae
- Genus names end in -virus
- Viral species: A group of viruses sharing similar genetic information and ecological niche (host)
  - Common names are used
- Subspecies are designated by a number

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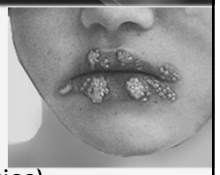
## Viral Taxonomy Example: Herpesvirus



- **Classification:**
  - Herpesviridae (Family)
  - Herpesvirus (Genus)
  - Herpes simplex type 1 / type 2 (Species)
- **Structure:**
  - non-seg., lin., dsDNA, helical, env.

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## Groupings Based on Transmission Route

- Not a taxonomic grouping
  - More than one family may be included in one transmission grouping

| Virus Group | Transmission Route |
|---|---|
| Enteric | Fecal-oral |
| Respiratory | Respiratory |
| Zoonotic | Animal to human |
| Sexually transmitted | Sexual contact |

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## Viral Multiplicaion Cycle - Steps

- Attachment
- Penetration
- Uncoating + Decapsidation
- Genome replication
- Transcription <-> Translation
  - mRNA and protein synthesis
- Assembly
- Release

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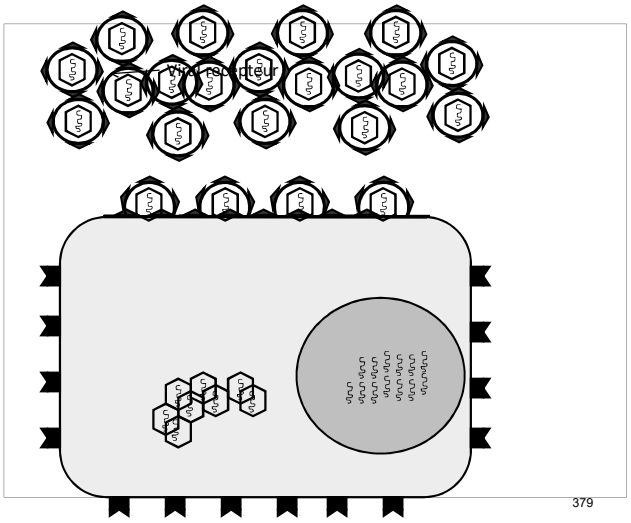


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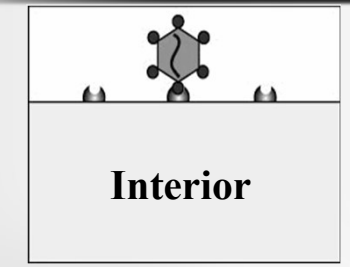
## Attachment and Penetration




- Bacterial viruses – Naked
  - Attachment to receptor
    - Receptor determines **tropism**
  - Injection of genome

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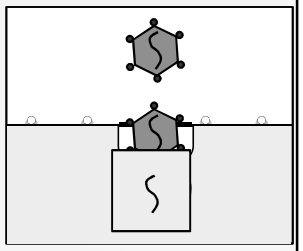
## Attachment and Penetration

- Naked viruses
  - Attachment to receptor
  - Endocytosis of naked virus
  - Release of genome



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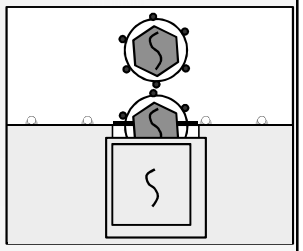
## Attachment and Penetration

- Enveloped viruses
  - Attachment to receptor
  - Endocytosis of env. virus
  - Uncoating
  - Release of genome



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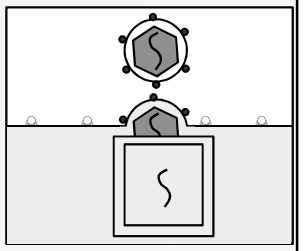
## Attachment and Penetration

- Enveloped viruses
  - Attachment to receptor
  - Fusion of envelope
  - Penetration of nucleocapsid
  - Release of genome



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## Genome Replication

- Generate copies of the original genome
  - Double stranded DNA or RNA = 2 strands; 1+ and 1-
    - Each strand acts as a template for the synthesis of opposing strand
  - Single stranded DNA or RNA = 1 strand; + or –
    - The synthesis of an identical copy of the original strand requires the synthesis of the strand of opposite polarity!!

      – + → - → + or - → + → -

      – + ~~→~~ + or - ~~→~~ -

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## Replication Enzymes

- Nucleic acid polymerases
  - Nucleic acid → nucleic acid
    - DNA dependent DNA Pol.: DNA→ DNA
    - RNA dependent DNA Pol.: RNA→ DNA
    - RNA dependent RNA Pol. RNA → RNA

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## Replication of DNA Genomes

- Small DNA viruses (ex. parvovirus):
  - Host's enzymes perform replication, transcription and translation
  - The viral DNA must be replicated in the nucleus
  - Viral replication requires actively growing cells
  - Host functions are not inhibited

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## Replication of DNA Genomes *(cont'd)*

- Large DNA viruses (ex. Herpesvirus)
  - Host enzymes are used to transcribe the genes required for replication of viral DNA
    - Viral DNA polymerase
  - Viral replication occurs in the nucleus
  - Require actively growing cells
  - Inhibit the host DNA synthesis

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## Replication of DNA Genomes *(cont'd)*

- Very large DNA viruses (ex. Poxvirus)
  - Nucleocapsid contains DNA and RNA polymerases
  - Viral genome is replicated in the cytoplasm
  - Do not necessarily require actively growing cells

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## Replication of SS RNA Viruses

**Positive or Negative strand**

- Positive strand:
  - Sequence is that of mRNA
  - Can be translated into proteins
- Negative Strand:
  - Sequence is complementary to that of mRNA
  - Cannot be translated

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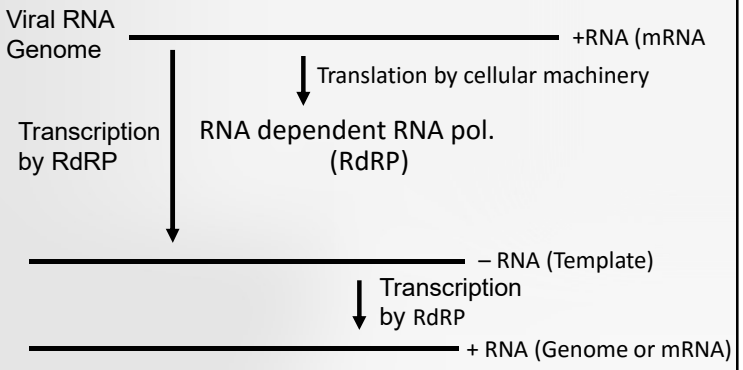
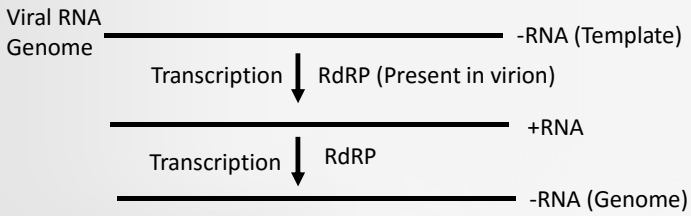
## Replication of Pos. RNA Genomes



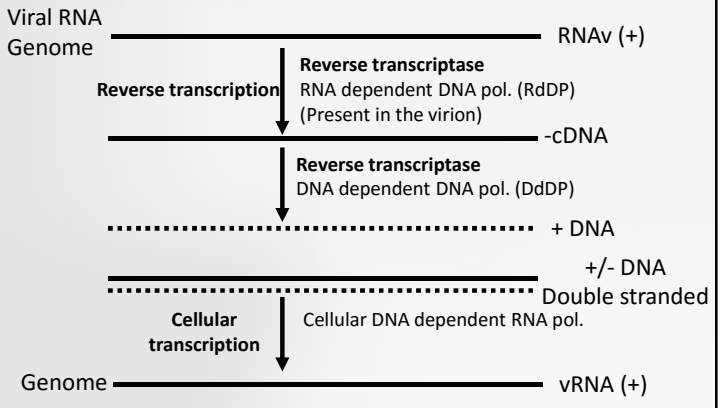

390

## Replication of Neg. RNA Genomes




391

## Replication of Retroviral Genomes – Pos. RNA




392

## Transcription <-> Translation

- Transcription
  - Generate viral mRNA
    - Small and large DNA viruses - Cell dependent process
      - Cellular DNA dep. RNA polymerase
      - Neg. strand of DNA transcribed into mRNA (pos.)
    - Very large DNA viruses – Viral dependent process
      - Viral DNA dep. RNA polymerase
      - Neg. strand of DNA transcribed into mRNA (pos.)

393

## Transcription <-> Translation *(Cont'd)*

- RNA viruses (double stranded)
  - Viral RNA dep. RNA polymerase
  - Neg. strand of RNA transcribed into mRNA (pos.)
- RNA viruses (Pos. single stranded)
  - Viral RNA dep. RNA polymerase
  - Pos. strand of RNA transcribed into neg. RNA (template)
  - Neg. RNA transcribed into pos. RNA (mRNA)
- RNA viruses (Neg. single stranded)
  - Viral RNA dep. RNA polymerase
  - Neg. RNA transcribed into pos. RNA (mRNA)

394

## Transcription <-> Translation *(Cont'd)*

- Translation
  - Always performed by cellular machinery
    - Synthesis of viral proteins
      - Capsid, polymerases, etc.

395

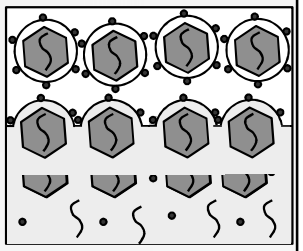
## Assembly and Release

- Capsid is assembled around the genome
- Disintegration of plasma membrane
- Release

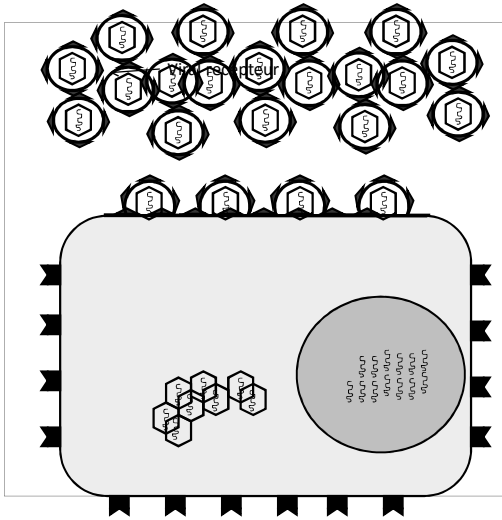
396

## Assembly and Release

- Synthesis of proteins of viral spikes
- Migration of proteins of spikes to the membrane
- Assembly of capsid
- Insertion of genome
- Migration and exocytosis of virions
  - Acquire envelope

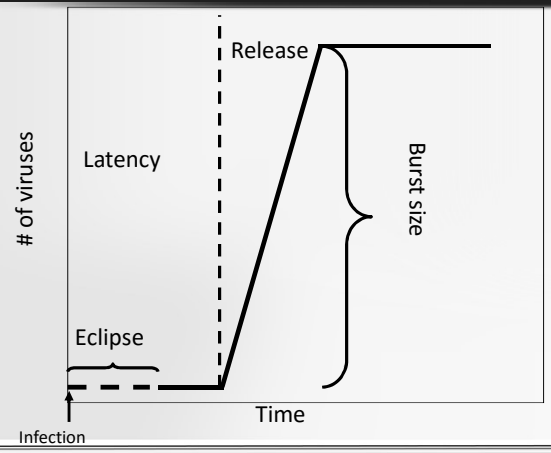


397




398

## Viral Growth Profile




399

## Viral Multiplicaion Cycle - Steps

- Attachment
- Penetration
- Uncoating + Decapsidation
- Genome replication
- Transcription <-> Translation
- Assembly
- Release

Eclipse

Latency

400

## Burst Size

- Definition:
  - Number of viruses generated by **one** infected cell following **one** complete infectious cycle
- Parameters:
  - Number of infected cells is determined according multiplicity of infection (M.O.I.)
    - M.O.I.: Number of infectious viruses available per **one** cell

401

## Multiplicity of Infection

Number of viruses = 4

M.O.I. = 4/6 = 0.67

or

4 of 6 cells will be infected

Number of cells = 6

•402
402

## Multiplicity of Infection

Number of viruses = 10

M.O.I. = 10/6 = 1.6

or

6 of 6 cells will be infected

Number of cells = 6

403

## Calculating the M.O.I.

- $10^8$ cells are exposed to $10^4$ viruses
  - What is the multiplicity of infection?
- How many cells will be infected?

404

## Calculating Burst Size

Number of viruses produced =10

or

10 viruses/4 infected cells

Thus burst size = 2.5 viruses produced/infected cell

Number of infected cells = 4

405

## Calculating Burst Size

- M.O.I.=0.0001
- Number of cells = $10^8$
- After 10 hours there are $10^6$ viruses
  - What is the burst size?

