Introduction to Microbiology

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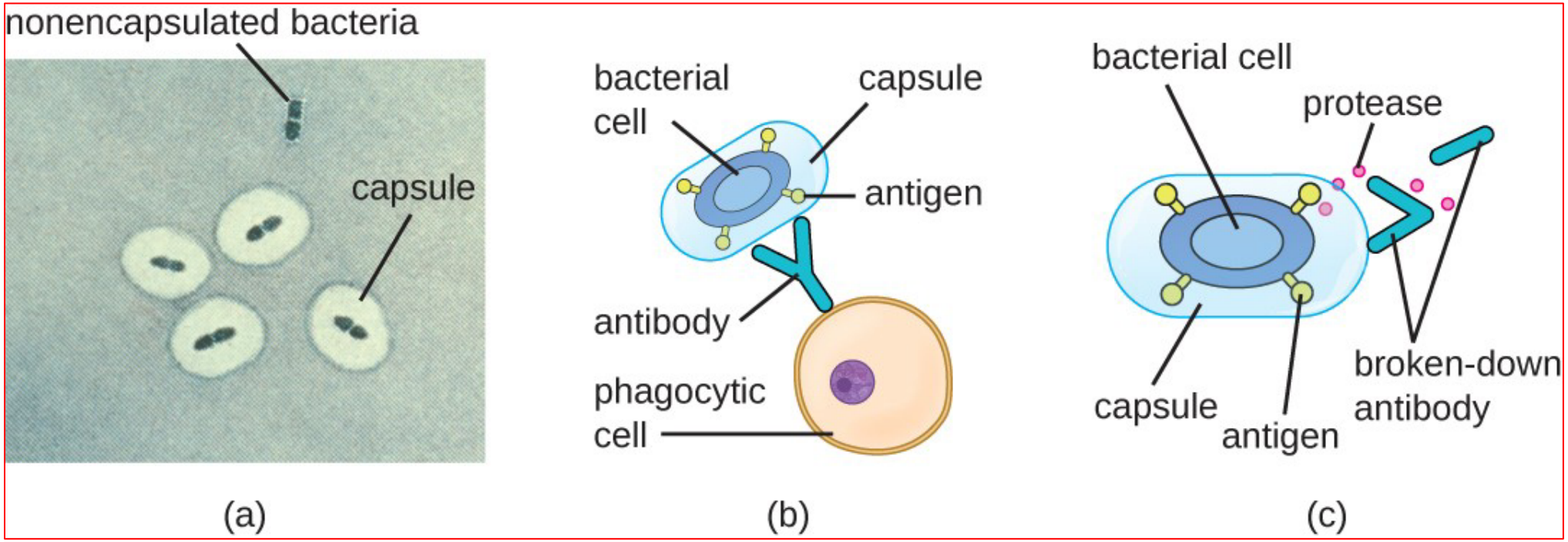
Lecture 10

Evasion of the host immune system

Pathogenic bacteria can evade phagocytosis in many ways, examples include **capsule production**, **Protein A in Staph aurues binds antibodies in an inactive manner.** Some bacteria produce proteins that **inhibit complement activation,** therby decresing immune signaling and opsonization* of bacteria**.** Intracellulaly some bacteria **inhibit phagolysosome fusion.**

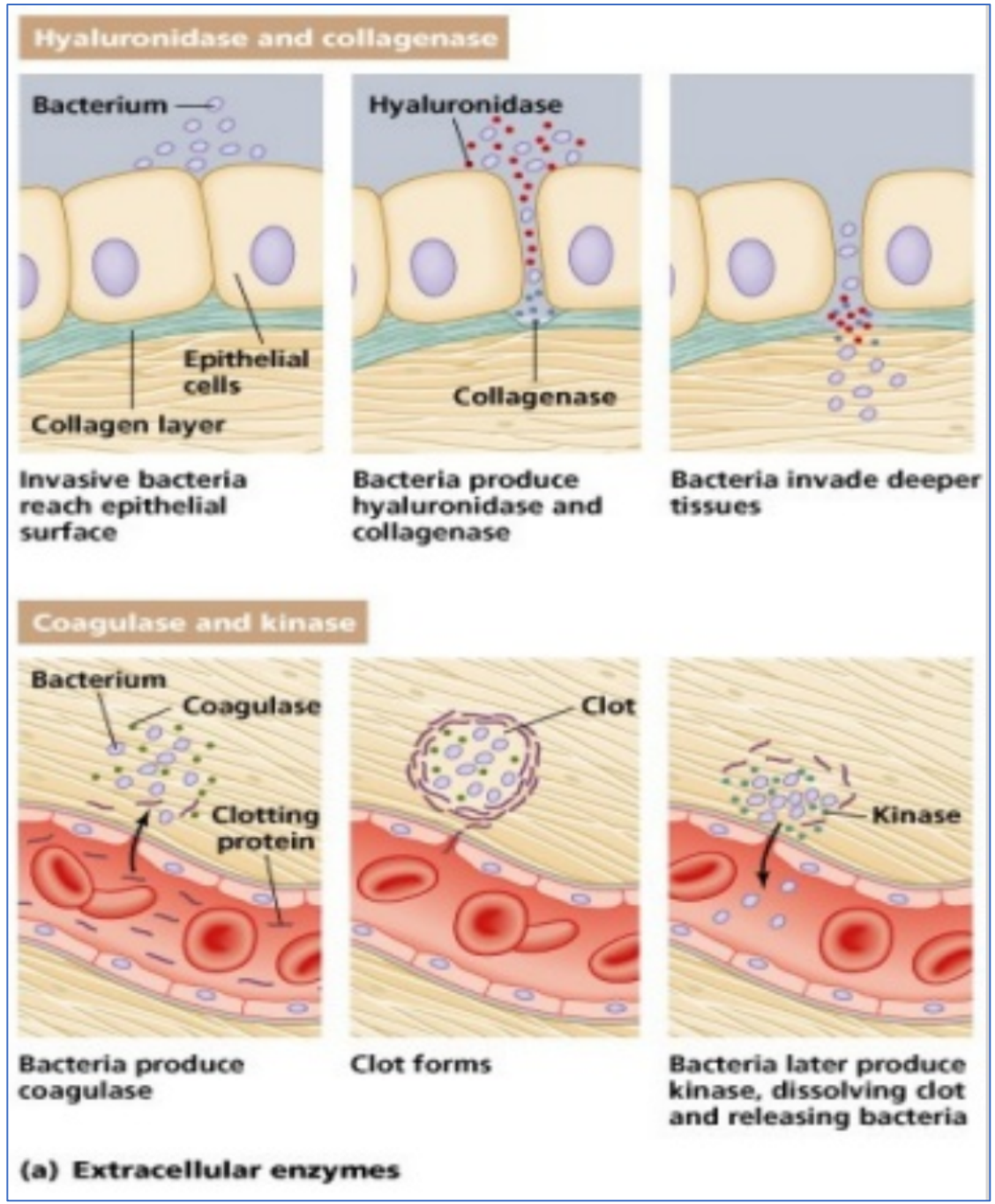


* **Opsonization** is the process in which bacteria is covered by substances to enhance phagocytosis. For example, antibodies bound on bacterial surface, as well as activated complement components depositing on bacterial surfaces are considered ”opsonins” since the make the bacteria easier to phagocytose.

Enzyme production

Pathogenic bacteria produce enzymes to degrade tissues and spread infection. E. g **Hyaluronidase and collagenase** are enzymes that hydrolyze hyaluronic acid and collagen respectively, constituents of the ground substance of connective tissue.

Bacteria produce **cytolysins which directly kill cells** usually by forming pores in their membranes **(e.g. hemolysins, leukocidins)**.



Pathogenicity islands

Chromosomal or extra chromosomal discrete genetic units that encode genes that aid in the virulence of a bacteria by coding for **adhesins, secretion systems (like type III secretion system), toxins, invasins, capsule synthesis, iron uptake systems** .

