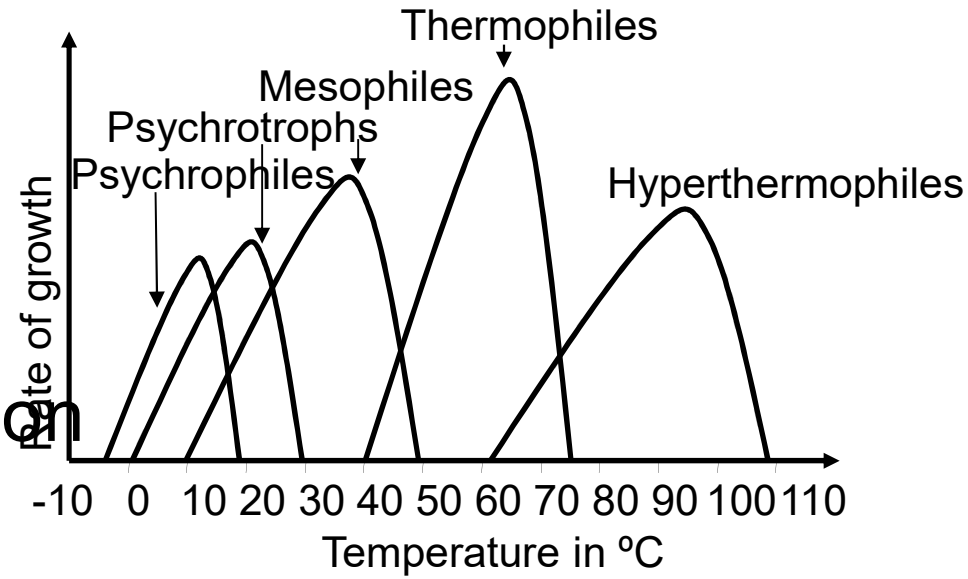
Microbiological Classification of Infectious Diseases

- Disease is a disturbance in the state of health
- Microbes cause disease in the course of stealing space, nutrients, and/or living tissue from their symbiotic hosts (e.g., us)
- To do this, microbes do most of the following:
  - Gain access to the host (contamination)
  - Adhere to the host (adherence)
  - Replicate on the host (colonization)
  - Invade tissues (invasion)
  - Produce toxins or other agents that cause host harm (damage)

Growth requirements

Physical

- Temperature
- pH
- Osmotic pressure
- Moisture & desiccation



Chemical

- Carbon source
- Nitrogen, sulfur phosphorus
- Oxygen

Temperature

Psychrophiles (cold loving)

- True psychrophiles (optimum growth at 15 ºC)
- Psychrotophs (optimum growth at 20-30 ºC)

Most pathogenic bacteria are mesophiles
And grow optimally at 37 ^{0}C
(human body temperature)

Mesophiles (moderate temperature loving)

Thermophiles (heat loving)

Hyperthermophiles (tolerate extreme temperatures)

pH

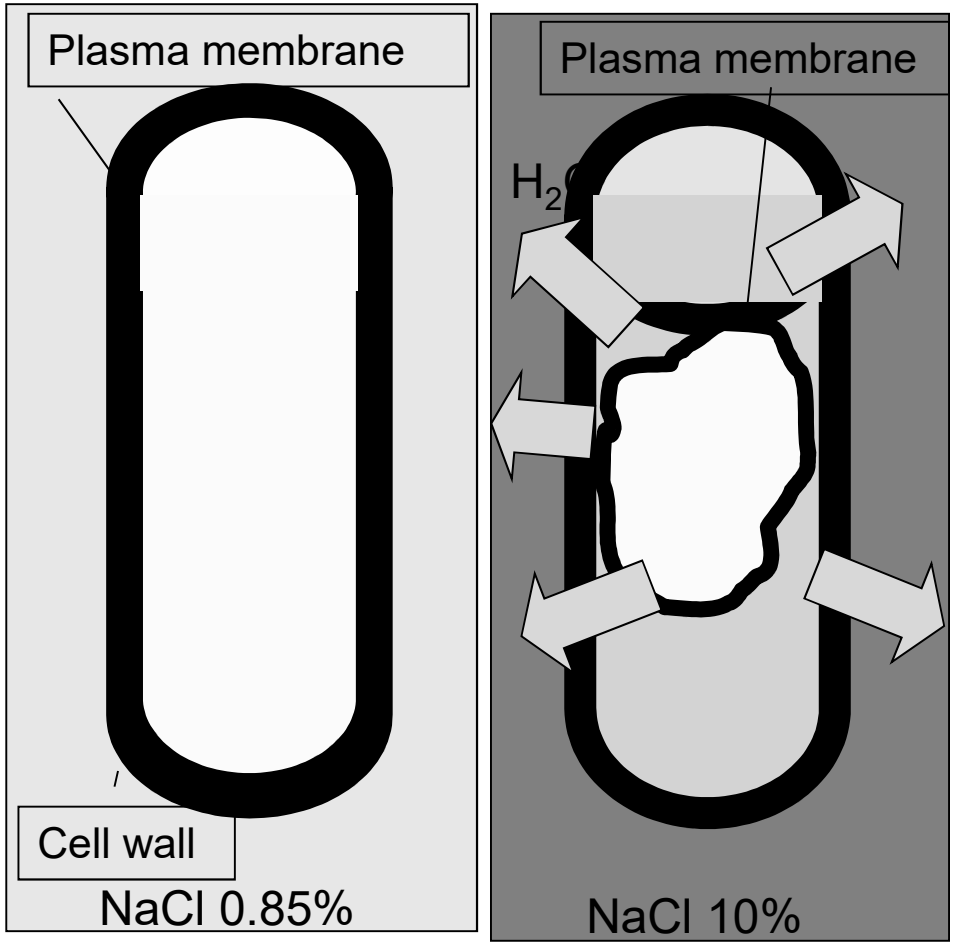
- Most medically important bacteria grow at neutral or slightly alkaline pH (7.2 to 7.6)
- Very few bacteria grow below pH 4
- Lactobacilli grow in acidic pH; cholera *vibrio* grow in alkaline pH
- Growth media includes chemical buffers to prevent acid production
- Foods are preserved by acids produced by bacterial fermentation

Osmotic pressure

High osmotic pressure (hypertonic) removes water causing plasmolysis – inhibits growth i.e. salt as preservative

Low osmotic pressures (hypotonic) cause water to enter and can cause lysis

Bacteria are more tolerant to osmotic variations because of the mechanical strength of the cell wall



Moisture and desiccation

Moisture is essential - 80% body weight is water

Effect of drying varies by organism

- *T pallidum*, gonococcus are very susceptible
- Tubercle bacilli, staphylococci may survive for weeks
- Bacterial spores survive several years

Lyophilization

- Freeze dry process that protects bacteria

Carbon

Chemo- and photo-autotrophs fix CO_2

Chemoheterotrophs obtain energy from organic compounds

Oxygen

Obligate aerobes

- Only aerobic growth, oxygen required

Facultative anaerobes (most human pathogens)