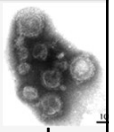
406

## Counting and Typing Viruses

- Direct count
  - Determines the total number of viruses
- Plaque assay
  - Determines the number of infectious viruses
- Hemagglutination assay
  - Determines the total number of viruses
  - Only possible with hemagglutinating viruses
    - Which have hemagglutinin – Ex. influenza
    - Infectious and non infectious
- Hemagglutination inhibition assay
  - Antigenic typing of hemagglutinating viruses

407

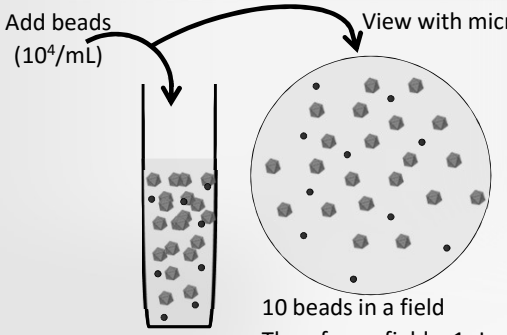
## Direct Counts



- Negative Staining
- Electronic microscope
- Does not discriminate between infectious and non-infectious particles
- Useful for viruses that cannot be grown in the lab
- Use beads at a known concentration to obtain an estimate of the volume of a field of vision

408

## Viral Direct Counts




10 beads in a field

Therefore a field = 1µL

21 virus in a field

Thus 21 virus/1µL = 2.1 X $10^4$ virus/mL

409

## Plaque Assay

- Infect monolayer of cells or bacterial lawn with different dilutions of virus
- Determine the number of plaques for each dilution
  - Each plaque is the result of a single viral particle infecting one cell initially
    - 1 **PFU**

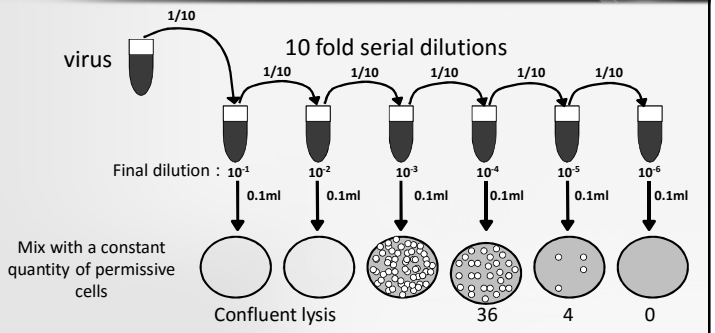
410

## Plaques

- Localized cytopathic effect
  - Cell lysis
- Each plaque represents an infection site
  - Each infection site is initiated by a single infected cell

411

## Plaque Assay



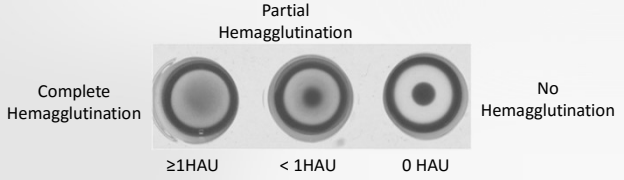

36 PFU for 0.1ml of a $10^{-4}$ dilution

Original conc. = 36PFU/0.1 X $10^4$ = 3.6 X $10^6$ PFU/ml

412

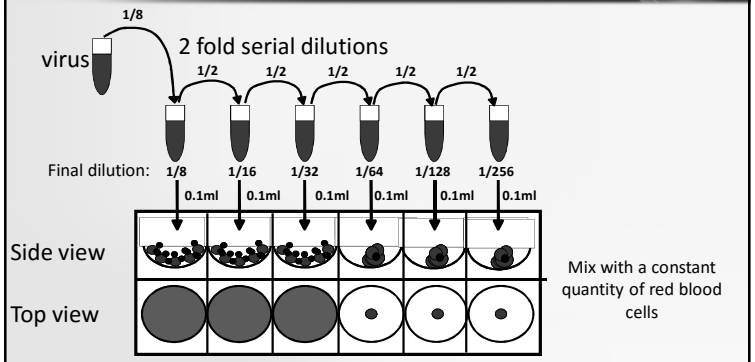
## Hemagglutination Assay

- Measures the minimum quantity of virus required to agglutinate all the red blood cells
  - 1 hemagglutination unit (HAU)




413

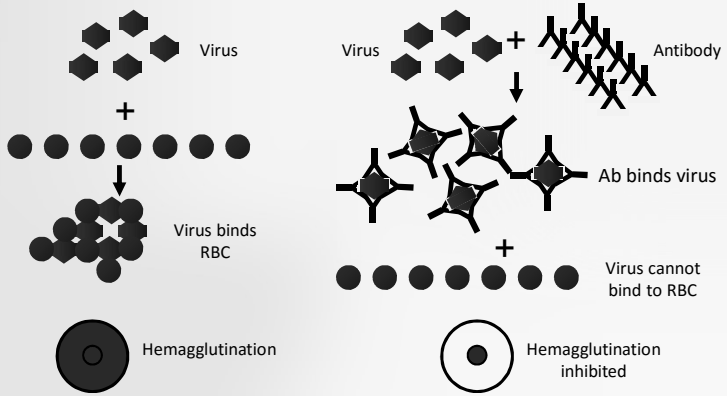
## Hemagglutination Assay



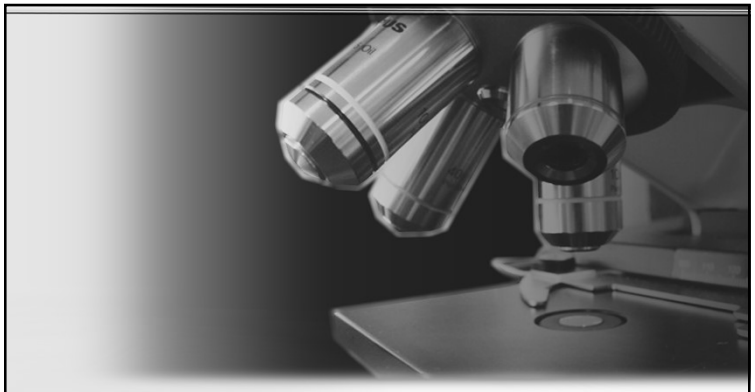

The most dilute sample with complete hemagglutination = 0.1ml of 1/32 = 1HAU
Original conc. = 1 HAU/0.1 X 32 = 320 HAU/ml

414

## Hemagglutination Inhibition Assay




415



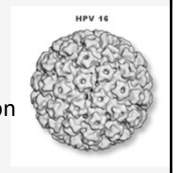
***Viruses Within Everyone's Reach***

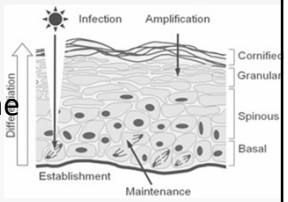
Human papilloma, Influenza and the Human Immunodeficiency Virus

416

## Human Papillomavirus

- Small naked icosahedral
  - 51 types known
    - Types 6, 11, 16 and 18 are the most common
- Tropism:
  - Differentiating cells of the epidermis
- Receptor:
  - unknown
- Double stranded DNA genome
  - Only 6 genes



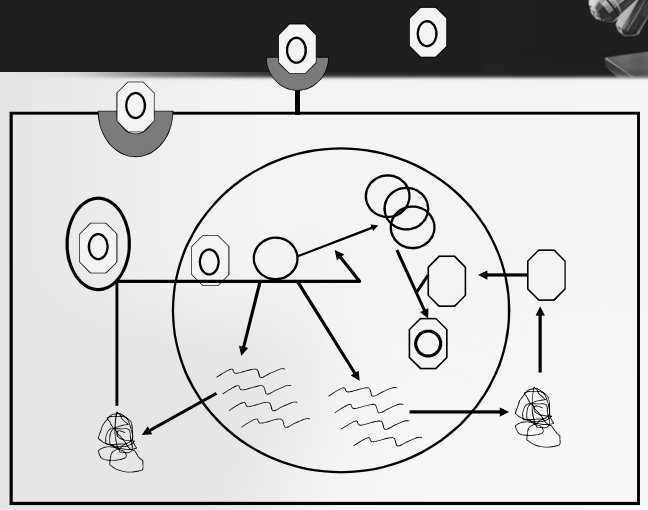




417

## HPV Infectious Cycle

1. Attachment to cellular receptor
2. Endocytosis
3. Penetration of enveloped virus in the nucleus
4. Fusion, uncoating, release of genome
5. Transcription and synthesis of early proteins
   - Non structural regulatory proteins
6. Recruitment and modification of host's DNA polymerase
7. Replication of viral genome
8. Transcription and synthesis of late proteins
9. Assembly of capsid
10. Packaging of genome
11. Cell lysis and viral release

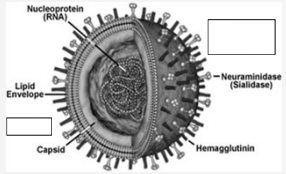
418



419

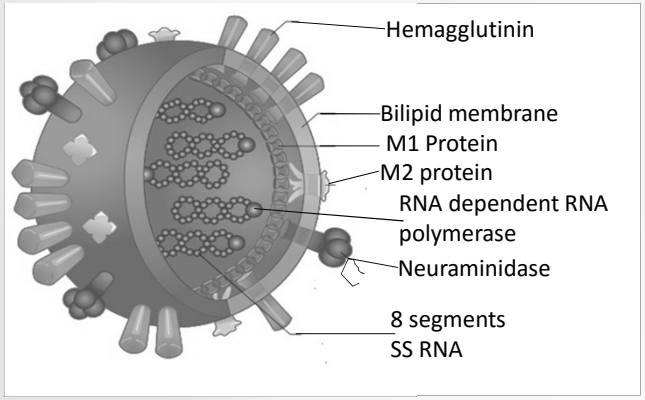
## Influenza

- Enveloped virus
- Three types
  - A, B and C
- Tropism:
  - Epithelial cells of the respiratory passage
- Receptor:
  - Sialic acid
- Segmented (-) RNA
  - 8 segments




420

## Influenza Virus - Anatomy




421

## Influenza Virus – Virion Proteins

- Hemagglutinin
  - Viral receptor
- M1 Protein
  - Scaffold protein - capsid
- M2 protein
  - Involved in uncoating
- Neuraminidase
  - Involved in release
- RNA dependent RNA polymerase
  - Required for viral replication

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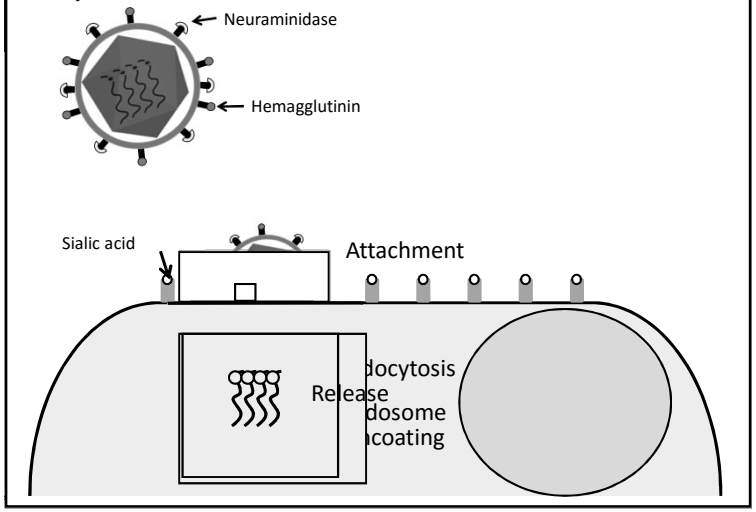
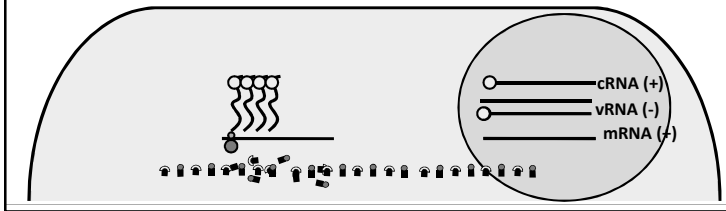
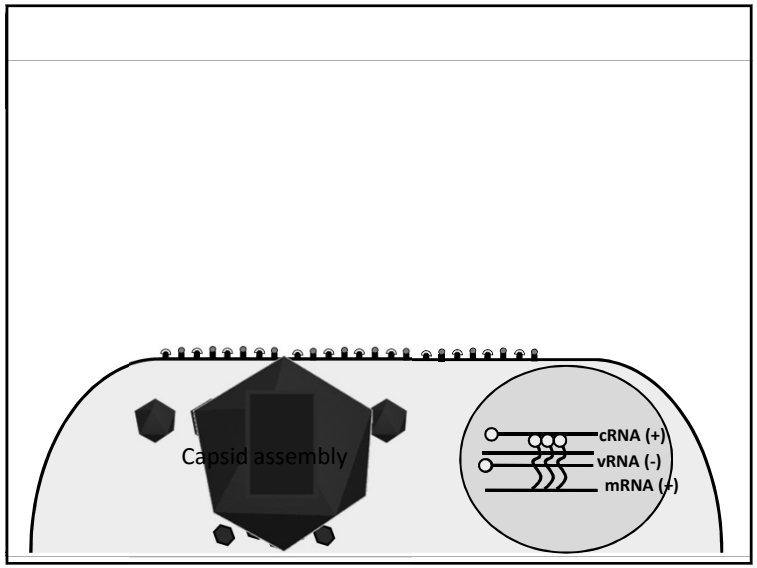
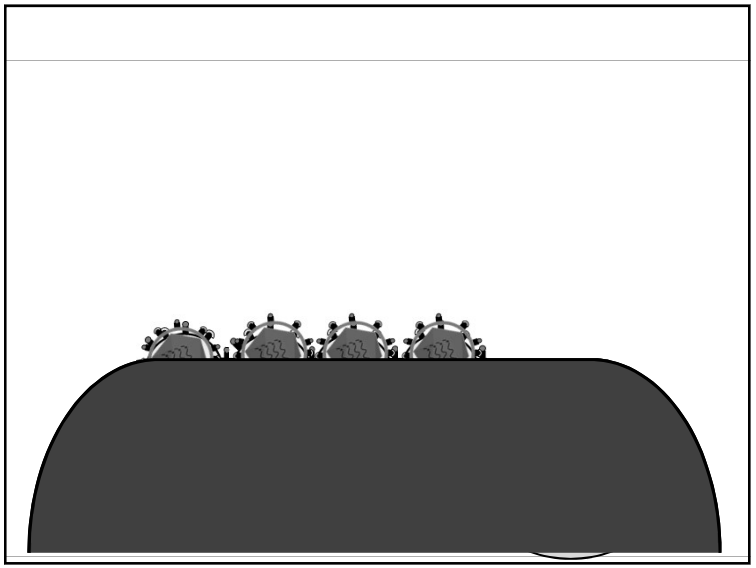
## Cycle Infectieux