Absent in non-pathogenic bacteria. Virulence genes are **usually activated by environmental cues (e.g. Temperature change).**

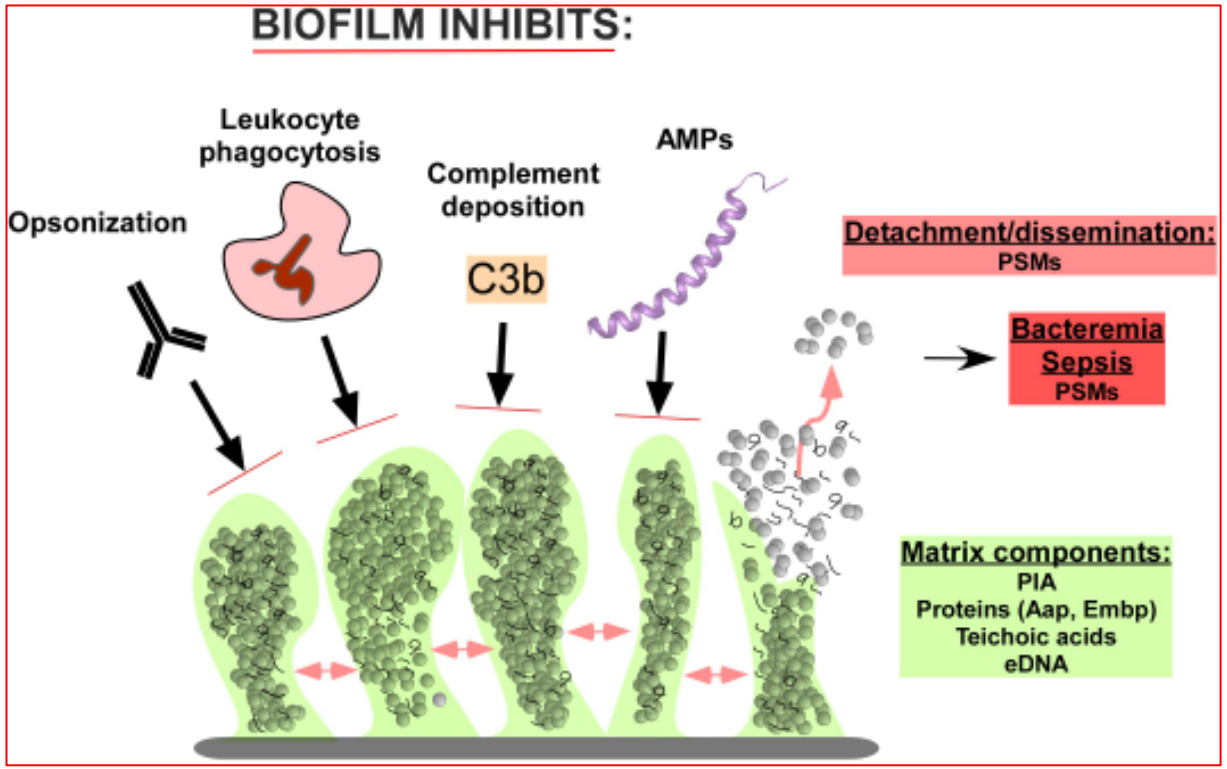
Commonly found on mobile genetic elements **(passed through plasmids, transformation, transduction, transposons**), the **G-C content** of pathogenicity islands is usually different from the rest of the genome.

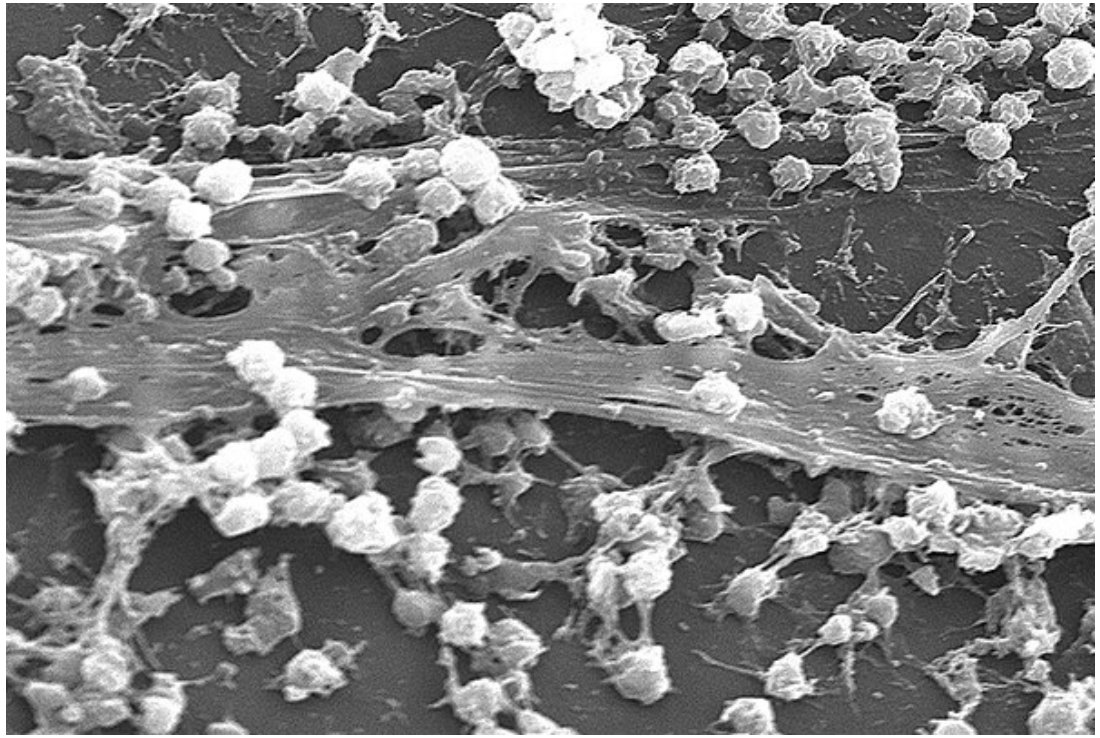
TABLE 9-2 Examples of Virulence Factors Encoded by Genes on Mobile Genetic Elements

Genus and Species	Virulence Factor and Disease
Plasmid encoded	
Escherichia coli	Heat-labile and heat-stable enterotoxins that cause diarrhea
Escherichia coli	Hemolysin (cytotoxin) of invasive disease and urinary tract infections
Escherichia coli and *Shigella* species	Adherence factors and gene products involved in mucosal invasion
Bacillus anthracis	Capsule essential for virulence (on one plasmid)
	Edema factor, lethal factor, and protective antigen are all essential for virulence (on other plasmids)
Phage encoded	
Clostridium botulinum	Botulinum toxin that causes paralysis
Corynebacterium diphtheriae	Diphtheria toxin that inhibits human protein synthesis
Vibrio cholerae	Cholera toxin that can cause a severe watery diarrhea

Bacterial communities / Biofilm and pathogenesis

A biofilm is an aggregate of interactive bacteria attached to a solid surface or to each other and encased in **EPS**.
The cells within the biofilm produce the EPS (extracellular polymeric substances) components, which are typically a polymeric conglomeration of **extracellular polysaccharides, proteins, lipids and DNA**
Biofilms may form on living or **non-living surfaces** and can be prevalent in natural, industrial **and hospital settings**.
Helps in persistence on surfaces, evasion of the immune response and antimicrobial resistance and dissemination.