- Greater growth in presence of oxygen

Obligate anaerobes

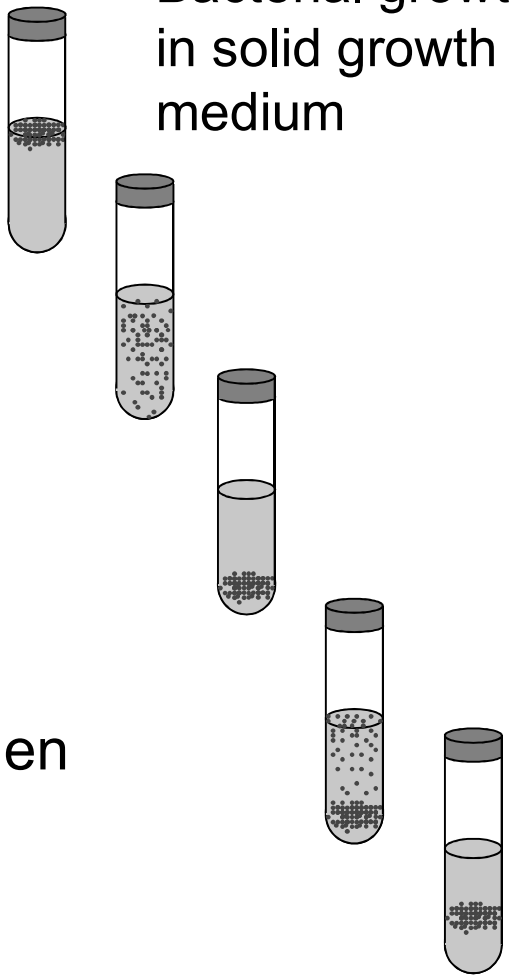
- Only anaerobic growth, cease with oxygen

Aerotolerant anaerobes (e.g., *C. perfringens*)

- Only anaerobic growth, continues with oxygen

Microaerophiles (e.g., *M. tuberculosis*)

- Only aerobic growth with little oxygen



Bacterial growth in solid growth medium

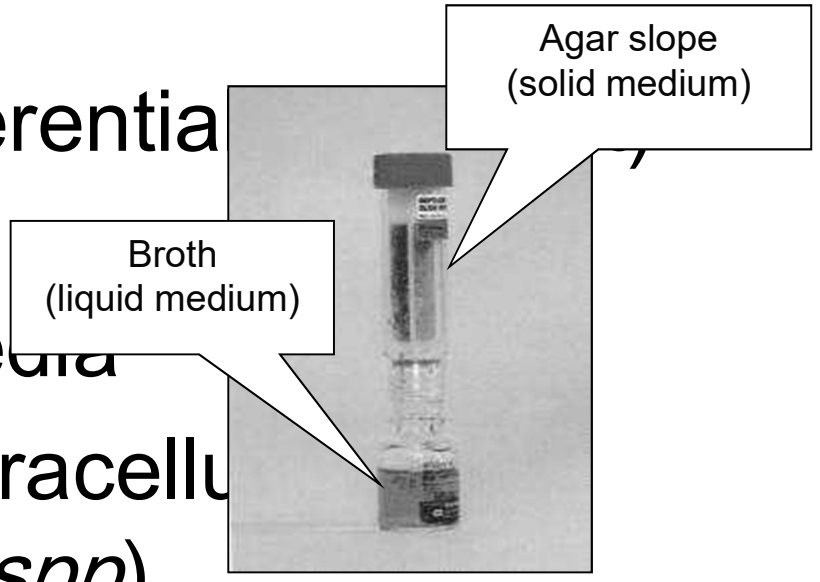
Types of bacterial culture media

Solid, semisolid, liquid, biphasic

Simple media, special media (enriched, selective,
enrichment, indicator/ differentia
synthetic media

Aerobic and anaerobic media

Cell culture for obligate intracellu
bacteria (e.g., *Chlamydia spp*)



Biphasic culture medium

Selective & differential media

Selective

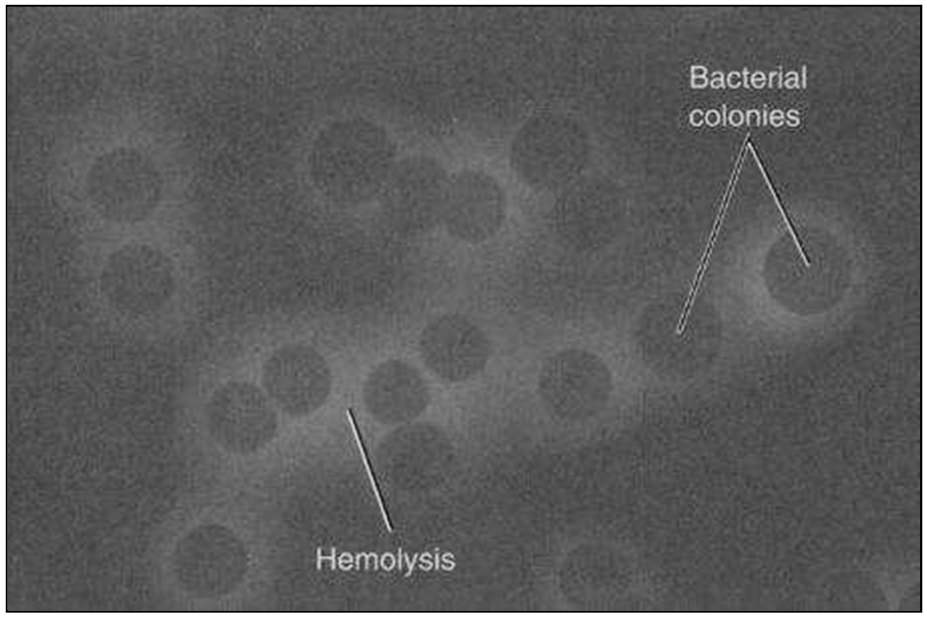
• Bismuth sulfite for *Salmonella typhi* (inhibits gram-positive and most gram-negative intestinal bacteria)