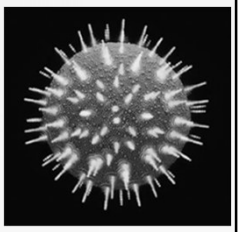





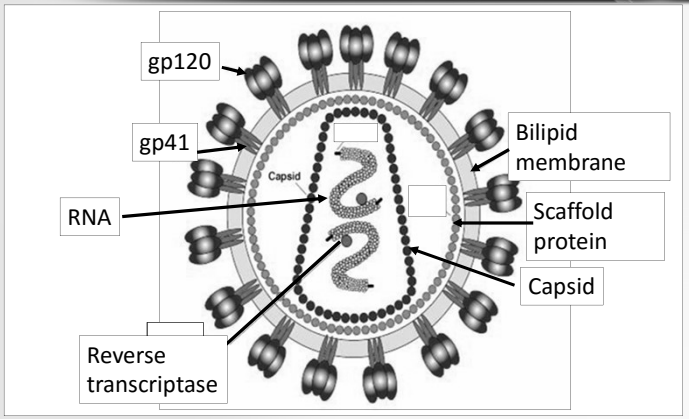
## HIV

- Enveloped icosahedral virus
- Tropism:
  - Cells of the immune system
    - T, monocytes and macrophage
- Receptor:
  - CD4
- +RNA Genome
  - 2 copies



427

## HIV- Anatomy




428

## HIV–Virion Proteins

- Capsid proteins
- Scaffold protein
- Viral receptor
  - GP120 and GP141
- Reverse transcriptase
  - RNA dependent DNA pol. and DNA dependent DNA pol.
- Integrase
  - Allows integration of viral genome into cellular genome
- Viral protease
  - Allows maturation of viral polyproteins

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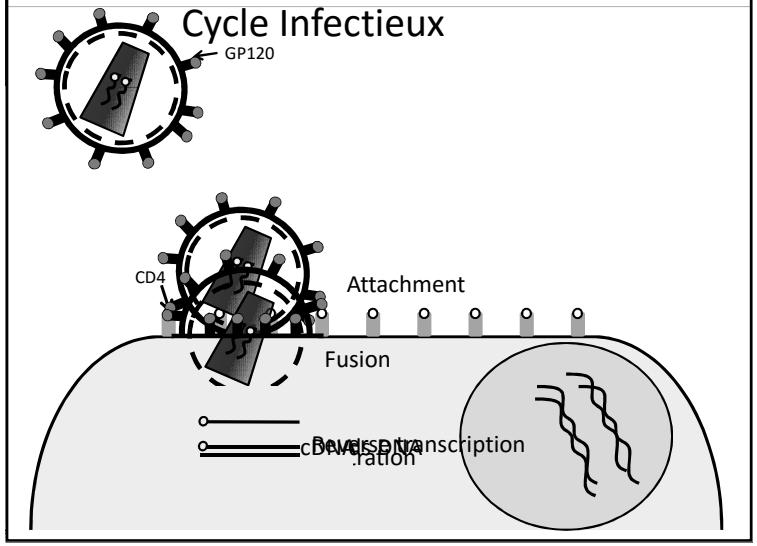
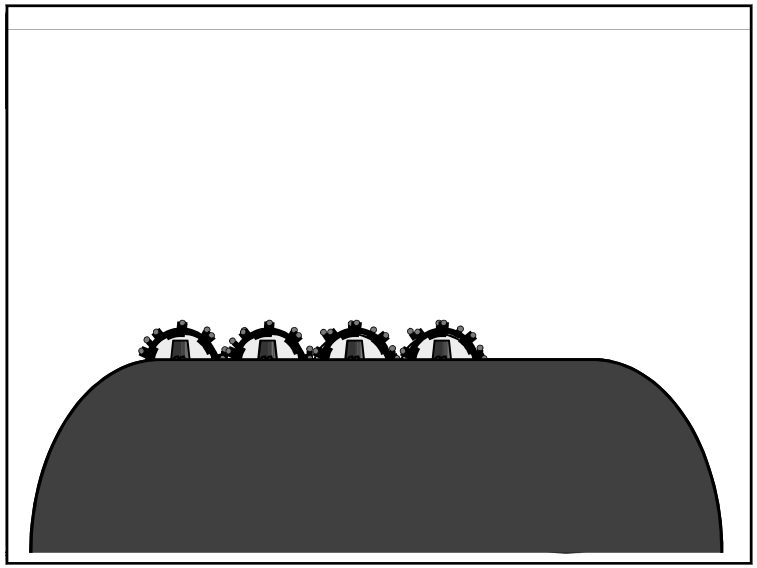
## Infectious cycle of HIV

1. Attachment of gp41/120 to CD4
2. Fusion of the envelope to cell membrane
3. Uncoating and release from capsid
4. Replication and integration of viral DNA genome
5. Transcription of mRNA by cell
6. Translation of polyprotein by the cell
   - gag, pol and env
7. Maturation of polyproteins by protease
   - gag → Capsid proteins
   - pol → Reverse transcriptase and integrase
   - env → gp120 and gp41
8. Capsid assembly
9. Packaging of genome
10. Exocytosis and release

430

## Cycle Infectieux





## Antiviral Drugs

- Several antivirals are prodrugs
  - They must be phosphorylated by viral enzymes to be activated
- Must act against an essential viral function
- Must have an acceptable toxicity for the host

434

## Antiviral Drugs

- Created to inhibit functions which are essential to the viral infectious cycle
  - Entry
    - Attachment
    - Penetration
  - Uncoating
  - Replication
  - Protein maturation
  - Assembly
  - Release

435

## Antiviral Drugs

- Current antivirals have no effect on viruses which are not reproducing!
- A host immune response is essential for recovery from a viral infection

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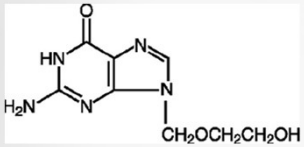
## Current Antivirals

- Purine and pyrimidine nucleoside analogs
  - Ex. Valacyclovir
    - Prodrug of acyclovir
    - Guanine analog
  - Inhibits polymerase and reverse transcriptase

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## Mode of Action of Acyclovir

Acyclovir + ATP —(Viral Kinase)→ Acyclovir phosphate

Acyclovir phosphate —(Cellular Kinase)→ Acyclovir triphosphate

Acyclovir triphosphate → Incorporated in DNA
Inhibits replication

Similar mechanisms for all nucleoside analogs

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## Current Antivirals

- Uncoating inhibitors
  - Ex. Amantidine
    - Inhibits M2 protein of influenza
- Release inhibitors
  - Ex. Oseltamivir (Tamiflu)
    - Inhibits neuraminidase of influenza
- Protein maturation inhibitors
  - Viral protease inhibitors (**PI**)
    - Ex. Atazanavir

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## Antiviral Drugs

- Non desirable characteristics associated with antivirals:
  - Low selective toxicity
  - High therapeutic dose
  - Low toxic dose
  - Low therapeutic index

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## Viroids

- Naked single circular strand of RNA that codes for a single protein
- No protein coat
- Replicated by RNA dep. RNA pol.
- Most infect plants
- Only one known to infect humans
  - Hepatitis D
    - Requires coinfection by Hepatitis B

441

## Prions

- "Infectious proteins"
- Normal proteins (PrPc) that get converted into an alternate configuration by contact with other prion proteins (PrPsc)
- No DNA or RNA
- Inherited and transmissible diseases
  - Spongiform encephalopathies
    - Sheep scrapie, Creutzfeldt-Jakob disease, Kuru, mad cow disease

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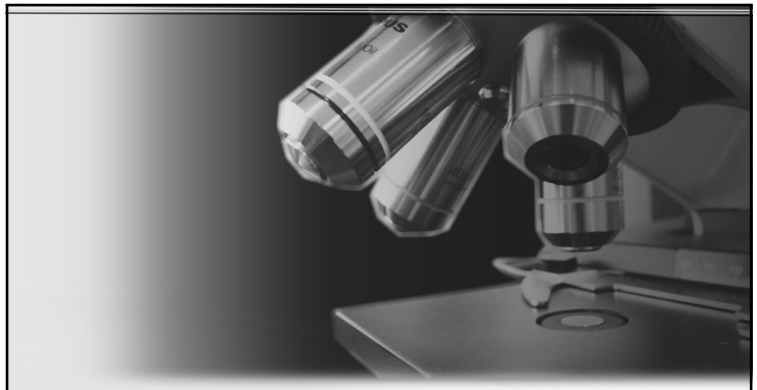

## *Immunology*

The Immune System

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## Immunity

- All the mechanisms used by the human body to protect itself against foreign invaders – The **antigens**
  - 3 lines of defense :
    - 1st – The barriers
      - **Innate system**– no education
      - Active at all times
      - Goal: prevent entry
    - 2nd –Phagocytic system
      - **Innate system** – no education
      - Prevent propagation
      - Destroy foreign entity
      - Recruit and activate the 3rd line of defense
    - 3rd - Acquired or adaptive system
      - **Must be educated - can learn**
      - Destruction/Neutralization specific to the entity
      - Prevent future invasions - memory

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## Characteristics of the Two Systems

**Innate**

- Antigen independent
- Immediate
- Not specific to the antigen
- No immunological memory

**Acquired**

- Antigen dependent
- Delayed
- Specific to antigen
- Immunological memory

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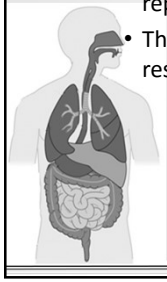
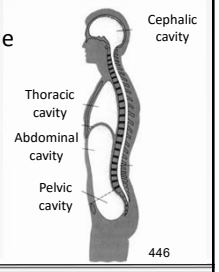
## 1st Line of Defense - Barriers

- Prevent entry inside the body
  - The interior is sterile unless there is an infection
    - In the man, all cavities represent the interior
    - In the woman, all the cavities, except for the pelvic cavity, represent the interior
    - The gastrointestinal tract and part of the respiratory tract are external



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## Barriers – The Skin

| Physical | Chemical |
|---|---|
| • Thick keratinized cells<br>• Sloughing of cells | • High salt concentration<br>• Fatty acids inhibit bacterial growth<br>• **Defensins**-peptide antibiotics<br>• Low pH<br>• Antimicrobial produces by microflora |

447

## Barriers – The Eye

| Physical | Chemical |
|---|---|
| • Tears<br>– Wash/elimination | • Tears-Lysozyme<br>• Lactoferrin<br>– Protein that binds iron<br>• Low $a_w$ |

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## Barriers – Respiratory and Gastrointestinal Tracts

| Physical | Chemical |
|---|---|
| • Nasal hairs and cilia<br>– Filtration<br>• Mucus secretions<br>– Traps particles<br>• Mucociliary escalator<br>• Sneezing and coughing<br>• Secretion of surfactants by A cells<br>– Prevents attachment | • Lysozymes<br>• Lactoferrin<br>• Antimicrobial peptides<br>• Thiocyanate secreted by salivary glands<br>• Gastric acids<br>• Bile salts<br>• Digestive enzymes<br>• Microflora |

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## Barriers – Natural Flora

- Microorganisms located within specific sites of healthy individuals
  - All external surfaces are colonized
  - Competition against pathogens
  - Source of pathogenic organisms (**Opportunists**)
  - Stimulates the immune system
    - Stimulates the production of antibodies against **shared epitopes** common to pathogens

450

## Innate system – 2nd Line of Defense

- Cells and serum substances predisposed for an immediate attack of antigens
  - Alternative complement cascade
  - Blood cells– Leucocytes
    - Polymorphonuclear cells – Granulocytes
    - Monocytes/macrophages
    - Natural killer cells
  - Inflammatory response

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## Alternative Complement Cascade

- Involves a collection of serum proteins
  - Complement proteins:
    - C2, C3, C4, C5, C6, C7, C8 and C9
  - Accessory proteins: B and P
- Initiated by binding to components common to a great variety of bacterial cells:
  - Alternative: Binding of C3b to lipid A or techoic acid

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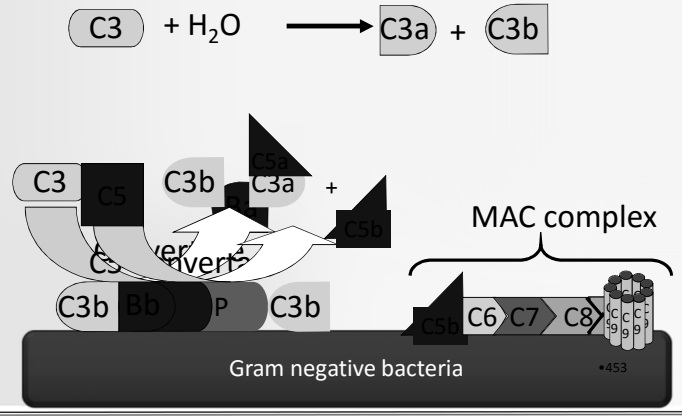
## Alternative Complement Cascade