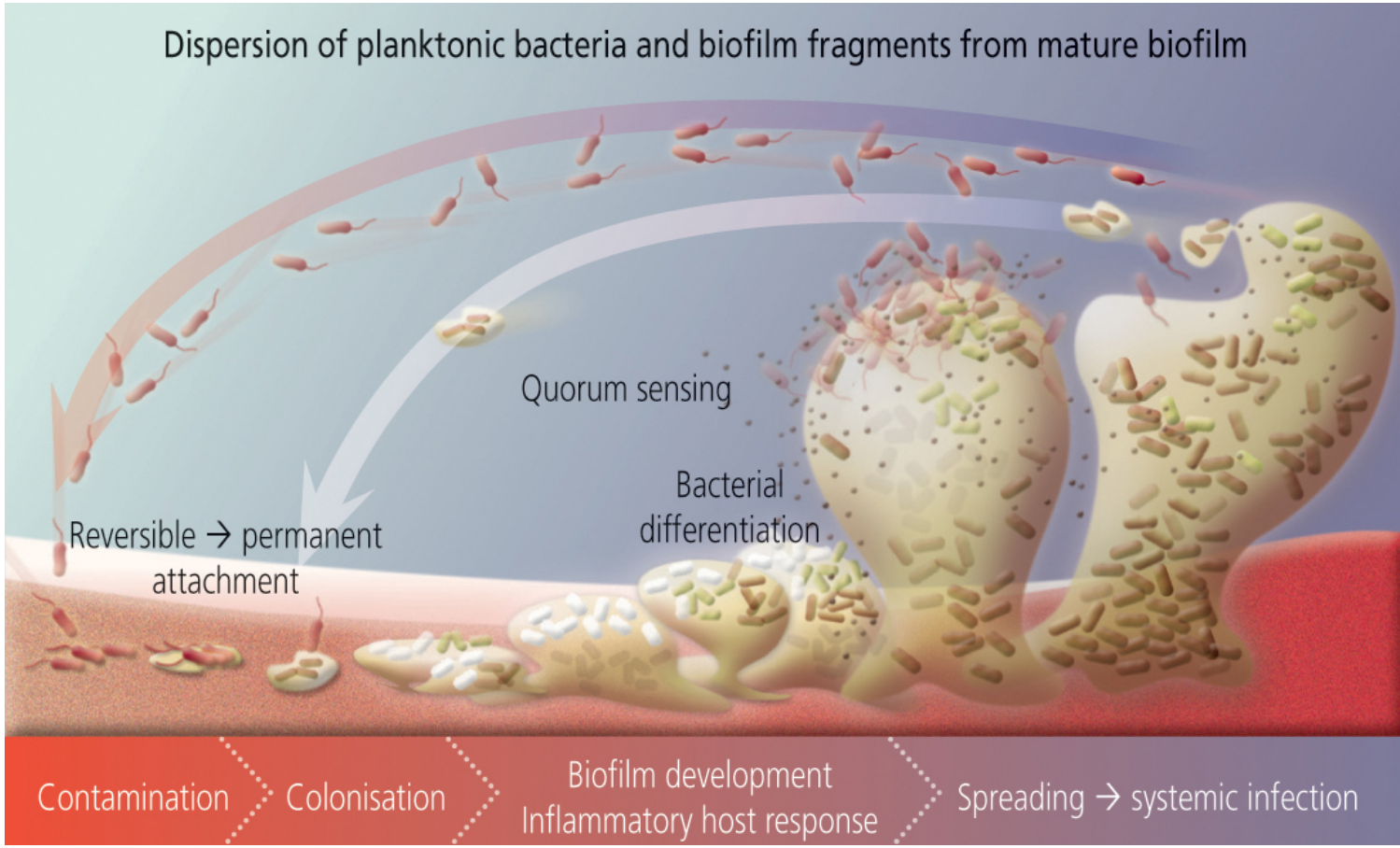




Prokaryotes / Communities

- Scientists had long held the view that bacterial cells behaved as self-sufficient individuals, unable to organize themselves into groups or communicate.

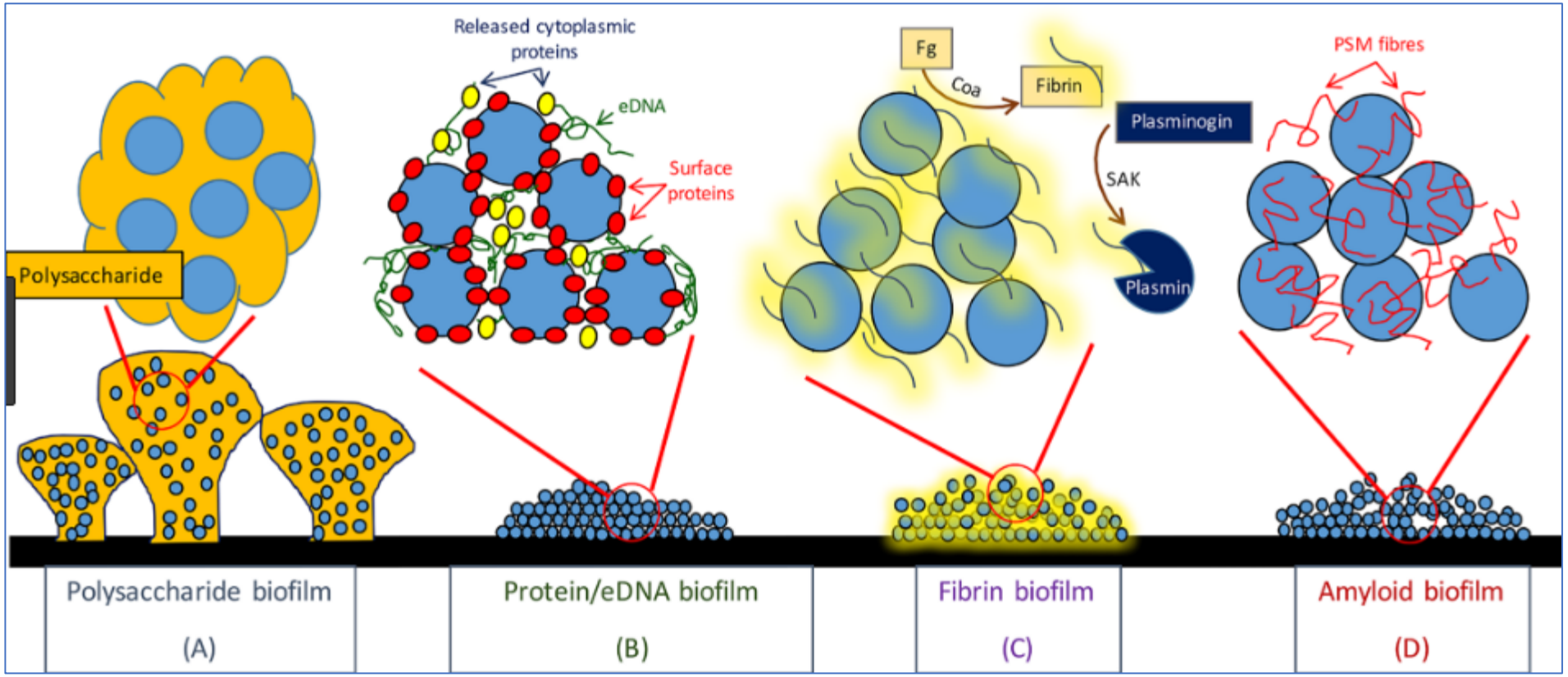
- But it was later discovered that **bacteria are found in communities that aid the survival of the whole**, through providing new characteristics. One important feature in bacterial communities is **biofilm** formation.