Differential

• Blood agar plates for *Streptococcus pyogenes*

Selective & differential

•Mannitol salt agar for *Staphylococcus aureus*

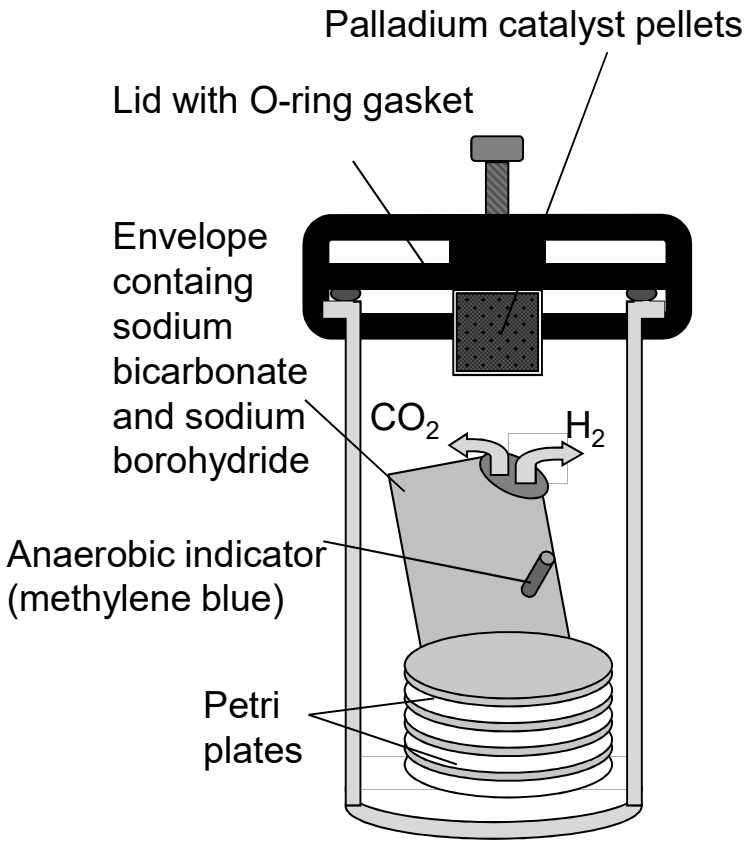


Type of hemolysis reaction aids identification of *S. pyogenes*

Anaerobic growth

Reducing media containing thioglycolate to deplete oxygen; cooked meat broth

Anaerobic jar, anaerobic chamber, anaerobic bags/ pouch

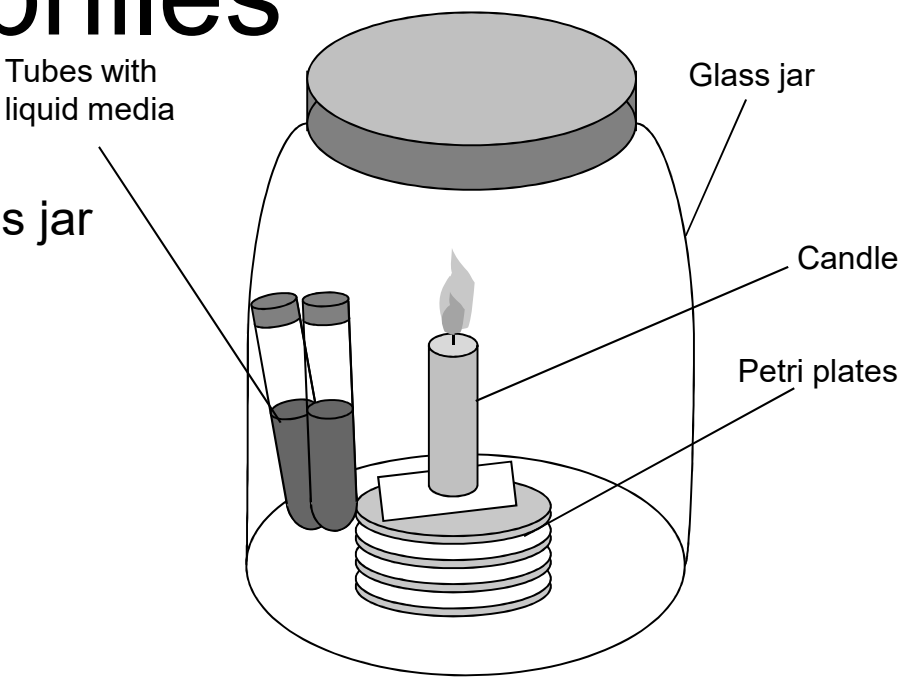


Capnophiles

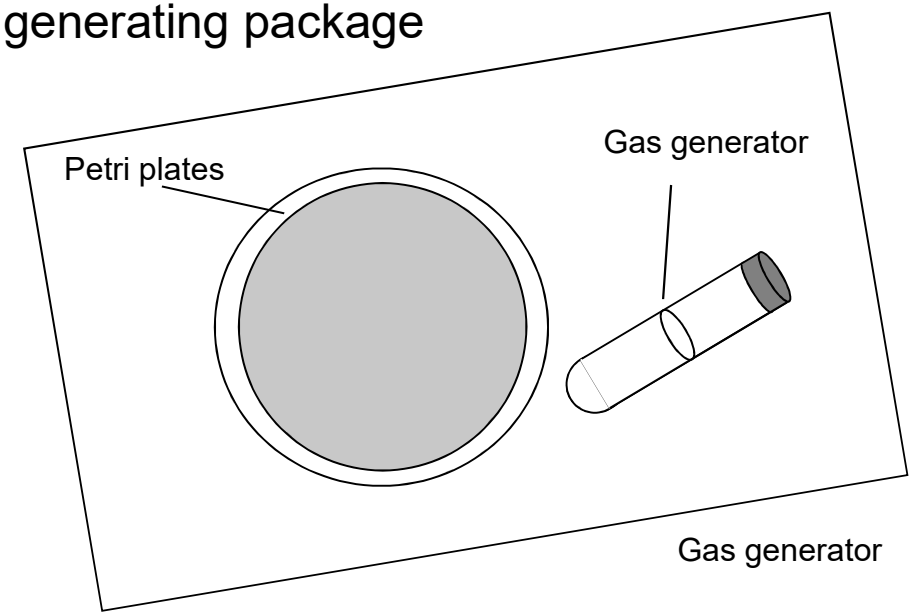
Capnophiles require high concentration of CO