## Consequences

- Opsonization
  - C3b and C4b
    - Bacteria, viruses and parasites
- Anaphylatoxins
  - C3a and C5a
    - Activate granulocytes and monocytes
      - Induce the inflammatory response
      - Degranulation
- Membrane attack complex (MAC)
  - Osmotic lysis
    - Gram negative bacteria – Allows lysozyme to reach cell wall
    - Parasites – Loss of solutes

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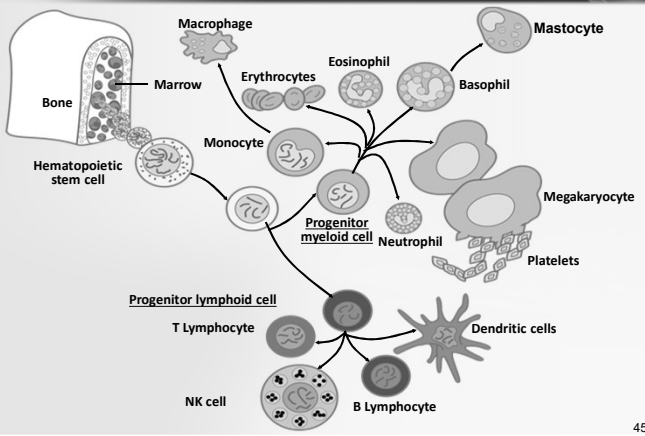
## Blood Cells




455

## Polymorphonuclear Cells - Granulocytes

- Neutrophil
  - Phagocytic cell
  - High concentrations are indicative of an infection
  - Normally absent in healthy tissues
- Eosinophil
  - Fights parasitic infections
  - Involved in allergic reactions
- Basophile/Mastocyte
  - Involved in allergic reactions

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## Roles of Granulocytes

- Destruction of invading entity
  - Phagocytosis
    - Have receptors which recognize opsonines
      - Enzymatic digestion
      - Chemical destruction
        - » Antiseptics: peroxide and hypochlorite
  - Release of granules
    - Inflammatory mediators
    - Digestive enzymes
    - Antiseptics: peroxide and hypochlorite
- Recruit non granular leukocytes
- Recruit lymphocytes – 3rd line of defense

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## Non Granular Leukocytes

- Monocytes/Macrophages/Dendritic cells
  - Phagocytes
    - Have receptors which recognize opsonines
  - **Antigen presenting cells (APC)**
    - Ingestion/Digestion/Presentation
      - Presentation of epitopes from exogenous antigens
      - Presentation on **MHCII**
      - Presentation **T helper** lymphocytes
    - Activate the 3rd line– Acquired response
- Natural killer cells (NK)
  - Lymphocyte which kills infected and tumor cells

458

## NK Cells

- Kill cells which lack or have low levels of **MHCI**
  - Virus infected cells
  - Cancer cells
- Two cell surface receptors
  - Inhibitory receptor
    - Interacts with MHCI of nucleated cells
  - Activating receptor
    - Interacts with stress induced glycoproteins

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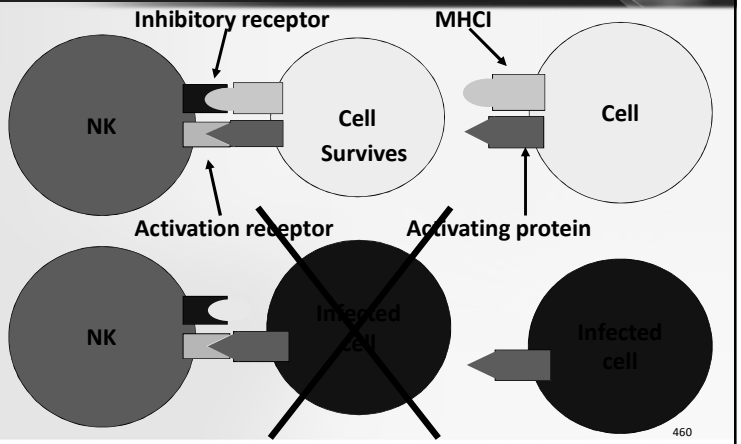
## NK Cell Activity




460

## Inflammatory Response

- Goals:
  - Neutralize or destroy the invader
  - Alert the third line of defense
- Initiators
  - Tissue damage
  - Infections
  - Toxins
  - Anaphylatoxins
- Symptoms
  - Redness, heat, pain, edema, loss of function

461

## Physiological Inflammatory Response

- Release of mediators by leukocytes:
  - Histamines
    - Cause vasodilatation and vasopermeability
      - Allows passage of leukocytes from the blood into the tissues
  - Prostaglandins
    - Act on the thermoregulatory center (hypothalamus)
      - Fever
  - Cytokines
    - Chemotaxis and activation of leukocytes

462

## 3rd Line of Defense – Acquired System

- Characteristics:
  - Specific
  - Acquires memory after a first encounter
  - Improvement of response for subsequent encounters (learns)
  - **Discrimination between "Self" and Non-Self"**

463

## Non-Self

- What is external and which gains access to my interior is probably non-self
- Most of my cells are labelled to identify myself
  - **MHCI** and **MHCII**
- My lymphocytes have receptors which recognize epitopes which I do not possess
  - BCR and TCR
- I label what is non-self with opsonins
  - Complement proteins and antibodies

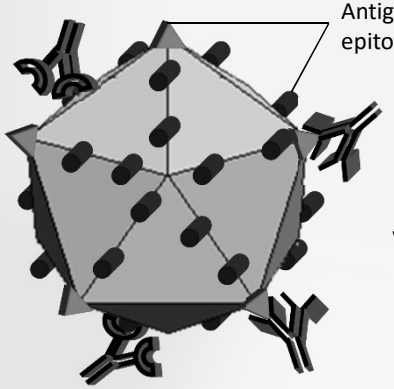
464

## Non-Self

- Antigens
  - Entities recognized by receptors of B (BCR) or T (TCR) lymphocytes
    - Exogenous antigens
      - Extracellular entities
    - Endogenous entities
      - Intracellular entities **synthesized** within the cell
  - Antigens have **epitopes** (or antigenic determinants) that interact with **paratopes** of BCR and TCR

465

## Non-Self – Antigen




**Virus=Antigen**

466

## Exogenous vs Endogenous Antigens

- Exogenous
  - Introduced into the body from outside
    - Includes infectious agents
      - Such as bacteria, viruses, fungi, protozoa, worms etc.,
    - Includes environmental substances
      - Such as foodstuff, pollen etc.
  - APCs actively intake exogenous antigens by phagocytosis and process into fragments
    - Presented on MHC class II molecules to TH cells

467

## Exogenous vs Endogenous Antigens

- Endogenous
  - Are generated within the cells due to normal cell metabolisms
    - Include self-antigens, tumor antigens, and viral antigens
  - May result from intracellular bacterial or a viral infection
  - Do not require phagocytosis
  - Fragments generated from degradation are presented by MHCI to Tc cells

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## Presentation

- Epitopes from antigens must be presented in order to be recognized as non-self

- **MHCI**
  - Present on most nucleated cells
    - Exceptions: red blood cells, neurons and spermatozoa
  - Presents epitopes from endogenous antigens to TCR of $T_c$ lymphocytes
- **MHCII**
  - Present only on APC
    - Monocytes, macrophages, dendritic cells, B lymphocytes
  - Presents epitopes from exogenous antigens to TCR of $T_H$ lymphocytes

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## 3rd line of defense – Two Fronts

| Humoral response | | Cellular response | |
|---|---|---|---|
| Target | Participants | Target | Participants |
| Exogenous Ag | $T_H$ Lymphocytes – TH2 | Endogenous Ag | $T_H$ Lymphocytes – TH1 |
| | B Lymphocytes | | $T_c$ Lymphocytes |
| | Antibodies | | NK cells |
| | Classical complement cascade | | |

470

## Humoral Response

- The humoral response needs **two** signals to be activated
  1. A $T_H$ (TH2) lymphocyte must **determine that an epitope** presented by **MHCII** of an APC from the innate system represents non-self
     - Consequence : $T_H$ is activated
  2. A $T_H$ (TH2) lymphocyte, which was activated, must **confirm** that an epitope presented by **MHCII** of a B lymphocyte represents non-self
     - Consequence : B lymphocyte is activated

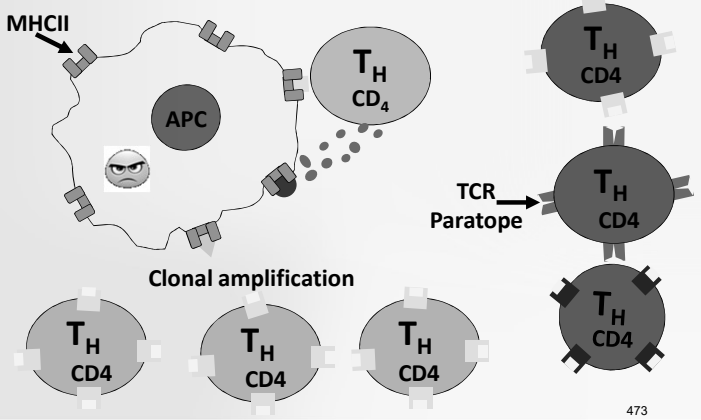
471

## $T_H$ Lymphocytes

- **One** $T_H$ Lymphocyte has TCRs with **one** paratope which can recognize **one** epitope presented by the **MHCII** of APCs
  - Macrophages, monocytes, dendritic cells and B lymphocytes
- Responsible for the discrimination of non-self


472

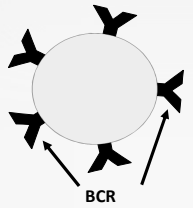
## 1. Presentation to $T_H$ Cells




473

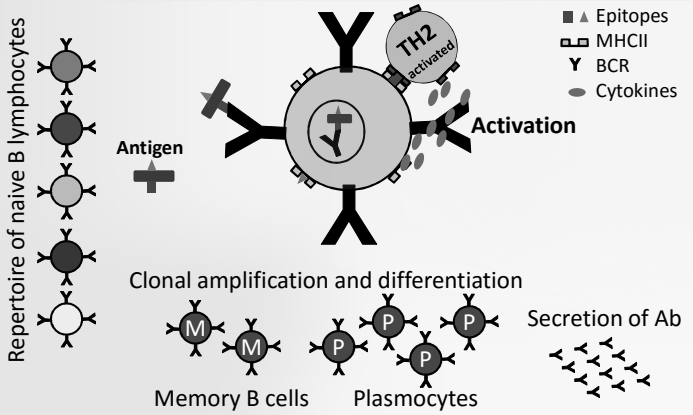
## B Lymphocytes

- Have **one** BCR which can recognize **one** epitope from exogenous antigens
- Do endocytosis of Ag-BCR complexes
- APC: Present epitopes on **MHCII**



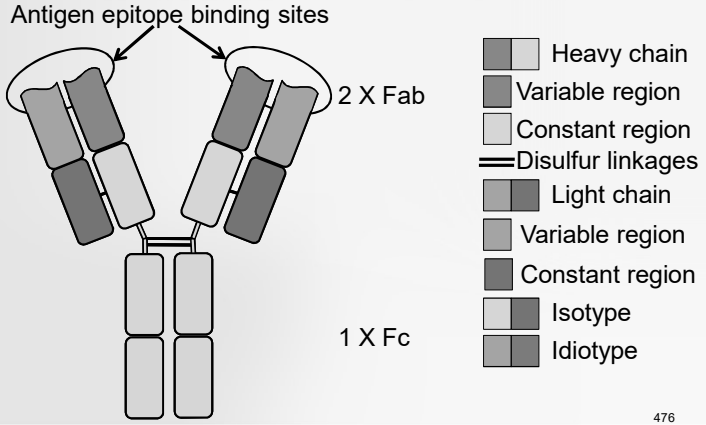

474

## 2. Activation of the Humoral Response




475

## Antibodies (Immunoglobulins)




476

## Antibodies *(cont'd)*

- Variable regions
  - Unique to each B lymphocyte
  - Ab Idiotype
  - Paratope region
  - Confers specificity towards epitope
- Constant regions
  - Ab Isotype
    - IgM, IgA, IgD, IgG, IgE
  - Confers mode of action of Ab

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## Isotypes

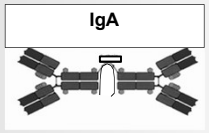


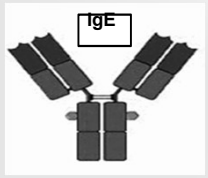


- Membrane bound: BCR
- Monomer; membrane bound: BCR
- Pentamer: Serum
  - First antibody secreted following a first encounter
- Serum antibody:
  - 80% of serum Ab
  - Crosses placenta

478

## Isotypes




- Dimer:
  - Associated with mucus linings
    - Urogenital tract
    - Respiratory tract
    - Gastrointestinal tract
  - Secreted in colostrum
- Monomer:
  - Membrane receptor of basophils and mastocytes

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## Modes of Action of Antibodies

| | |
|---|---|
| • Agglutination | IgM and IgA |
| • Neutralization | IgM, IgA and IgG |
| • Opsonization | IgG and IgM |
| • ADCC | IgG and IgM |
| • Degranulation of granulocytes | IgE |
| • Complement fixation | IgM and IgG |
| – Classical complement pathway | |