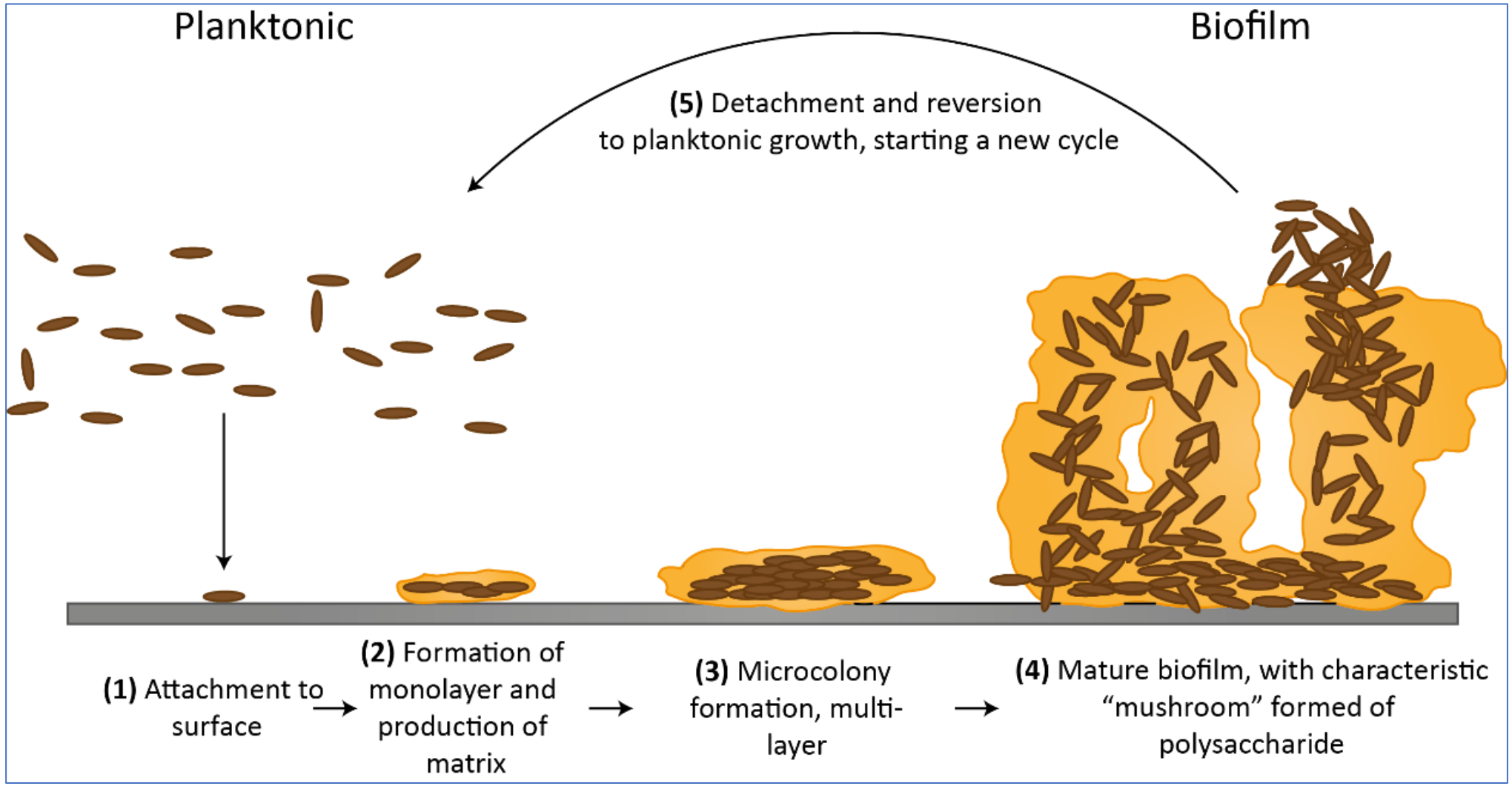
Prokaryotes / Communities

Biofilm is a community of microorganisms that are irreversibly linked to the surface, producing **extracellular polymeric substances (EPS**), and have modified properties in comparison to plankton (free living/not in a community) cells.

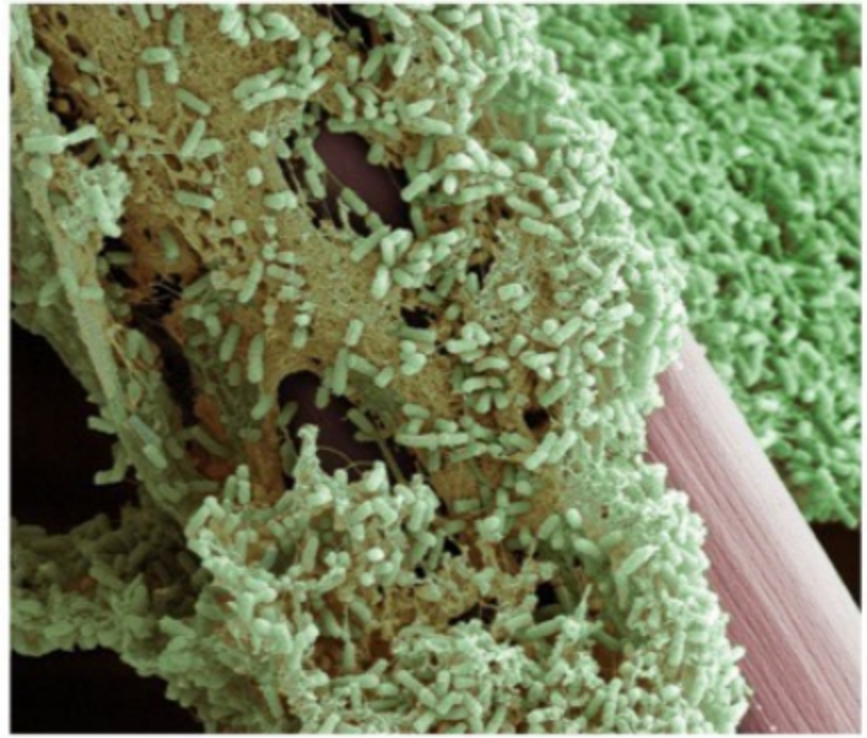
Within a biofilm, cells function co-ordinately **like a multicellular organism**.