e.g. *Brucella abortus*

A. Glass jar



B. CO_2 generating package



Culture methods

Streak culture

- Isolation of bacteria in pure culture from clinical specimen

Lawn culture

- Antimicrobial susceptibility testing (disc diffusion), bacteriophage typing

Liquid cultures

Stroke culture

- To obtain pure growth for slide agglutination; biochemical tests

Stab culture

- Maintenance of stock cultures

Pour-plate culture

- Quantification of bacteria in liquid cultures, urine sample

Culture methods

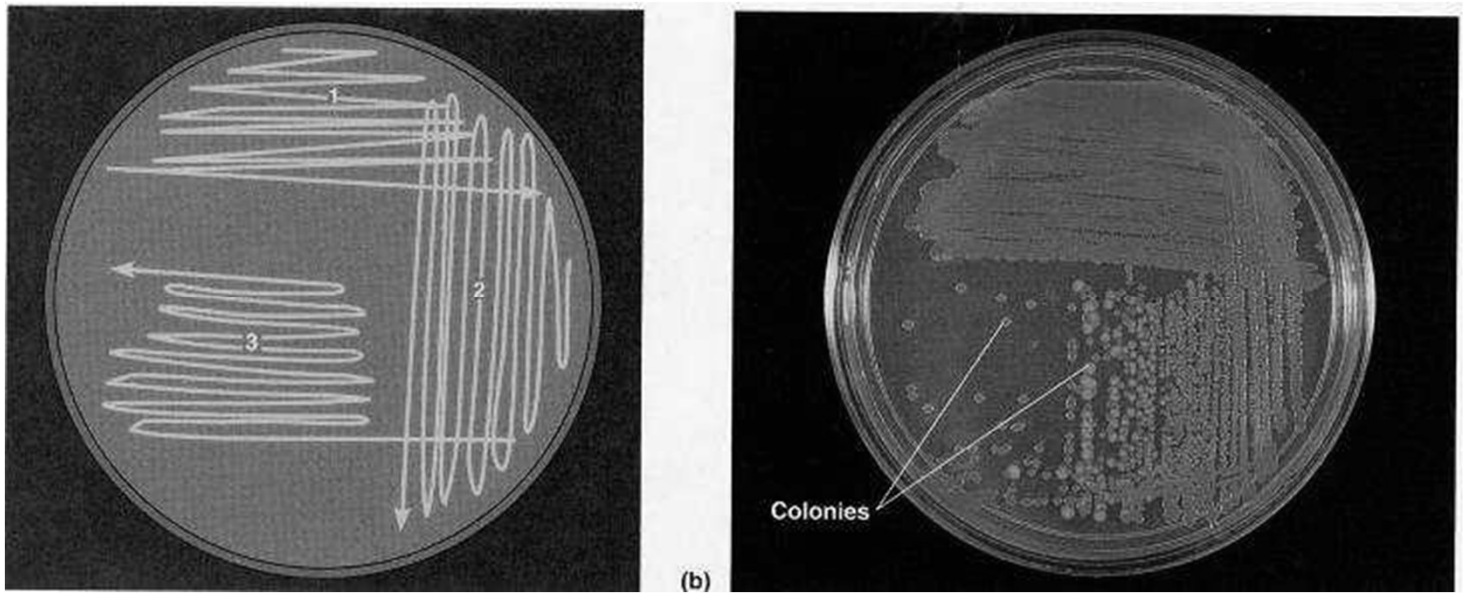
Continuous bacterial culture

Maintain a bacterial population at a constant density

- –Keeping a constant environment (oxygen, nutrient etc.)
- –Imitates the growth in the environment

Pure cultures, plate or any of the others

- In theory, each colony (~10^7) bacteria arises from a single bacterium deposited on the surface of the Petri dish
- Each colony consists of a pure clone of cells
- Best obtained on solid media; less sure in liquid media



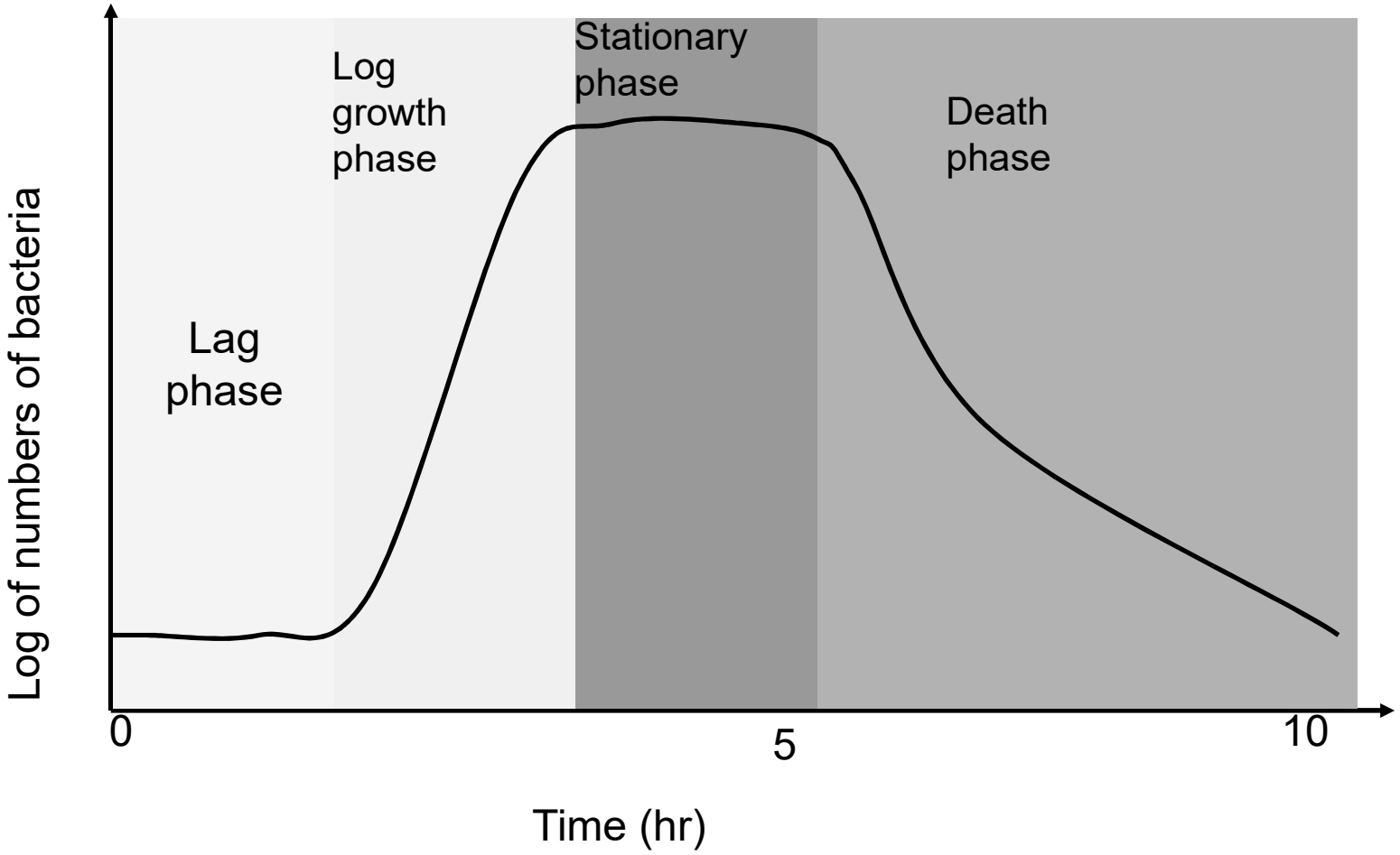
Bacteria grow by binary fission

In rich broth, the number of bacteria doubles every 30 minutes (generation time)

If start with 30 people in the room:

- In 30 minutes we would have 60 people
- In 60 minutes we would have 120 (very uncomfortable)
- In 90 minutes we would have 240 (suffocation)

Phases of bacterial growth



Inside the tissue: interpretation of the bacterial growth curve

Explosiveness of exponential growth

- Short generation time: small number of bacteria initiate a dangerous illness (e.g. acute meningococcal meningitis).
- Long generation time: tuberculosis bacillus causes chronic illness

Inside body tissues

- Bacteria are stressed
- Bacterial populations are rarely fully viable
- May cease growth but continue synthetic activities to meet adaptive stress

Non-growing bacteria can also be harmful:

- Immunogenic
- Production of toxins starts or accelerates during stationery phase
- Sporulation can release toxins

Rate of bacterial death

Death is exponential

- After 1’ – 10% remain alive
- After 2’ – 1% remain alive
- After 3’ – 0.1% remain alive

Effectiveness of antimicrobials

- Number of organisms – larger number longer to eliminate
- Environmental factors – organic materials reduce effectiveness
- Timing of exposure