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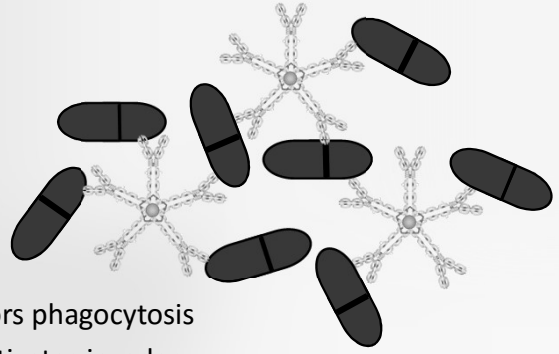
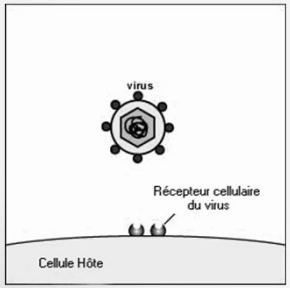
## Agglutination



- Favors phagocytosis
- Inactivates invader

481

## Neutralization




- Binding to essential components resulting in inactivation

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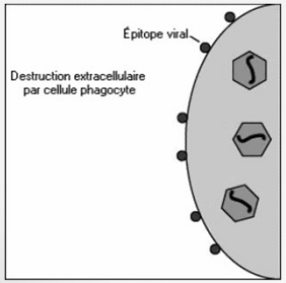
## Opsonization




- Fc regions are opsonins recognized by phagocytic cells

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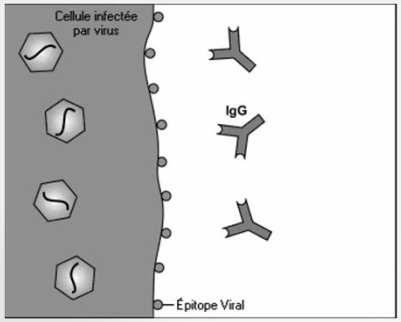
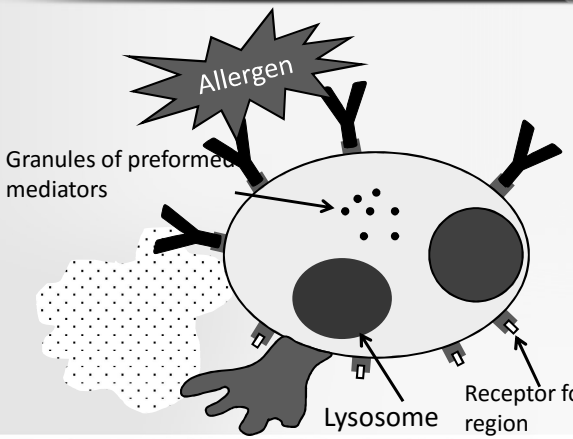
## Antibody Dependent Cell Mediated Cytotoxicity - ADCC




- Fc regions are recognized by receptors on NK cells

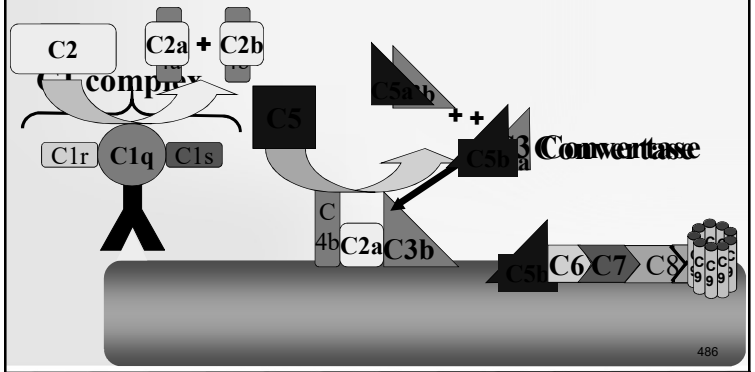
484

## Degranulation




485

## Classical Complement Cascade




486

## Consequences of the Classical Pathway

- Opsonization
  - C3b & C4b
- Anaphylatoxins
  - C3a & C5a
- MAC
  - Osmotic lysis

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## Immune Responses: 1st and 2nd Encounters

| Element | Primary response | Secondary response |
|---|---|---|
| Maximal response | Weaker | Stronger |
| Antigen dose required | Relatively high | Low |
| Delayed response | Between 10-21 days | Between 1-3 days |

488

## Cell Mediated Response

- The cell mediated response requires **two** signals to be activated
  1. A $T_H$ (TH1) lymphocyte must **determine** that an epitope presented by **MHCII** of an APC of the innate system represents non-self
     - Consequence : $T_H$ is activated
  2. A Tc (naive) lymphocyte must also **determine** that the epitope presented by **MHCI** of the same APC represents non-self
     - Consequence : Tc lymphocyte is armed

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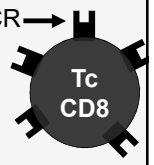
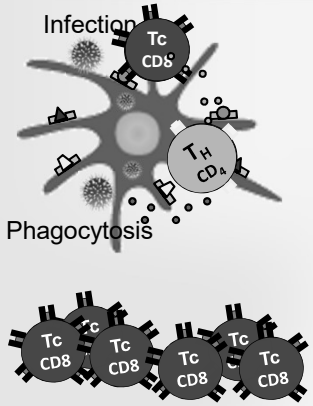
## Tc Lymphocytes




- Cellular receptor – TCR
  - Recognition of non-self presented by **MHCI**
  - **One** Tc lymphocyte has **one** TCR that can recognize **one** epitope complexed with **MHCI**
- Naïve Tc must be armed
  - **Two** signals are required :
    - Recognition by a TCR of a specific epitope complexed with **MHCI**
    - Activating cytokines produced by TH1

490

## Cell Mediated Response




Presentation of endogenous epitopes on MHCI

Presentation of exogenous epitopes on MHCII

Recognition by TCR of TH1 - activation

Recognition by TCR of Tc - arming

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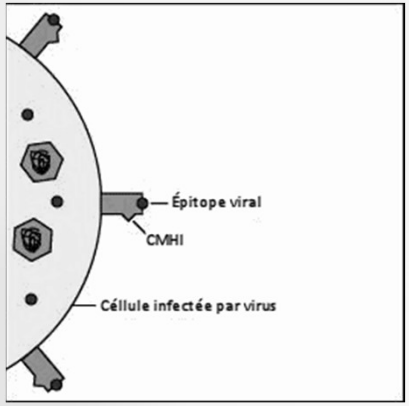
## Attack by Armed T Cell

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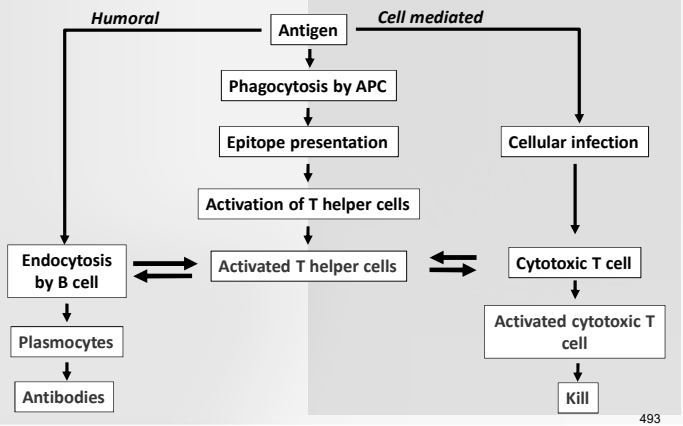
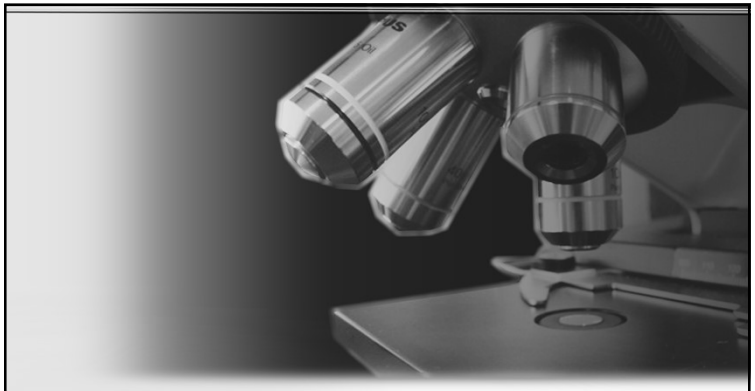
## Overview of Acquired Immune Responses




493



## *Attack of the Immune System - HIV*

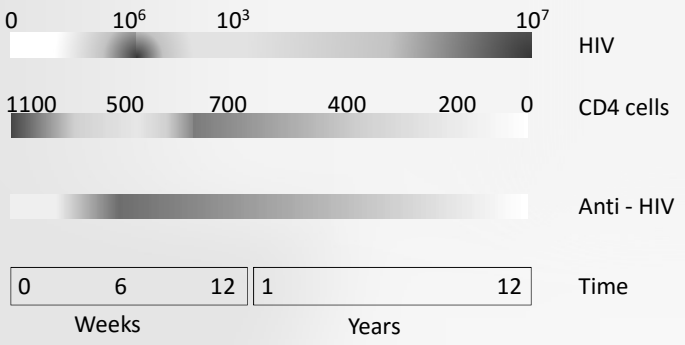
494

## HIV

- Viral receptor
  - Gp120
- Cellular receptor
  - CD4
    - T cells, monocytes and macrophages
- Preferential replication in activated T cells
- Lifespan of a T cell which is actively replicating HIV: 2.2 days

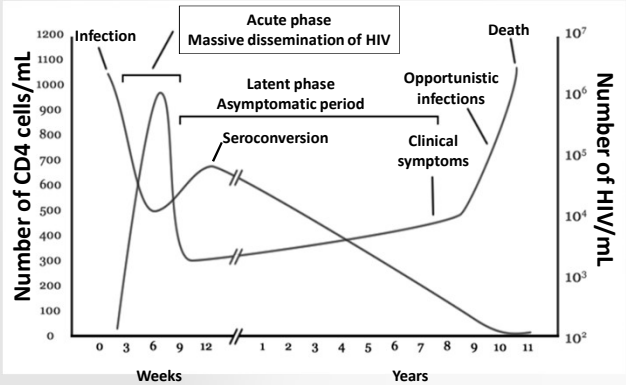
495

## HIV Infection Time Course




496

## HIV Infection Time Course




497

## HIV Infection Time Course

- Primary syndrome (6-12 weeks following the infection)
  - Symptoms similar to mononucleosis
  - CD4 count: Rapid drop 1000 → 500
- Clinical latent period (Up to 10 years)
  - Asymptomatic period
  - CD4 count: Progressive drop 650 → 0
    - From 500 – 200 : at risk of opportunistic infections
- AIDS
  - CD4 count below 200
    - From 200 – 50: High risk of severe opportunistic infections
    - Less than 50 : immuno-incompetence → death

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## *Acquired Immunity*

Vaccines

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## Immunizations

| Type of Immunity | Mode of acquisition |
|---|---|
| • **Active (Prophylactic)** | |
| ➢ Natural | ➢ Infection |
| ➢ Non intentional | |
| ➢ Artificial | ➢ Vaccination |
| ➢ Deliberate | |
| • **Passive (Therapeutic)** | |
| ➢ Natural | ➢ Ab transfer – mother to child |
| | ➢ Transplacental or colostrum |
| ➢ Artificial | ➢ Administration of Ab |

500

## Definitions

- Vaccine:
  - Suspension of attenuated or killed microorganisms or a fraction of these administered to induce an immune response and thus prevent the infectious disease
- Anatoxin:
  - Modified non toxic version of a toxin which retains its immunogenicity
- Adjuvant :
  - Compound added to vaccine preparations which increases the immune response

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## Content of Vaccine Preparations

- Proteins, polysaccharides, nucleic acids
- Preservative
  - Thiomersal (an organomercurial)
    - Antibacterial and antifungal
- Adjuvant
  - Aluminum salts
- Organism or some component of it

502

## Types of Vaccines

- Attenuated (live)
  - Less virulent, but live, version of a pathogen
- Inactivated (dead)
  - Bacteria or viruses killed with heat or formaldehyde
  - Anatoxin vaccines
  - Subunit vaccines
    - Protein or other purified component from the pathogen

503

## Goals of Vaccination

- Protecting the individual – Protection efficacy
  - Protect against infection
    - Prevent entry, growth, propagation, cell entry
  - Protect against disease
    - Prevent damages (symptoms) caused by pathogen or product thereof
- Protecting the population – Herd Immunity
  - Restrict propagation between individuals

504

## Attenuated Vaccines (*live*)

- Attenuated or eliminated virulence
  - Attenuation is a consequence of mutations
    - Ex. Measles, mumps, rubella, influenza, tuberculosis
- Advantages:
  - High efficacy
  - Mimics infection
  - Structures remain unchanged
- Disadvantages:
  - May induce symptoms
  - May cause the disease in immunodepressed individuals
  - Can revert to wild type

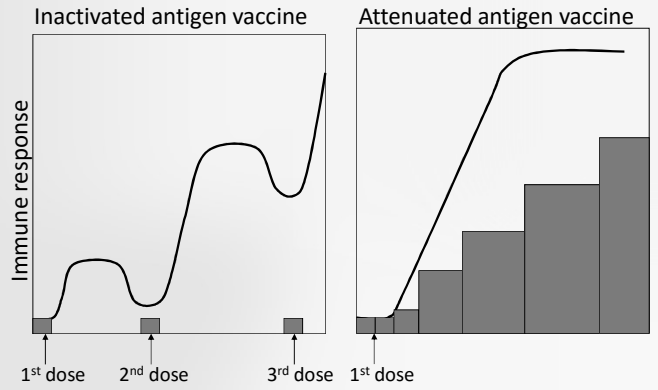
505

## Inactivated Vaccines

- Inactivation by physical methods
  - Heat treatment
  - Formaldehyde treatment
    - Anthrax, Cholera, Pertussis, Influenza
- Advantage:
  - Very safe
- Disadvantages:
  - Antigenic structures may have changed
  - Short term and weak immunity
  - Does not mimic infection
  - Allergic reactions
  - Booster shots usually required
  - Adjuvants required