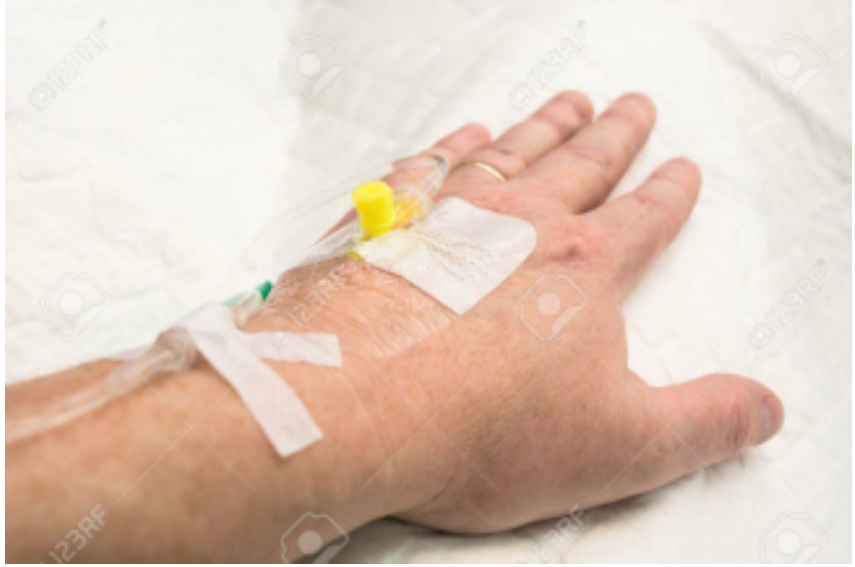
Prokaryotes / Communities

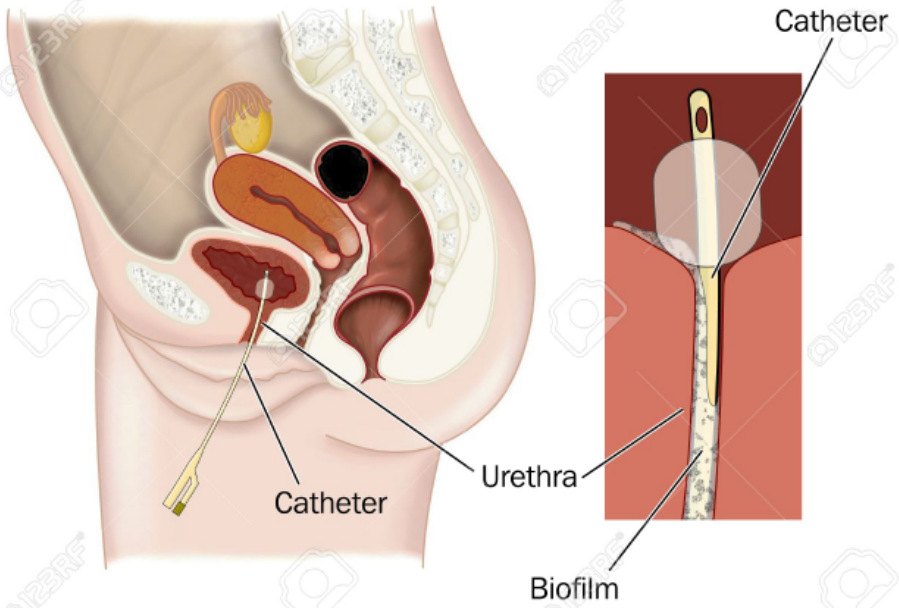


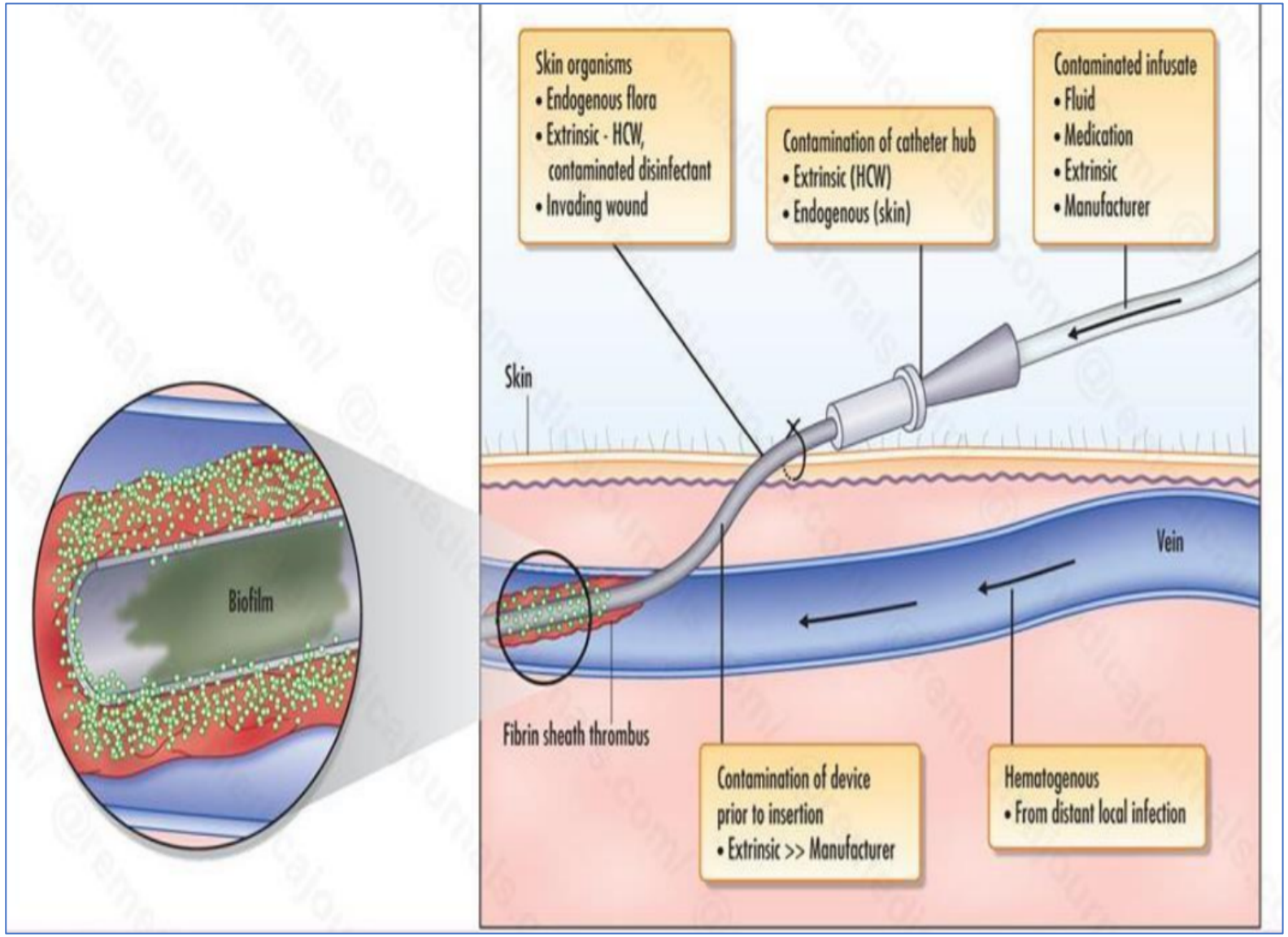
Biofilm on a toothbrush bristle

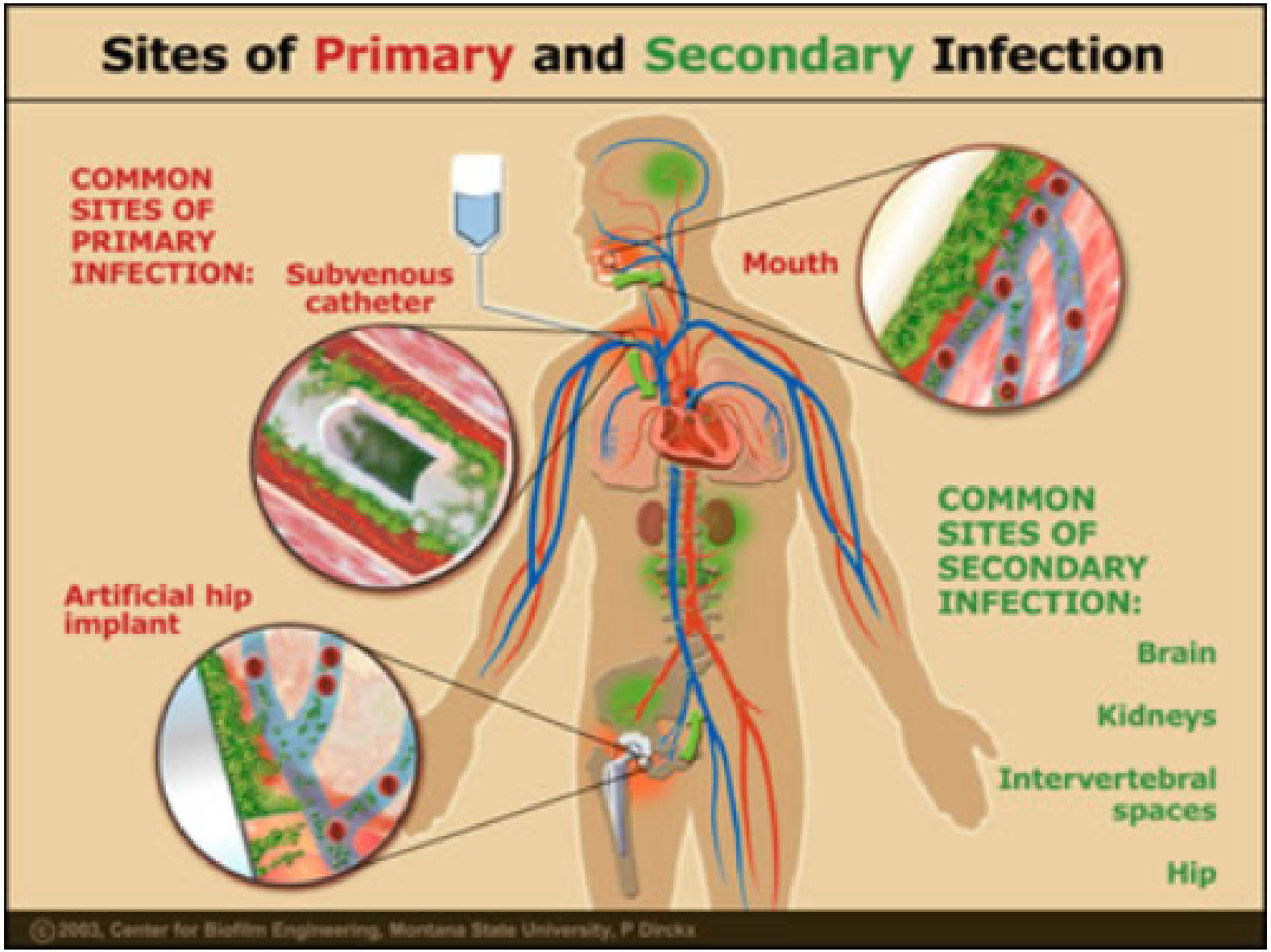


Biofilm on a toothbrush bristle (higher magnification)