Bactericidal versus bacteriostatic

Bactericidal drugs (e.g. beta-lactams)

- Kill growing bacteria without the action of humoral/cellular immune response

Bacteriostatic drugs (e.g. tetracyclin)

- Prevent growth of susceptible bacteria that must be killed by host

Growth on bio-films can dramatically reduce the effectiveness of antibiotic therapy

Measurement of cell growth

Measure total counts

- Measure both viable and non-viable bacterial cells
- Direct microscopy using Gram stain; automated cell counter

Measure viable counts

- Measure only viable cells
- Pour plate cultures to give quantitative number of viable bacteria

Measurement of cell growth

Semi-quantitative methods

- Give less accurate but working estimate of bacterial load to aid in decision making
- Semi-quantitative urine culture; MPN test for water bacteriology

Quantitative methods

- Give accurate estimate of bacterial number; more exact applications
- Vaccine production

Rapid cultivation & automation

Lysis centrifugation system

- Pre-treatment of blood culture

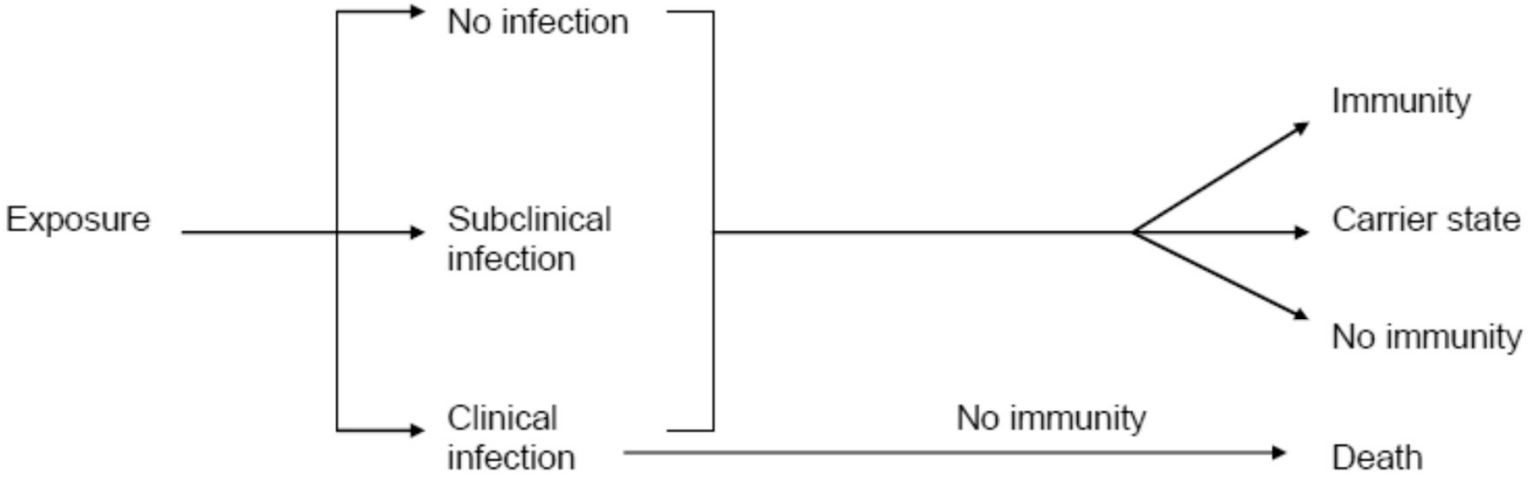
Instrument-based systems

- Periodic and continuous monitoring systems; growth detected by:
  - Colorimetric or fluorescent detection of CO2
  - Consumption of gasses
  - Fluorescent detection of growth

Bioluminescence assay for viable organisms

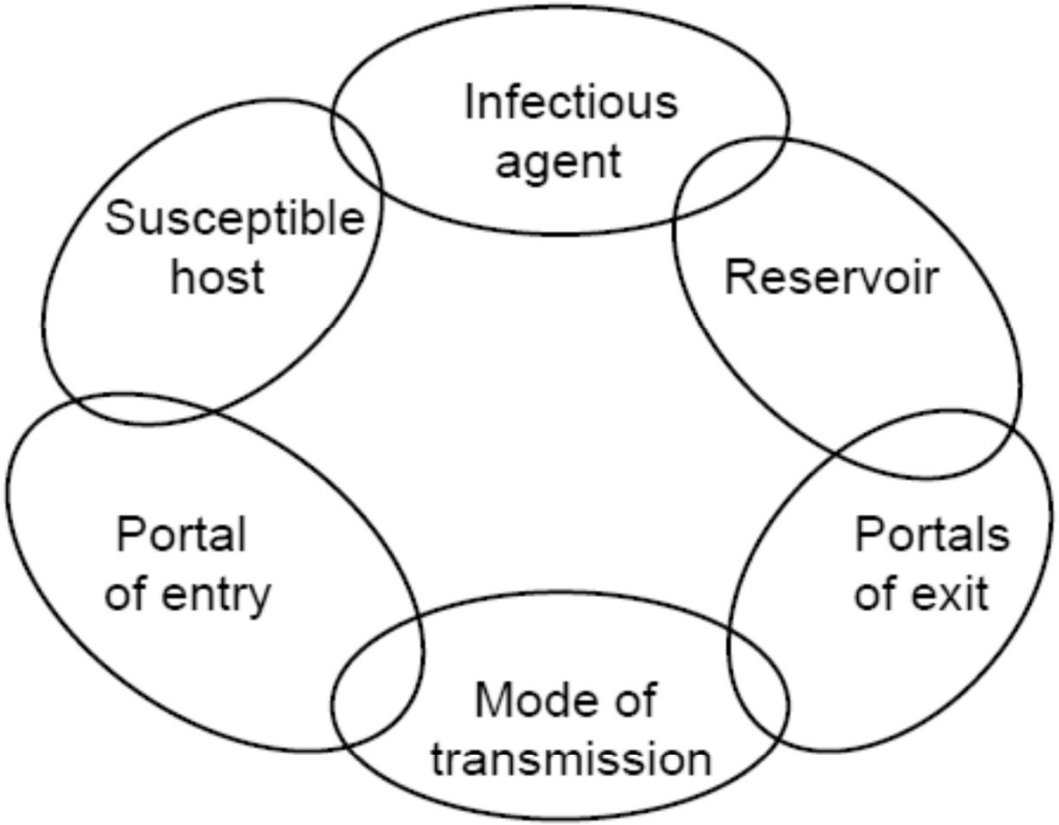
Colorimetric filtration (urine screening)

The different outcomes of an exposure to an infectious agent



Kramer A, et al. Principles of infectious disease epidemiology. In *Modern Infectious Disease Epidemiology*, Kramer A, et al (eds). Springer Science + Business Media, 2010; p 92 (modified from Giesecke 1994)

The chain of infection



Kramer A, et al. Principles of infectious disease epidemiology. In *Modern Infectious Disease Epidemiology*, Kramer A, et al (eds). Springer Science + Business Media, 2010; p 91