506

## Immune Response – Inactivated Vs Attenuated




507

## Smallpox Vaccine

- Infectious agent: Smallpox Virus
  - Single host: Humans
- Vaccine: Vaccinia virus (cowpox)
  - Live virus (infectious, attenuated)
  - The vaccine strain could transmit itself from immunized to non immunized individuals!!
  - Allowed the eradication of the virus and its disease
    - Last case 1977

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## Influenza Vaccines

- 2 versions
  - Inactivated
    - 2 type A strains and 1 type B strain
    - Virus is grown in chicken embryos and then killed
    - Combined treatment of heat and formaldehyde
    - Only induces a humoral response
  - Attenuated (LAIV)
    - Attenuated by cold adaptation at 25°C
    - Induces a humoral as well as a cell mediated immunity

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## DPT Vaccine

- D - Diphteria
  - *Corynebacterium diphtheriae*
    - Diphteric anatoxin
- P – Pertussis (whooping cough)
  - *Bordetella pertussis*
    - Pertussis antigen
- T - Tetanus
  - *Clostridium tetani*
    - Tetanic anatoxin

510

## Gardasil - HPV

- Preparation of the L1 capsid protein
  - Protein L1 auto-assembles generating non-infectious virus like particles - **VLP**
  - The VLPs induce a strong humoral response
    - Protects against the infection not the disease
    - Non-therapeutic


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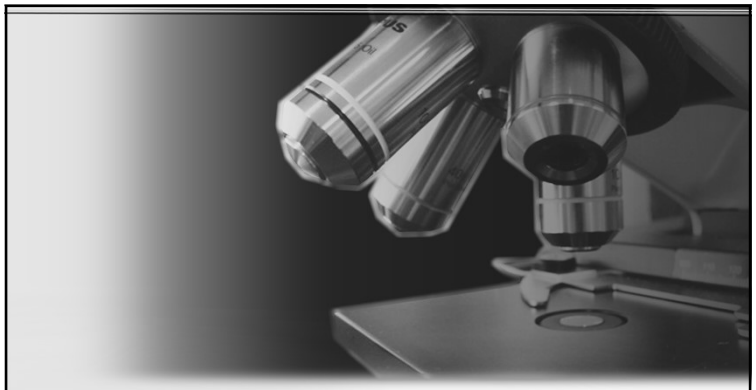
## MMR Vaccine

- Multivalent live attenuated vaccine against measles, mumps and rubella
  - Measles RNA virus propagated in human cells
  - Mumps RNA virus propagated in chick embryos
  - Rubella RNA virus propagated in human cells
- Efficacy 95%
- Lifelong Immunity

512



# *Medical Microbiology*

Host-Pathogen Relationship

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## What is a Disease?

- Any change to a healthy state in which the whole or part of the body of the host is not in perfect balance
  - **Infectious** - A diseased state due to the presence of a pathogen or its products
  - **Non Infectious -** A diseased state due to non-living causes
    - Genetics, poisoning, environmental, etc.

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## The Pathogen and the Infection

- Pathogen :
  - Any organism which has the ability to cause an infectious disease
- Infection :
  - State when a pathogen grows and multiplies in the host
    - An infection can cause or not cause a disease
    - **Infection is not synonymous with disease**

515

## Types de Pathogens

- Primary pathogens
  - Cause disease after infection
  - Are not normally associated with the host
    - Ex. TB (Mycobacterium tuberculosis), Influenza
- Opportunistic pathogens
  - Cause illness in certain circumstances
  - Can be part of the natural flora
    - Ex. *Enterococcus faecium, Candida albicans* (natural flora)
    - Ex. *Pseudomonas aeruginosa, Serratia marcescens* (Environmental microorganisms)

516

## Classes of Microbial Pathogens

- Bacteria (Primary and opportunistic)
  - Ex. Primary: TB; Opportunistic : *E.coli*
- Fungi (Mostly opportunistic)
  - Ex. Yeast (candidosis)
- Protozoa (Primary)
  - Ex. *Plasmodium spp*. (malaria), *Trypanosoma spp*. (sleeping sickness)
- Virus (Primary)

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## The Infectious Disease

- How does it establish itself?
- 3 Requirements :
  - A susceptible host
  - A pathogenic agent
  - A favorable environment for the pathogen

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## To Become a Disease Causing Agent

- 7 Commandments
  - Find an appropriate host
  - Obtain access to the interior
    - Penetration
  - Find a site of establishment
    - Adherence
  - Multiplication
  - Cause harm
  - Exit
  - Transmission to a new host

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## Penetration

- Penetration of skin
  - Most difficult barrier to penetrate
  - Penetration is dependent on trauma that destroys skin integrity
    - Scratch, cut, needles, insect bite, etc.
- Penetration of mucous membranes
  - Most common route of entry
    - Ingestion, inhalation, sexual contact (urogenital tract or anus)

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## Adherence

- Pathogen must adhere to host cells to establish infection
  - Bacterial adherence factors:
    - **Adhesins - L**ocated at the top fimbriae
    - **S-layer** – Outer protein layer similar to capsule
    - **Glycocalyx or capsule** – Outer polysaccharide layer
    - **LPS layer**
    - **Techoic acid**

521

## Harming the Host

- Production of poisons such as toxins and enzymes which damage or kill cells and tissues
- Direct invasion and destruction of host cells
- Initiate an immune response of the host, which leads to symptoms

522

## Toxins

- Endotoxin:
  - Structural component of the membranes of Gram-negative bacteria (LPS)
    - Only toxic if it is released
      - Cell lysis
- Exotoxins:
  - Proteins synthesized and secreted by bacteria
    - Not a structural component

523

## Properties of Toxins

| Endotoxins | Exotoxins |
|---|---|
| – Thermostable | – Thermolabile (60-80$^{o}$C) |
| – Low toxicity (mg/Kg) | – High toxicity (µg/Kg) |
| – Inflammatory<br>• Non specific | – Effects associated to specific symptoms |
| – Weakly immunogenic | – Highly immunogenic |
| – Pyrogenic (fever) | – Non pyrogenic |

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## Endotoxins

- Lipopolysaccharide:
  - Structural component of gram negative bacteria
    - Lipid A
  - Only active when released as a consequence of cell lysis
  - Causes **endotoxemia** :
    - Free endotoxin in the blood stream
  - Cause **Septicemia** (septic shock)
    - Systemic inflammatory response

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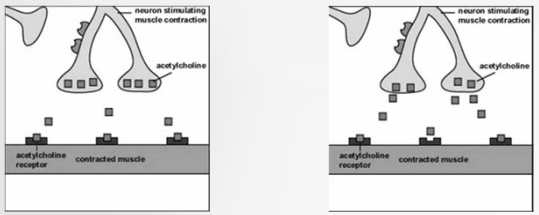
## Classes of Exotoxins

- Neurotoxins
  - Interfere with synaptic transmission of neurons
- Enterotoxins
  - Interfere with the reabsorption of water by mucosa
    - Respiratory and intestinal tracts
- Cytotoxins
  - Inhibit specific cellular functions
    - Ex. Protein synthesis

526

## Neurotoxins (*cont'd*)

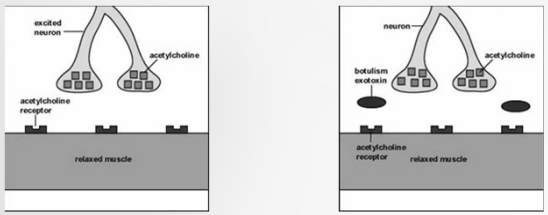
- Tetanic toxin (*Clostridium tetani*)
  - Inhibits neurotransmitter secretion by inhibitory neurons
  - Causes spastic paralysis




527

## Neurotoxins (*cont'd*)

- Botulinum toxin *(Clostridium botulinum)*
  - Inhibits discharge of acetylcholine
  - Cause flaccid paralysis




528

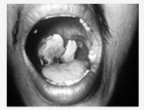
## Enterotoxins - Cholera Toxin

- The toxin stimulates the production of cAMP
- An increase in cAMP levels causes loss of electrolytes and water from cells lining the intestine
- Causes very abundant watery diarrhea (1L / h)
  - Severe dehydration
  - Death (18 hours to days)

529

## Cytotoxins – Diphtheria Toxin

- Produced by *Corynebacterium diphteriae*
  - Inhibits protein synthesis causing cellular death:
    - Localized (mucous membrane of the throat)
      - Degeneration of the epithelial cells of the throat
      - Inflammation and edema
      - Pseudomembrane formation
    - Systemic
      - Heart failure
      - Inhibition of the nervous system - paralysis



530

## Exit

- Damage caused to the host facilitates exit
  - Ex. *Vibrio cholerae* causes diarrhea
  - Ex. *Bordetella pertussis* causes coughing
- May use natural bodily functions
  - Speech, urine, semen, vaginal secretions
- May use a vector
  - Insect bite, needles

531

## Consequences of the Infectious Disease

- The result of the infectious disease depends
  - Properties of the host
    - The immune response
  - Properties of pathogen
    - Virulence

| The host is sick<br>He dies | The host is sick<br>He recovers | The host is not sick<br>Infection without disease |
|---|---|---|
| Defenses failed | Defenses eventually worked | Defenses worked |
| No immunological memory | No or poor immunological memory | Immunological memory from a previous encounter |
| Highly virulent agent | Virulent agent | Non-virulent agent |

532

## Virulence

- Virulence is quantitative term referring to pathogen's disease-causing ability
  - Measure of the severity of the damage caused to the host
  - High virulence = high pathogenicity
- Level of virulence is a function of both properties of the pathogen and of the host

533

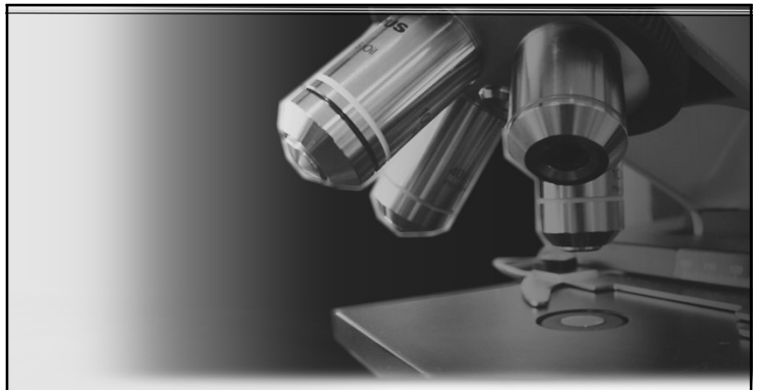
## Measure of Virulence

- Infectious dose ($ID_{50}$)
  - Minimum number of organisms to cause the disease in 50% of hosts
    - Apparition of symptoms
- Lethal dose ($LD_{50}$)
  - Minimum number of organisms required to kill 50% of hosts

***Bacillus anthracis***

| Dose / Route of entry | $ID_{50}$ | $LD_{50}$ |
|---|---|---|
| **Skin** | **10-50** | **50 - 250** |
| **Inhalation** | **1 000 - 8 000** | **8 000 – 10 000** |
| **Ingestion** | **25 000-100 000** | **150 000-500 000** |

534

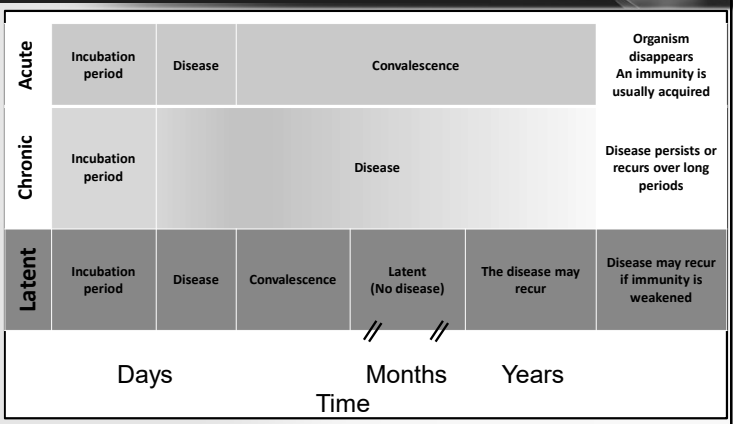


## *Host:Pathogen Relationship*

The Infectious Disease

535

## Classification of Diseases by Duration




536

## Classification of Infectious Diseases

- As a function of the site of infection
  - Local
    - Confined to a specific area of the body
  - Systemic
    - Infection with a generalized distribution in the tissues
- As a function of the order of declaration
  - Primary
    - Initial infection in a healthy individual
  - Secondary
    - Occurs in a person weakened by a primary infection

537

## Progression of the Acute Disease

- Incubation period:
  - Includes meeting, the penetration, adherence and growth
  - Average duration of 2-3 days, several weeks or months
- Prodromal period:
  - Precedes the expression of specific symptoms
    - Ex. Headache, dizziness, gastrointestinal pain
  - Represents the beginning of the pathogenic activity