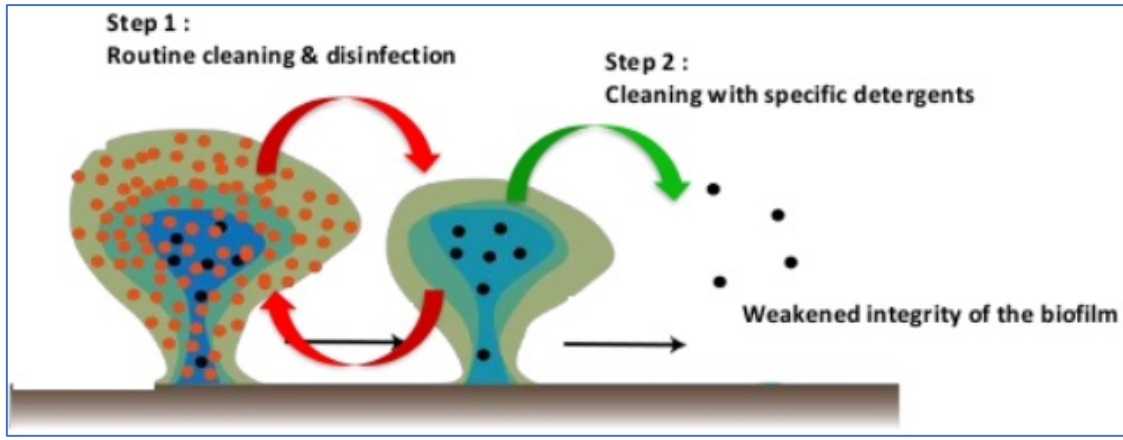

Catheter
Urethra
Catheter
Biofilm


Skin organisms
• Endogenous flora
• Extrinsic - HCW, contaminated disinfectant
• Invading wound
Contamination of catheter hub
• Extrinsic (HCW)
• Endogenous (skin)
Contaminated infusate
• Fluid
• Medication
• Extrinsic
• Manufacturer
Skin
Biofilm
Vein
Fibrin sheath thrombus
Contamination of device prior to insertion
• Extrinsic >> Manufacturer
Hematogenous
• From distant local infection


Sites of Primary and Secondary Infection
COMMON SITES OF PRIMARY INFECTION:
Subvenous catheter
Mouth
Artificial hip implant
COMMON SITES OF SECONDARY INFECTION:
Brain
Kidneys
Intervertebral spaces
Hip
© 2003, Center for Biofilm Engineering, Montana State University, P Dirckx

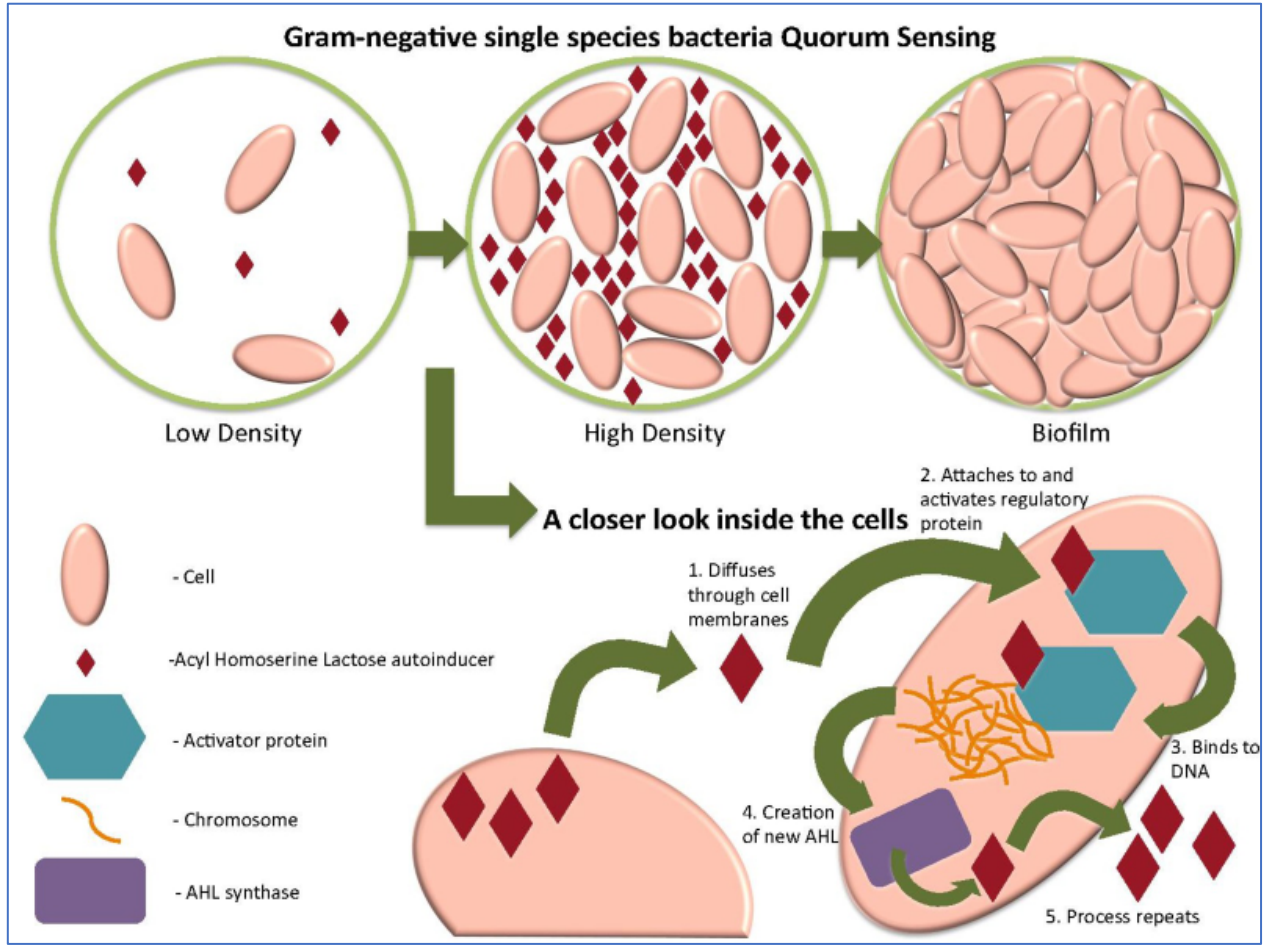
“ Factors that affect the efficacy of both disinfection and sterilization include:

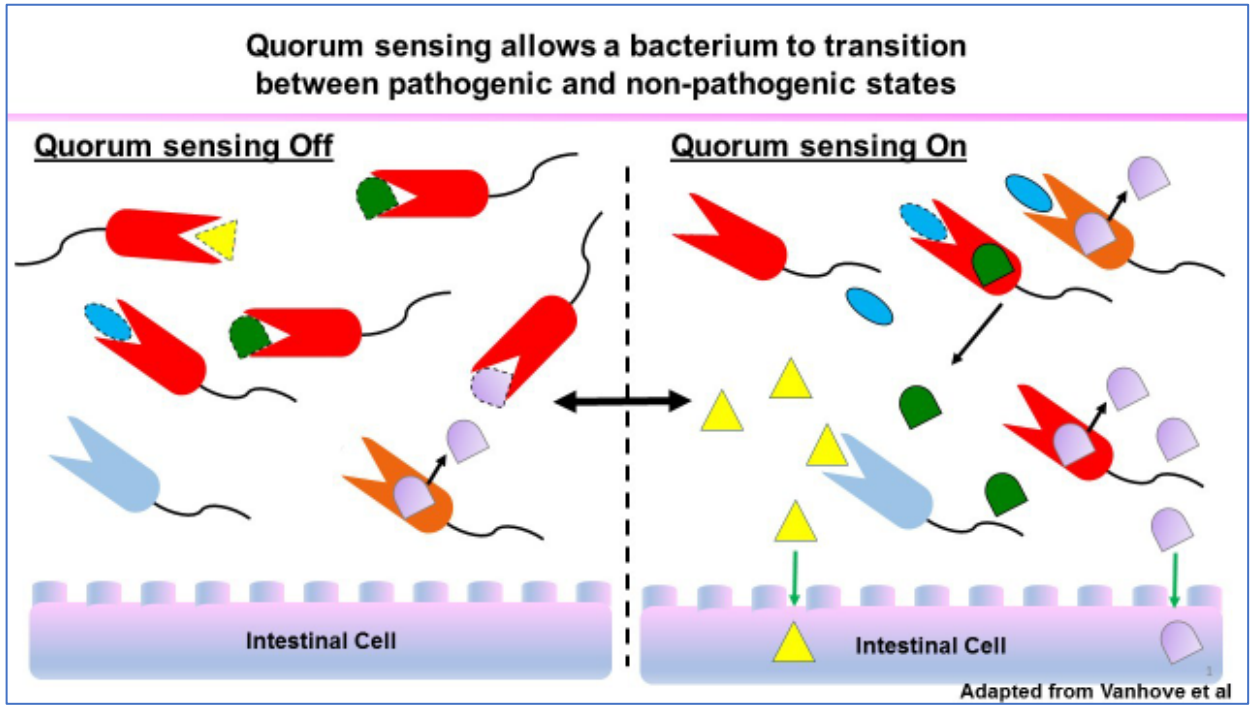
- prior cleaning of the object;
- organic and inorganic load present;
- type and level of microbial contamination;
- concentration of and exposure time to the germicide;
- physical nature of the object (e.g., crevices, hinges, and lumens);
- presence of biofilms;
- temperature and pH of the disinfection process”



Taken from the Centers for Disease Control and Prevention (CDC) Guideline for Disinfection and Sterilization in Healthcare Facilities (2008)

Bacterial communities / Quorum sensing and pathogenesis





Koch's Postulates:

① The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

② The microorganism must be isolated from a diseased organism and grown in pure culture.

③ The cultured microorganism should cause disease when introduced into a healthy organism.

④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

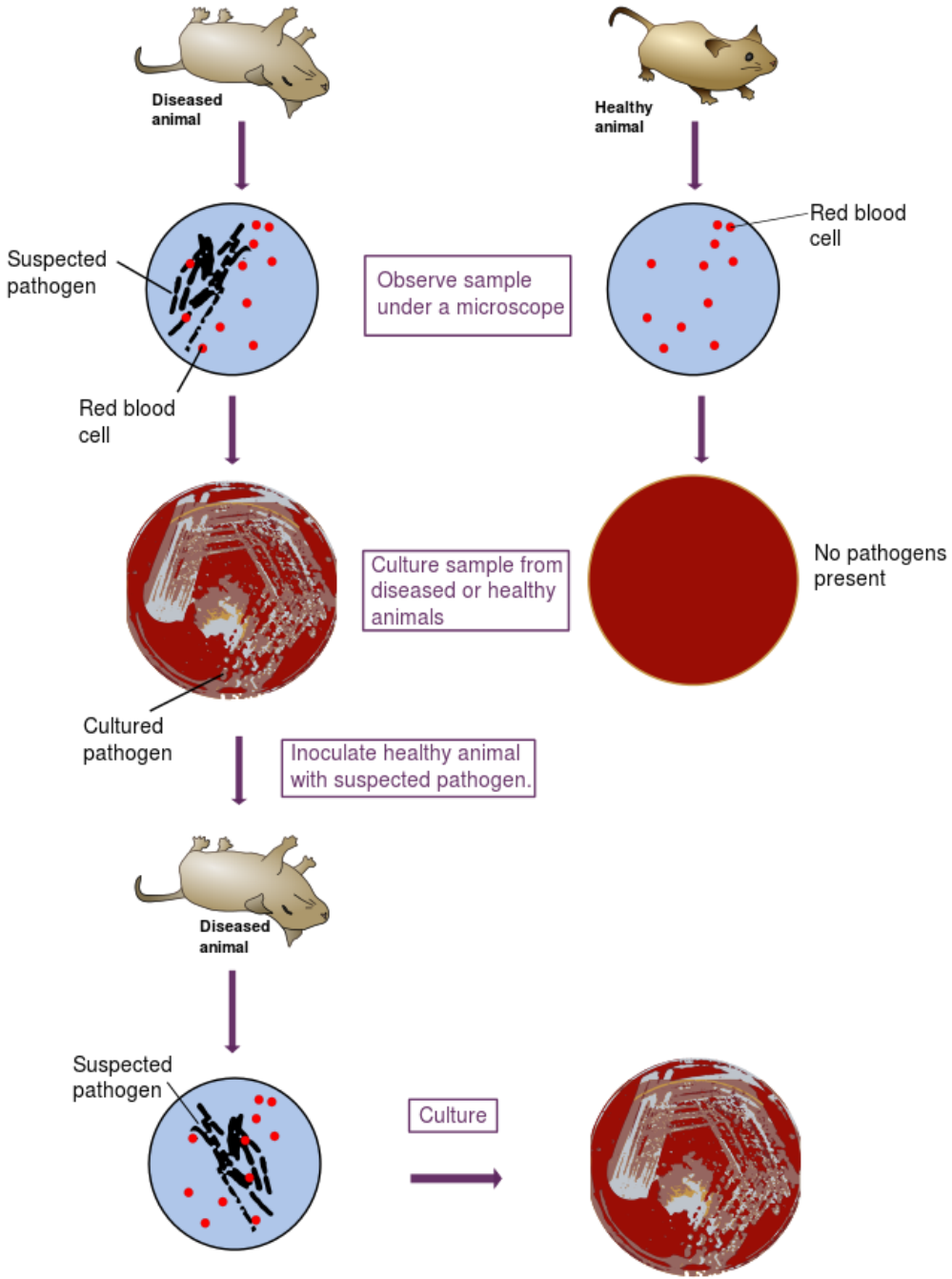


TABLE 9-1 Guidelines for Establishing the Causes of Infectious Diseases

Koch's Postulates	Molecular Koch's Postulates	Molecular Guidelines for Establishing Microbial Disease Causation
1. The microorganism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed. 2. The microorganism should be grown in pure culture in vitro (or outside the body of the host) for several generations. 3. When such a pure culture is inoculated into susceptible animal species, the typical disease must result. 4. The microorganism must again be isolated from the lesions of such experimentally produced disease.	1. The phenotype or property under investigation should be significantly associated with pathogenic strains of a species and not with nonpathogenic strains. 2. Specific inactivation of the gene or genes associated with the suspected virulence trait should lead to a measurable decrease in pathogenicity or virulence. 3. Reversion or replacement of the mutated gene with the wild-type gene should lead to restoration of pathogenicity or virulence.	1. The nucleic acid sequence of a putative pathogen should be present in most cases of an infectious disease and preferentially in anatomic sites where pathology is evident. 2. The nucleic acid sequence of a putative pathogen should be absent from most healthy control participants. If the sequence is detected in healthy control participants, it should be present with a lower prevalence as compared with patients with disease and in lower copy numbers. 3. The copy number of a pathogen-associated nucleic acid sequence should decrease or become undetectable with resolution of the disease (eg, with effective treatment) and should increase with relapse or recurrence of disease. 4. The presence of a pathogen-associated nucleic acid sequence in healthy subjects should help predict the subsequent development of disease. 5. The nature of the pathogen inferred from analysis of its nucleic acid sequence should be consistent with the known biologic characteristics of closely related organisms and the nature of the disease. The significance of a detected microbial sequence is increased when microbial genotype predicts microbial morphology, pathology, clinical features of disease, and host response

Optical Microscopy

- Historically, the microscope first revealed the presence of bacteria and later the secrets of cell structure. Today it remains a powerful tool in cell biology.

- The basic components of light microscopes consist of a light source used to illuminate the specimen positioned on a stage, a condenser used to focus the light on the specimen, and two lens systems **(objective lens** and **ocular lens)**

- **Resolving power** is the distance that must separate two point sources of light if they are to be seen as two distinct images. The best brightfield microscopes have a resolving power of **approximately 0.2 μm**, which allows most bacteria, **but not viruses**, to be visualized.