BIOLOGIC CHARACTERISTICS OF INFECTIOUS AGENTS

- ***Infectivity*** – the ability to infect a host
- ***Pathogenicity*** – the ability to cause disease in the host
- ***Virulence*** – the ability to cause severe disease in the host
- ***Immmunogenicity*** –the ability to induce an immune response in the host

Infectious Disease Terms

Infectious dose – number of organisms needed to successfully infect

Latent period - exposure to infectiousness interval

Incubation period – interval from exposure to clinical symptoms

Infectious period – interval during which host can transmit infection

Reproductive rate – ability of an agent to spread in populations

Virulence

Pathogenicity

Immunogenicity

Outbreak – limited spread

Endemic – usually present; steady prevalence

Epidemic – rapid spread

Pandemic – occurring across countries and in multiple populations

Important Terms

Table 19.1 Terms Used in the Study of Infectious Diseases

Term	Definition
Bacteremia	Bacteria circulating in the bloodstream
Colonization	Establishment and growth of a microorganism on a body surface
Disease	Noticeable impairment of body function
Immunocompromised	A host with weaknesses or defects in the innate or adaptive defenses
Inapparent infection	Infection with no obvious symptoms
Infectious disease	Disease caused by an infecting microorganism or virus
Latent infection	Infection in which the infectious agent is present but not active
Opportunistic pathogens	Organisms that cause disease only when introduced into an unusual location or into an immunocompromised host
Parasite	An organism that benefits at the expense of another organism, the host
Pathogen	Any disease-causing microorganism or virus
Pathogenic	Disease-causing
Primary infection	Infection in a previously healthy person
Secondary infection	An additional infection that occurs as a result of a primary infection and that occurs during or immediately following the primary infection
Septicemia	Acute illness caused by infectious agents or their products circulating in the bloodstream
Systemic infection	Widespread infection through blood or lymph
Toxemia	Toxin circulating in the bloodstream
Viremia	Viruses circulating in the bloodstream
Virulence determinants	Attributes of a microorganism or virus that promote pathogenicity

MODES OF TRANSMISSION

- Direct
  - Droplet
  - Aerosol
  - Skin to skin
- Indirect
  - Fomites (clothes, blankets, door handles etc)
  - Vectors (e.g. mosquitoes)
  - Food and water
  - Intermediate hosts (e.g. snails)

Measures of Disease Occurrence

Measure	Description
Prevalence	Number or proportion of persons with a specific disease at a specific time point in the population
Incidence	Number or proportion of persons developing a specific disease during a time period
Morbidity	Ambiguously used: prevalence or incidence
Mortality	Number or proportion of persons dying during a time period
Fatality rate	Proportion of persons dying from a specific disease among all persons with the disease
Attack rate	Proportion of cases developing the disease among all persons who were exposed to the disease

Mikolajczyk R. Methods and concepts of epidemiology. In *Modern Infectious Disease Epidemiology*, Kramer A, et al (eds). Springer Science + Business Media, 2010; p 193

CLINICAL RESPONSES TO INFECTION BY AN AGENT

- Inapparent infection – no clinical symptoms generated
- Carrier state – usually no clinical symptoms but host can transmit infection for long periods
- Clinical symptoms
  - Mild disease
  - Severe disease
  - Residual impairment
  - death

CLASSIFICATION OF INFECTIOUS AGENTS (1 of 2)

- Bacteria – survive on appropriate media, stain gram-positive or -negative
- Viruses – obbligate intracellular parasites which only replicate intracellularly (DNA, RNA)
- Fungi – non-motile filamentous, branching strands of connected cells
- Metazoa – multicellular animals (e.g.parasites) with complicated life cycles often involving several hosts

CLASSIFICATION OF INFECTIOUS AGENTS (2 of 2)

- Protozoa – single cell organisms with a well-defined nucleus
- Rickettsia – very small bacteria spread by ticks
- Prions – unique proteins lacking genetic molecules
- *Chlamydia* – bacteria lacking cell walls

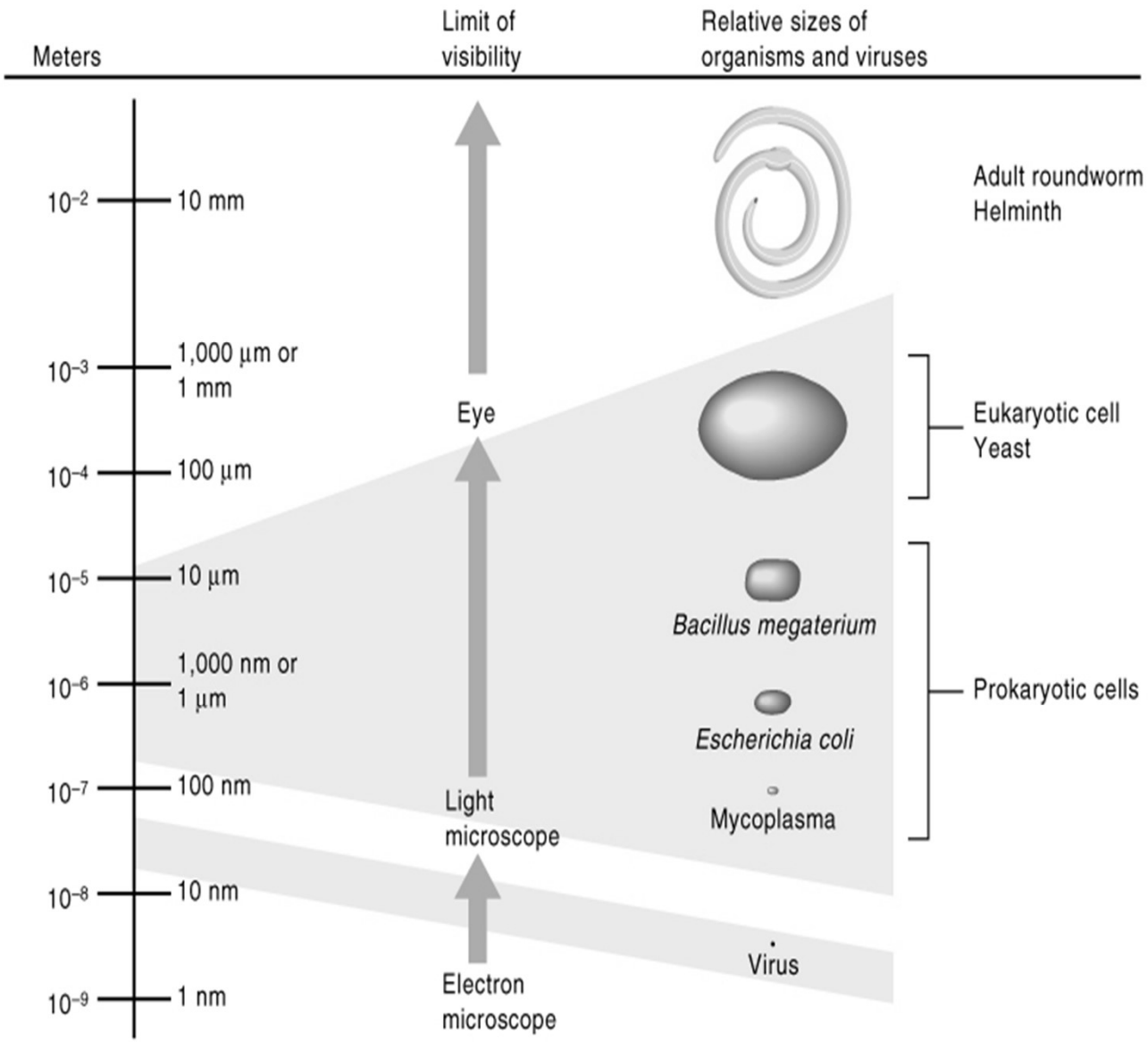
Size Comparison of Microbes

The basic unit of length is the meter (m), and all other units are fractions of a meter.