538

## Progression of the Acute Disease

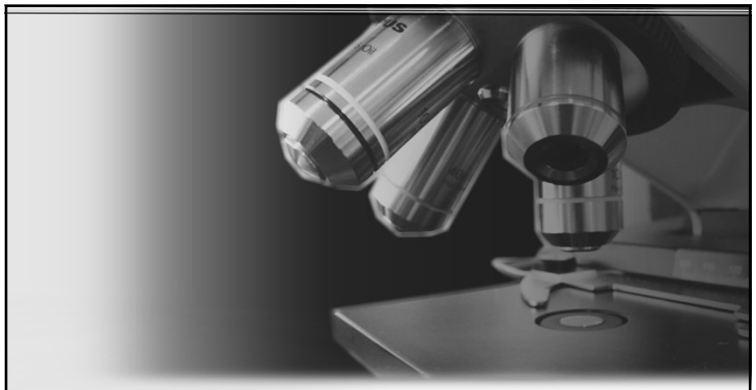
- Acute period
  - Interactions between pathogen and host are maximal
    - Active contribution of the inflammatory response
    - Specific and non-specific symptoms
      - 1st encounter
        - » The acquired immune response is initiated, there are no antibodies present
      - 2nd encounter
        - » High level of activity of the acquired system
        - » High levels of antibodies

539

## Progression of the Acute Disease

- Convalescence:
  - Recovery from the acute period
  - Decrease of specific and non-specific symptoms
  - Antibody level is at its peak

540



## *Clinical Microbiology*

Diagnostic

541

## Diagnostic

- Establish and confirm the etiology of the disease
- Track the progression of the infection in the patient

542

## Methods

- Microscopy and biochemical tests
- Immunological
  - Precipitation test
  - Agglutination test
  - Complement fixation test
  - ELISA
  - Immunochromatography
- Molecular
  - Hybridization
  - PCR and RT-PCR

543

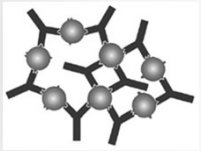
## Microscopy and Biochemical

- Microscopy
  - Gram Stain
  - Acid fast stain
- Biochemical tests
  - Identification based on metabolic characteristics
    - Ex. Oxygen Requirements
    - Carbon sources used
    - Oxidative or fermentative metabolism
    - Metabolic byproducts

544

## Immunology – Precipitation Tests

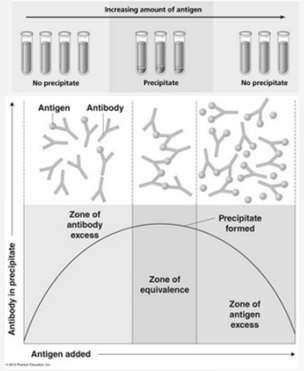
- Principal
  - When antibodies react with multiple epitopes on soluble antigens, there is formation of networks which generate an insoluble precipitate
  - Precipitation reactions can take place in solution or in gels such as agar



545

## Phenomena of Zones

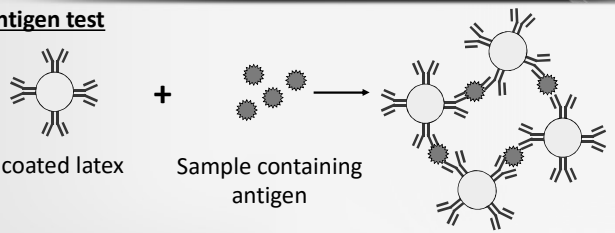
- The precipitate formation is influenced by the concentration of Ag - Ab
- Used for the detection of Ab
  - Ex. Khans Test for Syphilis




546

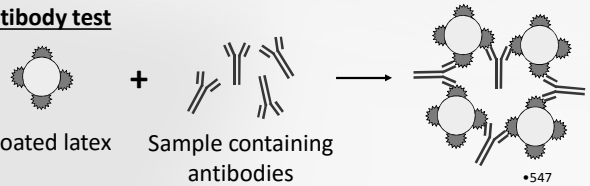
## Latex Agglutination

**Antigen test**



Ab coated latex + Sample containing antigen

**Antibody test**



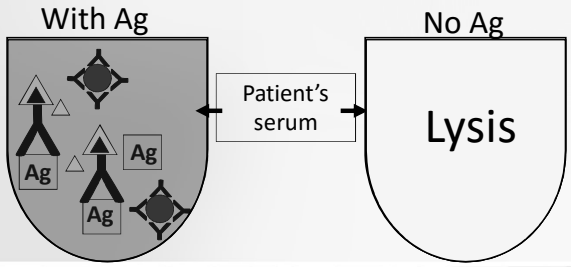
Ag coated latex + Sample containing antibodies

•547

547

## Complement Fixation

- Method
  - Add Ab against Ag to detect
  - Add a limiting concentration of complement
  - Add IgG sensitized RBC




548

## Complement Fixation *(cont'd)*

- Test that determines whether complement was used as a result of specific binding of Ab to Ag
  - Complement used – No lysis of RBC
    - Complement fixation
    - Antigen present
  - Unused complement – lysis of RBC
    - No complement fixation
    - Antigen absent
- Test can be performed quantitatively
- Moderate sensitivity

549

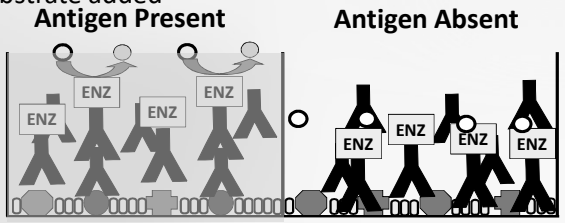
## Dosage by ELISA

- Used to detect the presence of antibodies or antigens
  - Very sensitive
  - Quantitative
  - Quick

550

## ELISA –Antigen Detection

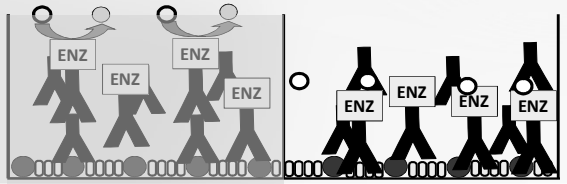
Serum (source of Ag) Added to wells
Blocking agent added
Ab against Ag added
Wash
Detecting Ab added
Wash
Substrate added




551

## ELISA –Antibody Detection

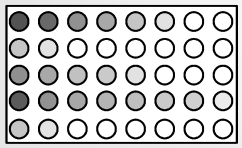
Target Ag for Ab to be detected added to wells
Blocking agent added
Test serum added
Wash
Detecting Ab added
Wash
Substrate added




552

## Interpretation of Results

Assay for Ag of virus X



ELISA plate

Dilutions

| | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 |
|---|---|---|---|---|---|---|---|---|
| Standard | 3.8 | 1.9 | 0.8 | 0.4 | 0.2 | 0.1 | 0.04 | 0.03 |
| Patient 1 | 0.1 | 0.05 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Patient 2 | 0.8 | 0.4 | 0.2 | 0.1 | 0.05 | 0.03 | 0.03 | 0.03 |
| Patient 3 | 3.6 | 3.0 | 1.6 | 0.8 | 0.4 | 0.2 | 0.1 | 0.05 |
| Neg. control | 0.12 | 0.06 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |

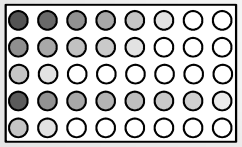
Absorbance readings

Conclusions:
Patient 1 is not infected
Patients 2 & 3 are infected
Patient 3 has an Ag load 8X higher

553

## Interpretation of Results

Assay for Ab against virus X



ELISA plate

Dilutions

| | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 |
|---|---|---|---|---|---|---|---|---|
| Standard | 3.8 | 1.9 | 0.8 | 0.4 | 0.2 | 0.1 | 0.04 | 0.03 |
| Patient 1 | 2.5 | 1.2 | 0.6 | 0.3 | 0.15 | 0.07 | 0.03 | 0.03 |
| Patient 2 | 0.1 | 0.05 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Patient 3 | 3.6 | 3.0 | 1.6 | 0.8 | 0.4 | 0.2 | 0.1 | 0.05 |
| Neg. control | 0.12 | 0.06 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |

Absorbance readings

Conclusions:
Patient 1 & 3 are seropositive
Patients 2 is seronegative

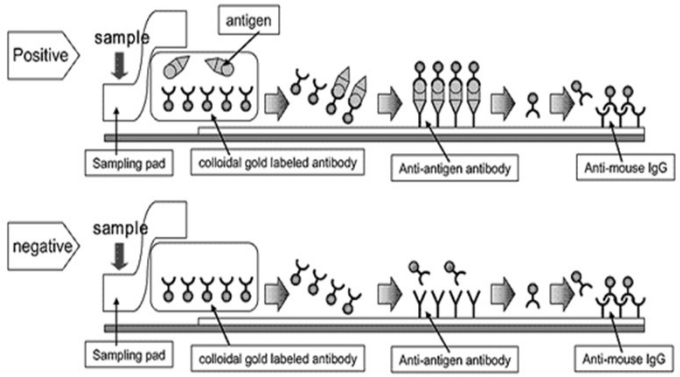
554

## Immunochromatography

- Same principal as the ELISA
  - Qualitative rather than quantitative
  - Detects interaction of a specific antibody against an antigen
    - Colorimetric Method
  - Basis of several rapid tests
    - ≤ 15 minutes
    - Used for bacterial and viral pathogen detection
    - Can also be used for antibody detection

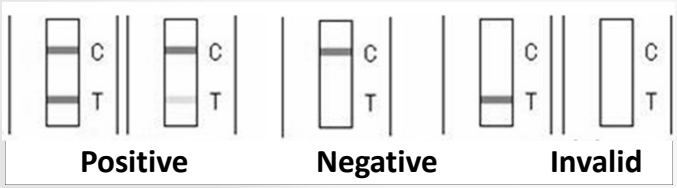
555

## Principal of Immunochromatography




556

## Interpretation




557

## PCR and RT-PCR

- PCR
  - Allows exponential amplification of a specific sequence from genome DNA
    - Based on the replication of DNA
- RT-PCR
  - Allows exponential amplification of a specific sequence from RNA genome
    - Same as PCR, but requires an initial step to convert RNA into DNA
      - Reverse transcriptase reaction

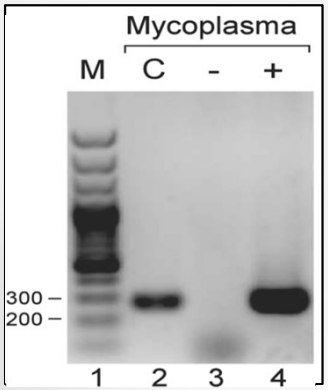
558

## Uses of PCR and RT-PCR

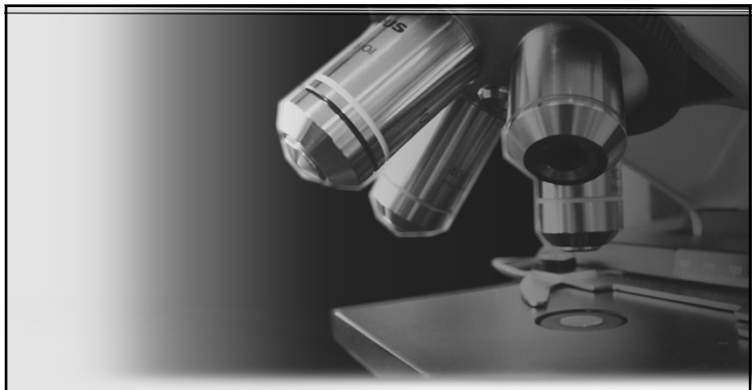
- Detection pathogens that are difficult or cannot be grown in the lab
  - Ex. Mycoplasma
- Early detection of low level pathogens
  - Ex. HIV
- Detection of latent infections
  - Ex. EBV
- Rapid screening of tissue samples
  - Ex. CMV in organ transplants

559

## PCR




560



## *Epidemiology*

561

## Definitions

- **Epidemiology**: Branch of medicine that describes the occurrence, distribution and types of diseases in populations for distinct time periods
- Epidemiology is the study of who, what, when, where and how as they relate to outbreaks of infectious diseases

562

## Definitions *(cont'd)*

- Infectious disease
  - Disease caused by an infectious agent
    - Ex. Common cold
- Subclinical disease
  - State in which the infection caused tissue damage, but with no clinical signs or symptoms
- Contagious disease
  - Direct or indirect transmission from an infected person
    - Ex. Influenza – The flu
- Transmissible disease
  - Transmission by non-natural means from an infected person
    - Ex. Food intoxication – *S. aureus*

563

## Measures of the Occurrence of a Disease

- Cumulative incidence or attack rate
  - Type of incidence applied to a population that is observed over a defined time period
  - Measure of the risk of developing the disease
  - **Measure of transmissibility**

$$CI = \frac{\text{Nº of } \textbf{new} \text{ cases of the disease during the specified time}}{\text{Population at risk } \textbf{during} \text{ the time period}} \times 1\,000$$

564

## Attack Rate: Example

- During the first week of April 2002, the unit of Public Health was called to investigate more than 20 reports of people suffering from gastroenteritis after eating at the Parramatta restaurant. An investigation was conducted and all customers who ate at the restaurant during the week were interviewed. They found 2,000 customers who ate at the restaurant, including 400 who fell ill
  - What was the attack rate?

Attack rate = 400/2000
= 0.2 X 1 000 = 200/1 000 customers

565

## Measures of the Occurrence of a Disease

- Prevalence :
  - Fraction of population at risk that is affected (new cases and preexisting cases) by the disease over a defined time period
    - **Measure of pathogenicity**
  - Varies as a function of
    - Incidence or attack rate of the condition
    - Average duration of the condition
      - Duration is influenced in turn by the recovery rate and mortality rate

566

## Prevalence *(Cont'd)*

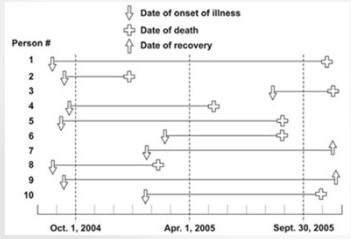
$$P = \frac{\textbf{Total N}^{o}\textbf{ (new + preexisting)}\text{ of cases of the disease during the specified time period}}{\text{Population at risk }\textbf{during}\text{ the time period}} \times 1\,000$$

- Ex. In a survey of 1,150 women who gave birth in Maine in 2000, 468 of them reported having a common cold during their pregnancy
  - Calculate the prevalence of the common cold in this group
    - Numerator = 468 cases of the common cold
    - Denominator = 1,150 women
  - Prevalence = (468 / 1,150) × 1000 = 4.07 cases/1 000

567

## Cumulative Incidence Vs Prevalence

- This figure represents 10 new cases of illness over about 15 months in a population of 20 persons. Each horizontal line represents one person and the duration of illness.