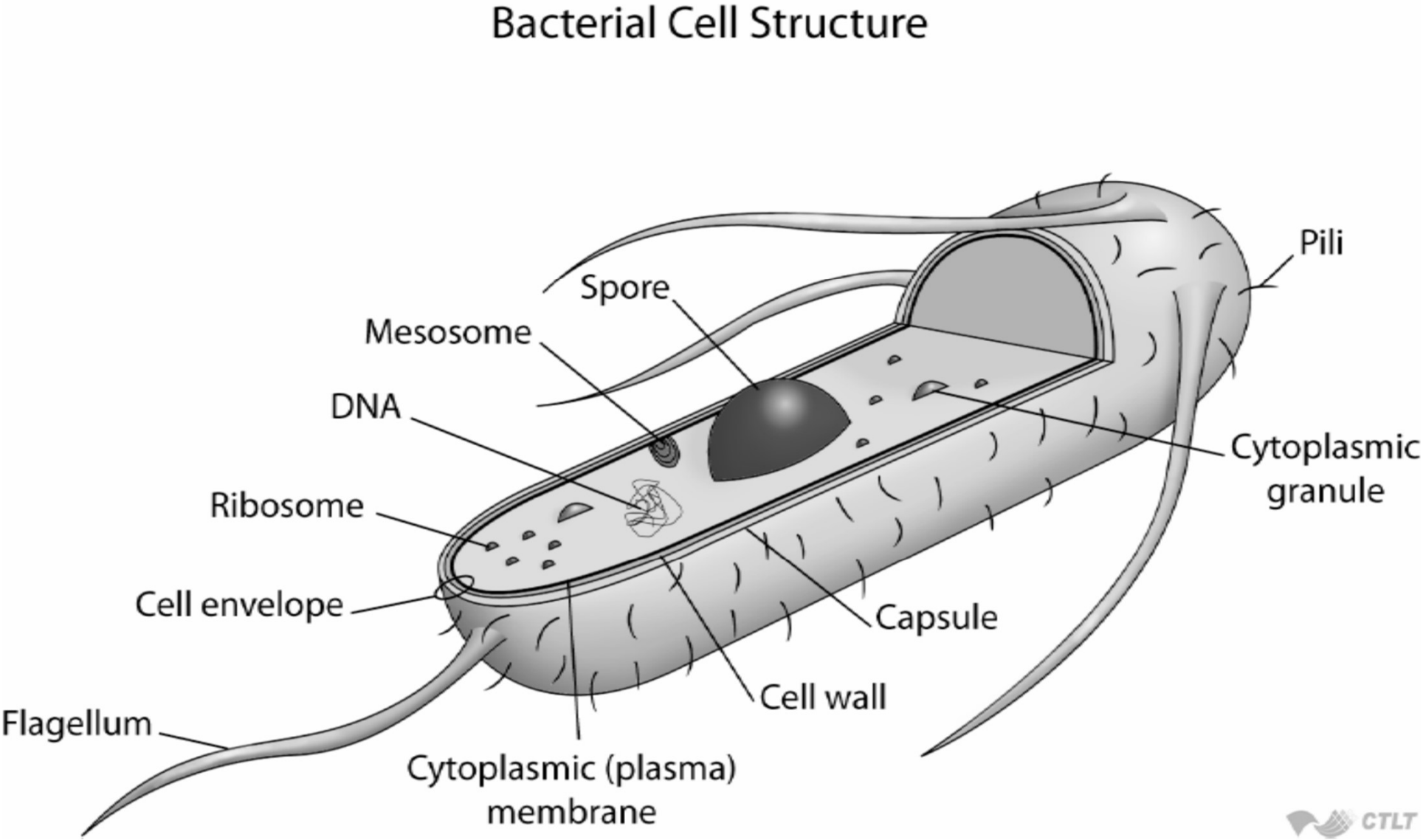
nanometer (nm) = 10^{-9} meter = .000000001 meter
micrometer (μm) = 10^{-6} meter = .000001 meter
millimeter (mm) = 10^{-3} meter = .001 meter
1 meter = 39.4 inches

These units of measurement correspond to units in an older but still widely used convention.

1 angstrom (Å) = 10^{-10} meter
1 micron (μ) = 10^{-6} meter



Bacterial Cell Structure



James D. Dick, PhD, Johns Hopkins University

Taxonomy of Bacteria

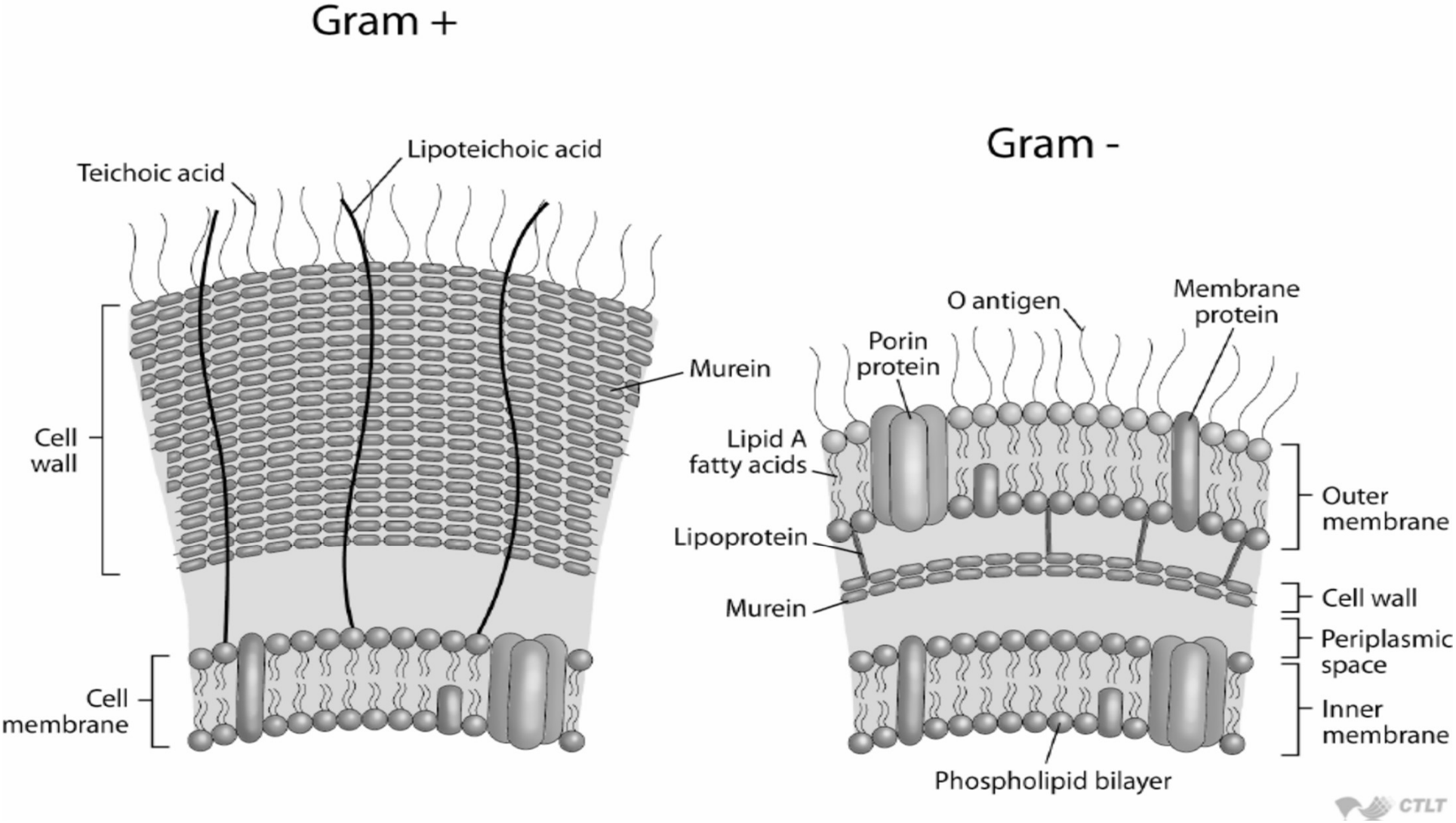
Table 10.1 Taxonomic Ranks of the Bacterium *Escherichia coli*