Calculate the cumulative incidence and prevalence for the period of Oct. - Sept

568

## Cumulative Incidence Vs Prevalence

- Cumulative incidence
  - Numerator = number of new cases between October 1 and September 30; = 4 (the other 6 all had onsets before October 1, and are not included)
  - Denominator = 20
  - **Cumulative Incidence** = (4 / 18) × 1000 = 220 new cases per 1000 population
- Prevalence
  - Numerator includes anyone who was ill any time during the period; 10 persons were all ill at some time during the period
  - **Prevalence** = (10 / 20) × 1000 = 500 cases per 1000 population

569

## Measures of the Occurrence of a Disease

- Incidence rate
  - Number of new cases in a population divided by the total units of time of each individual is observed in the population at risk
  - **Measure of infectivity**
    - Probability of a pathogen to establish an infection

$$IR = \frac{\text{Nº of new cases of the disease for the specified time period}}{\text{Sum of the amount of time during which each person was at risk (Person-years)}}$$

570

## Calculating the Number of Person-Years

- Example 1 :
  - At the beginning of a study, with a duration of 5 years, it was determined that among a population of 100 people, 30 were at risk of a particular disease

| Year of study | Number of cases |
|---|---|
| 1 | 2 |
| 2 | 0 |
| 3 | 1 |
| 4 | 0 |
| 5 | 3 |

Number of person-years =
(2 pers. X 1 year) + (1 pers. X 3 years) + (27 pers. X 5 years)
= 140 **person-years**

571

## Calculating the Number of Person-Years

- Example 2:
  - The incidence of duodenal ulcer was examined in 14 subjects who used a particular drug
    - 4 subjects began the study in January 1990
    - In December 1994, two subjects left the study
    - 10 subjects joined the study in January 1995
    - The study was completed in December 1996

Number of person-years =
(2 pers. X 5 years) + (2 pers. X 7 years) + (10 pers. X 2 years)
= 44 **person-years**

572

## Calculating the Number of Person-Years

- Example 3:
  - The incidence of influenza was examined in a population of 1000 individuals during the period of Jan. - April 2013

| Month of study | Number of cases |
|---|---|
| J | 12 |
| F | 25 |
| M | 100 |
| A | 200 |

(12 pers. X 1/12) + (25 pers. X 2/12) + (100 pers. X 3/12) + (863 pers. X 1/12) = 102.1 person-years

573

## Prediction

- Over a period of 3 years, among 150 people who consumed seafood, 10 contracted a food infection. If on average 25% of a population of 10 000 inhabitants consume seafood, how many people / year will contract a food infection following the consumption of seafood?

574

## Solution

- Incidence rate:
  - Nº of person-years = 150 pers. X 3 years= 450 P-Y
  - Nº of new cases = 10
  - I.R.= 10/450= 0.02 cases/person-years
- Prediction:
  - Nº of persons at risk = 0.25 X 10 000 = 2 500
  - Nº of persons predicted to contract a food infection:
    - 0.02 X 2 500 = 50 persons/year

575

## Measures of the Occurrence of a Disease

- Mortality rate :
  - **Measure of virulence**

$$\frac{\text{Number of deaths resulting from a disease}}{\text{Number of individuals with the disease}}$$

576

## Relative Risk

- Often we need to know the association between a result and factors (eg, age, sex, race, smoking status, etc.)
  - Relationship between the probability of contracting a disease when exposed to a factor, and the likelihood of contracting the disease when not exposed to this factor

577

## 2 x 2 Table: Calculating the Association

| | **Result** | |
|---|---|---|
| **Exposure** | Yes | No |
| Yes | *a* | *b* |
| No | *c* | *d* |

578

## 2 x 2 Table

*a* = Number of exposed persons with the result
*b* = Number of exposed persons without the result
*c* = Number of persons not exposed with the result
*d* = Number of persons not exposed without the result

**********

*a* + *b* = Total number of exposed persons
*c* + *d* = Total number of persons not exposed
*a* + *c* = Total number of persons with the result
*b* + *d* = Total number of persons without the result
*a* + *b* + *c* + *d* = Total population at risk

579

## Calculating the Relative Risk

- The relative risk is the risk of the disease in the exposed group divided by the risk of disease in the unexposed group

$$RR = \frac{a/(a + b)}{c/(c + d)}$$

580

## Relative Risk : Example

| | *Diarrhea*? | |
|---|---|---|
| Pink hamburger | Yes | No |
| Yes | 23 | 10 |
| No | 7 | 60 |

$$RR = \frac{a / (a + b)}{c / (c + d)} = \frac{23 / 33}{7 / 67} = 7.0$$

581

## Interpretation of the RR

- = 1 – Indicates that is no association
- > 1 – Indicates a positive association
- < 1 – Indicates a negative association
  - Ex.
    - If RR = 5
      - The people exposed are 5 times more likely to have the result as compared to people who have not been exposed
    - If RR = 0.5
      - The people exposed are 2 times less likely to have the result as compared to people who have not been exposed
        - » Protective effect
    - If RR = 1
      - The people exposed are not more or less likely to have the result as compared to people who have not been exposed

582

## Problem

| | Cases of Salmonellosis for April 2008 | | |
|---|---|---|---|
| Age group | 4-11 (n = 2050) | 12-19 (n= 4000) | 20-49 (n=3950) |
| Nº of cases | 19 | 32 | 12 |
| % who consumed peanut butter | 20 | 35 | 6 |

- Is there an association between the consumption of peanut butter and Salmonellosis?
- If so, which age group is more at risk?

583

## Types of Outbreaks

- Sporadic
  - Occasional incidence
  - No defined pattern
- Endemic
  - Regular incidence maintained at a low rate
- Epidemic
  - Sudden increase above the predicted rate
- Pandemic
  - Epidemic disease of which the incidence is world wide

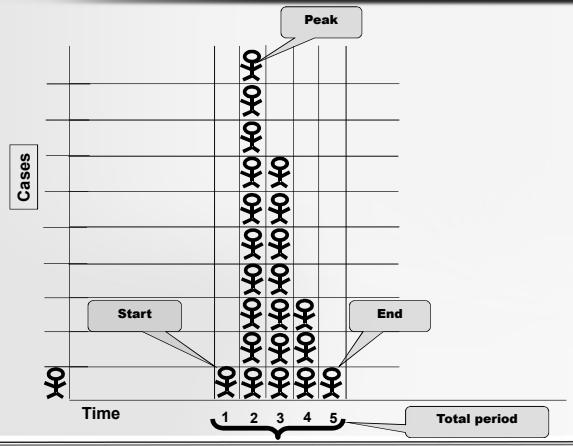
584

## Epidemiological Profiles

- Epidemics linked to a common source
  - Sudden increase in the number of individuals afflicted followed by a progressive decline
- Epidemic Propagation
  - Slow increase in the number of people afflicted
    - Typical of contagious diseases
  - Index-Case: First person that can be found to have contracted the disease

585

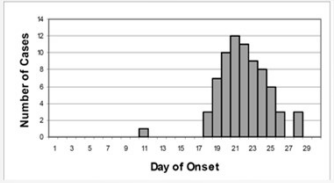
## Characteristics of the Curve




586

## Epidemics Linked to a Common Source

- People are exposed to the same source for a short defined period of time
- The shape of the curve shows a rapid increase with a defined peak, followed by a gradual decline

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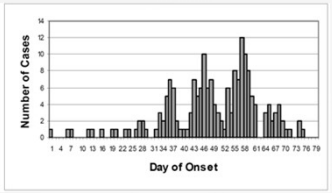
## Epidemic Propagation

- One case of disease is the source of the infection
  - Subsequent cases then act as sources for subsequent infections
- The shape of the curve contains a series of successively larger peaks
- This trend may continue until
  - The number of susceptible people is exhausted
  - Herd immunity is acquired
  - Control measures are implemented

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## Basic Reproduction Number - $R_0$

- Definition: Expected number of secondary cases produced by a single infection in a completely susceptible population
  - If $R_0 < 1$: the infection will die out in the long run
  - If $R_0 > 1$: the infection will spread in a population
- Factors that affect $R_0$
  - Probability of infection when a susceptible and infected individual meet
  - Rate of contact between susceptible and infected
  - Duration of infectiousness

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## Herd Immunity

- Resistance of a community or group of people to a particular disease
- Herd immunity implies group protection beyond that afforded by the protection of immunized individuals
  - Immunized individuals protect non immunized individuals
    - $R_0 < 1$

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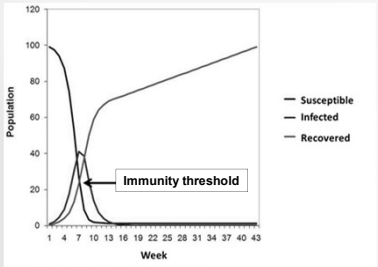
## Herd Immunity Threshold

- Proportion of immune individuals in a population above which a disease may not persist

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## Determining Herd Immunity Threshold

- How many people must be immunized to acquire herd immunity?
  - $V_c = (1-1/R_0)/E$
    - $V_c$: Critical minimum proportion to be vaccinated
    - $E$: Percentage of immunized individuals which are protected (Measure of vaccine efficiency)
  - Need to reach an $R_0 < 1$

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## Determining Herd Immunity Threshold

- Example
  - A given disease has $R_0$ of 3 and vaccination provides 100% protection. How many people must be immunized to acquire herd immunity?
  - $V_c = (1-1/R_0)/E$
  - $V_c = (1-1/3)/1$
  - $V_c = (1-1/R_0)/E$
  - $V_c = 0.66$; therefore 66% need to be immunized

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## Source of the Infectious Agent

- Inanimate reservoirs
  - Some pathogens are found mainly in non-living habitats
  - ex. *Clostridium tetani*, found in soil
- Animate reservoirs
  - The pathogen is not usually found in nonliving habitats
  - ex. Virus; obligate parasite

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## Human Reservoirs

- Active carrier
  - Individual who suffers from the disease and which expresses the symptoms associated it
- Incubating carrier
  - Healthy individuals who harbor the pathogen
  - The individual will be sick at a later date
- Convalescent carrier
  - Individual who has recovered from the illness
  - Expresses no symptoms
  - Harbors a large number of live pathogens
- Healthy carrier
  - Individual was never sick
  - Harbors the pathogen

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## Animal Reservoirs

- Healthy carriers
  - Does not cause the disease in animals
  - Can be a resident of the natural flora
    - Ex. *E. coli, Salmonella*
- Diseased carrier
  - Causes the disease in the animal
  - The disease can be transmitted to humans
    - **Zoonosis**
      - Disease that can be transmitted from animals to humans naturally

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## Bacterial Zoonosis

| Bacteria | Animal host | Disease |
|---|---|---|
| *Mycobacterium bovis* | Livestock | Tuberculosis |
| *Yersinia pestis* | Rodents | Bubonic plague |
| *Bacillus anthracis* | Livestock | Anthrax |
| *Borrelia burgdorferi* | Cervidae | Lime disease |
| *Chlamydia psittacosis* | Birds | Psittacosis |

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## Control of the Reservoir

- Destruction of the reservoir
  - Domestic animals
    - Ex. Bovine tuberculosis, mad cow disease
  - Wild animals (Very difficult)
    - Rabies, Nile virus
  - Humans (impossible)
  - Inanimate reservoirs
    - Elimination or treatment is possible
      - Ex. Treatment of water

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## Control of Transmission

- Airborne transmission
  - Isolate sick patients
  - Wearing of a mask (frequent in Japan)
  - Filtration system
- Transmission by contact
  - Frequent hand washing
  - Minimize contacts
  - Use of condoms
  - Use of disinfectants
- Transmission by ingestion
  - Chlorine treatment of water
  - Treatment of waste water
  - Cooking food
  - Use of food preservatives

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## Quarantine

- Goal:
  - Eliminate/restrict propagation
  - The goal is not to save the diseased!
- Diseases for which there is an international agreement which allows quarantine :
  - Smallpox
  - Cholera
  - Plague
  - Yellow fever
  - Typhoid fever
  - SARS


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## Nosocomial Diseases

- Infection acquired in a hospital that was not present or incubating upon admission
- Persons at risk of nosocomial infections:
  - Patients
  - Personnel
  - Visitors

## Nosocomial Vs Community Acquired

- Greater risk of acquiring a nosocomial infection compared to a community acquired infection; why?
  - Host defenses depressed by underlying disease or treatment, malnutrition, age
  - Anatomic barriers breached (IV's, catheters, surgery, etc.)
  - Exposure to more virulent pathogens
    - Ex. Many multiple resistance organisms

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## Source of Pathogens

- Reactivation of latent infection
  - TB, herpes viruses
- Endogenous:
  - Normal flora of the skin, respiratory Tract, GI tract
- Exogenous
  - Inanimate environment
  - Animate environment
    - Hospital staff, visitors, other patients

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## Preventive Measures

- Hand washing :
  - Before and after contact with a patient
  - Before and after preparing/handling/serving food or medicine
  - After contact with contaminated items
  - After satisfying one's personal needs
  - Before leaving the work area
- Wearing gloves
- The mask
- Isolation of patients

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