Formal Rank	Example
Domain	*Bacteria*
Phylum	*Proteobacteria*
Class	*Gammaproteobacteria*
Order	*Enterobacteriales*
Family	*Enterobacteriaceae*
Genus	*Escherichia*
Species	*coli*
Strain	O157:H7

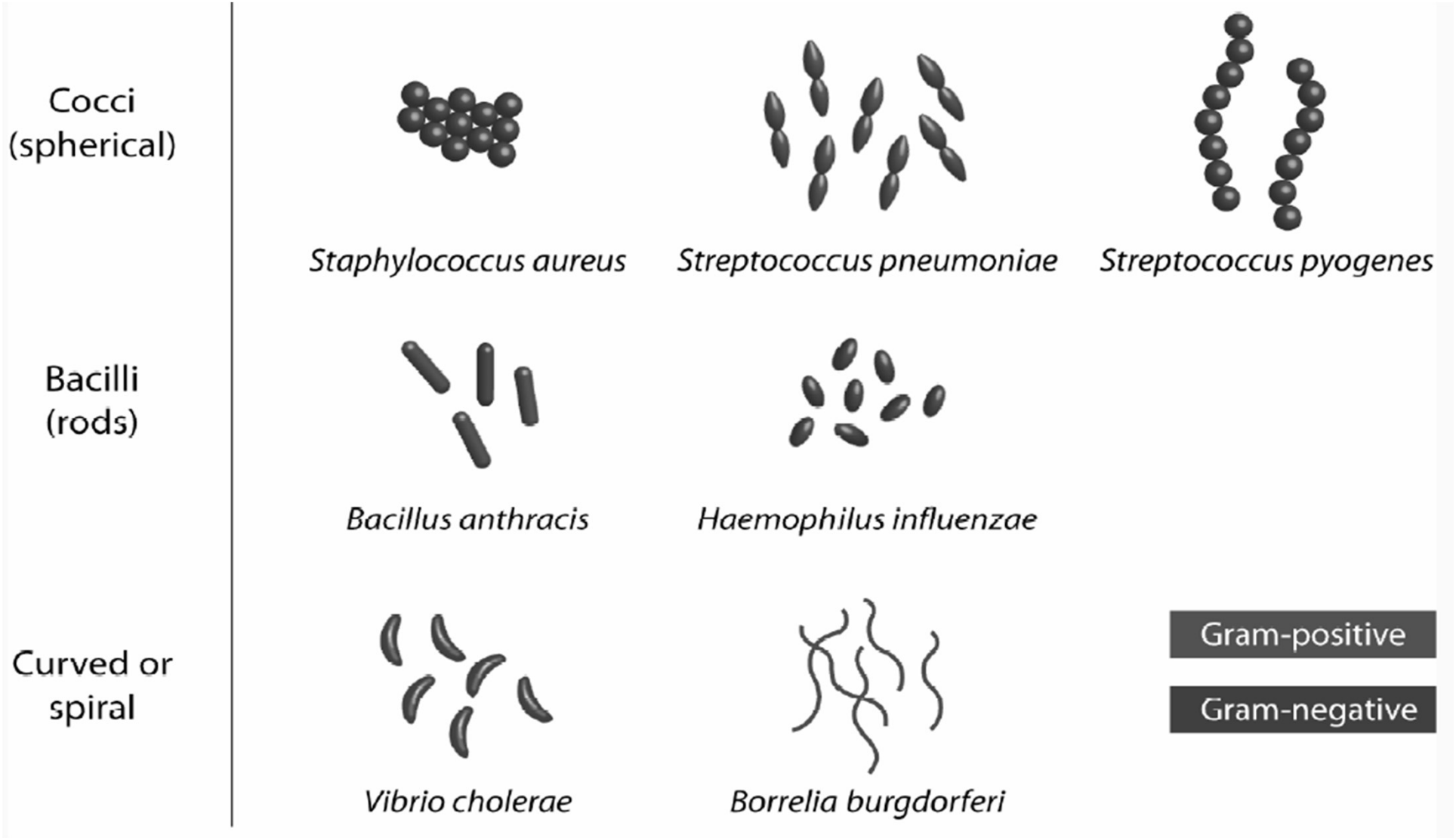
Microbiological Classification of Infectious Diseases

- Bacteria are classified by their Gram stain characteristics.
- Gram staining is the application of a crystal violet dye to a culture of bacteria. Bacteria that retain the color of the dye are called Gram positive; bacteria that don't are Gram negative.
  - The Gram stain attaches to peptidoglycan in the bacterial cell wall.
    - In Gram-negative bacteria, the peptidoglycan layer is protected by an outer membrane.

Microbiological Classification of Infectious Diseases



Microbiological Classification of Infectious Diseases



Microbiological Classification of Infectious Diseases

- Viruses are acellular, obligate intracellular organisms.
- The complete infectious virus is termed a virion.
- The virion consists of the specific nucleic acid (DNA or RNA) surrounded by a protein coat (capsid).
  - Some viruses are enveloped which means that they possess a lipoprotein coat that surrounds the capsid and is acquired from infected host cell membrane.
  - Viruses that lack an envelope are “naked.”