- The resolving power is greatest when **oil** is placed between the objective lens (typically the 100× lens) and the specimen, because **oil reduces the dispersion of light**.

- Three different objective lenses are commonly used: low power (**10-fold magnification**), which can be used to scan a specimen; **high dry (40-fold**), which is used to look for large microbes such as parasites and filamentous fungi; and **oil immersion (100-fold**), which is **used to observe bacteria, yeasts (single-cell stage of fungi), and the morphologic details of larger organisms and cells**. Ocular lenses can further magnify the image (generally 10-fold to 15-fold).

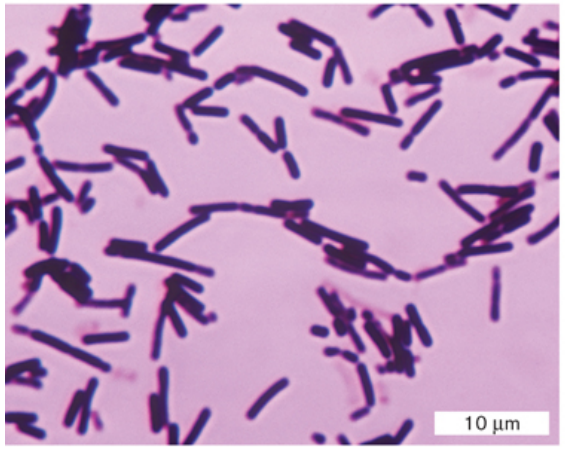
Brightfield (Light) Microscopy

- A typical microscope that uses transmitted light to observe targets at high magnification.

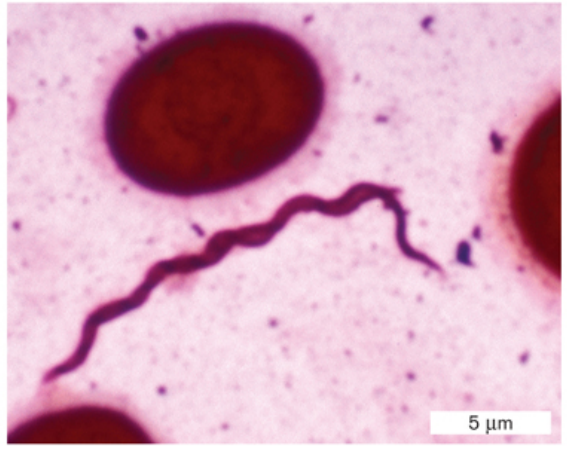
- In order to view a specimen under a brightfield microscope, the light rays that pass through it must be changed enough in order to interfere with each other (or **contrast**) and therefore, build an image.

- At times, a specimen will have a refractive index very similar to the surrounding medium between the microscope stage and the objective lens. When this happens, the image can not be seen. In order to visualize these biological materials well, **they must have a contrast caused by the proper refractive indices, or be artificially stained**. Since staining can kill specimens, there are times when darkfield microscopy is used instead.

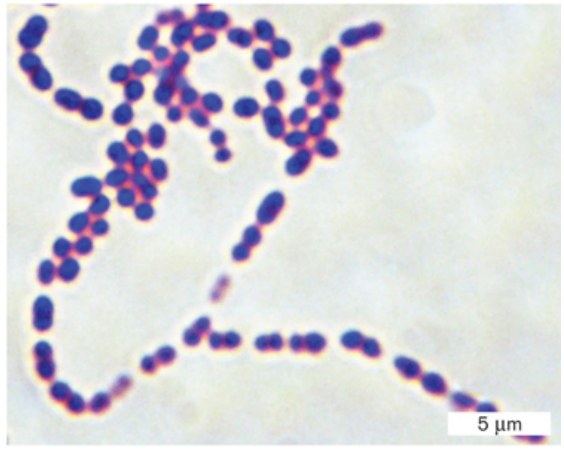
A. Filamentous rods (bacilli).
Lactobacillus lactis, Gram-positive bacteria (LM).



C. Spirochetes.
Borrelia burgdorferi, cause of Lyme disease, among human blood cells (LM).



E. Cocci in pairs (diplococci).
Streptococcus pneumoniae, a cause of pneumonia. Methylene blue stain (LM).

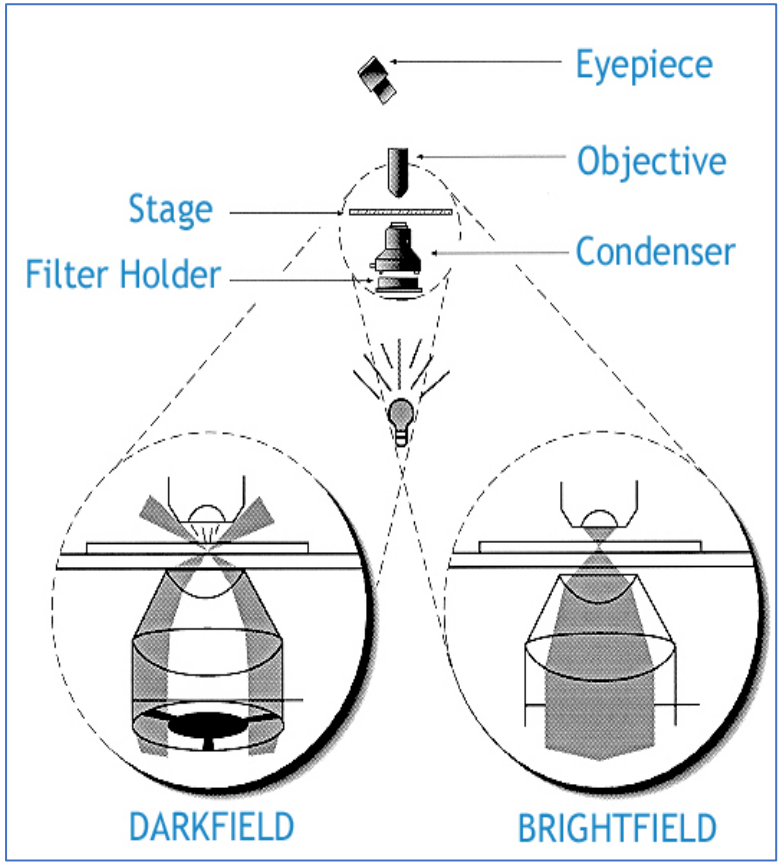


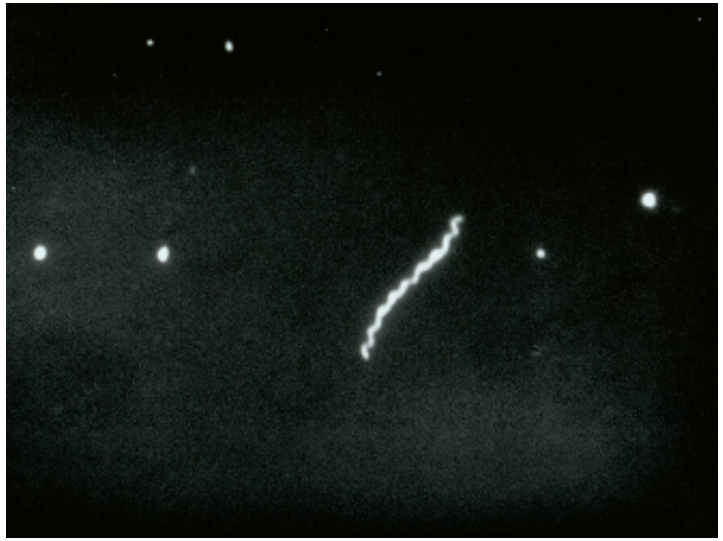
Darkfield Microscopy

- The dark-field microscope is a **light microscope in which the lighting system has been modified** to reach the specimen from the sides only.

- This creates a “dark field” that contrasts against the highlighted edge of the specimens and results when the oblique rays are reflected from the edge of the specimen upward into the objective of the microscope.

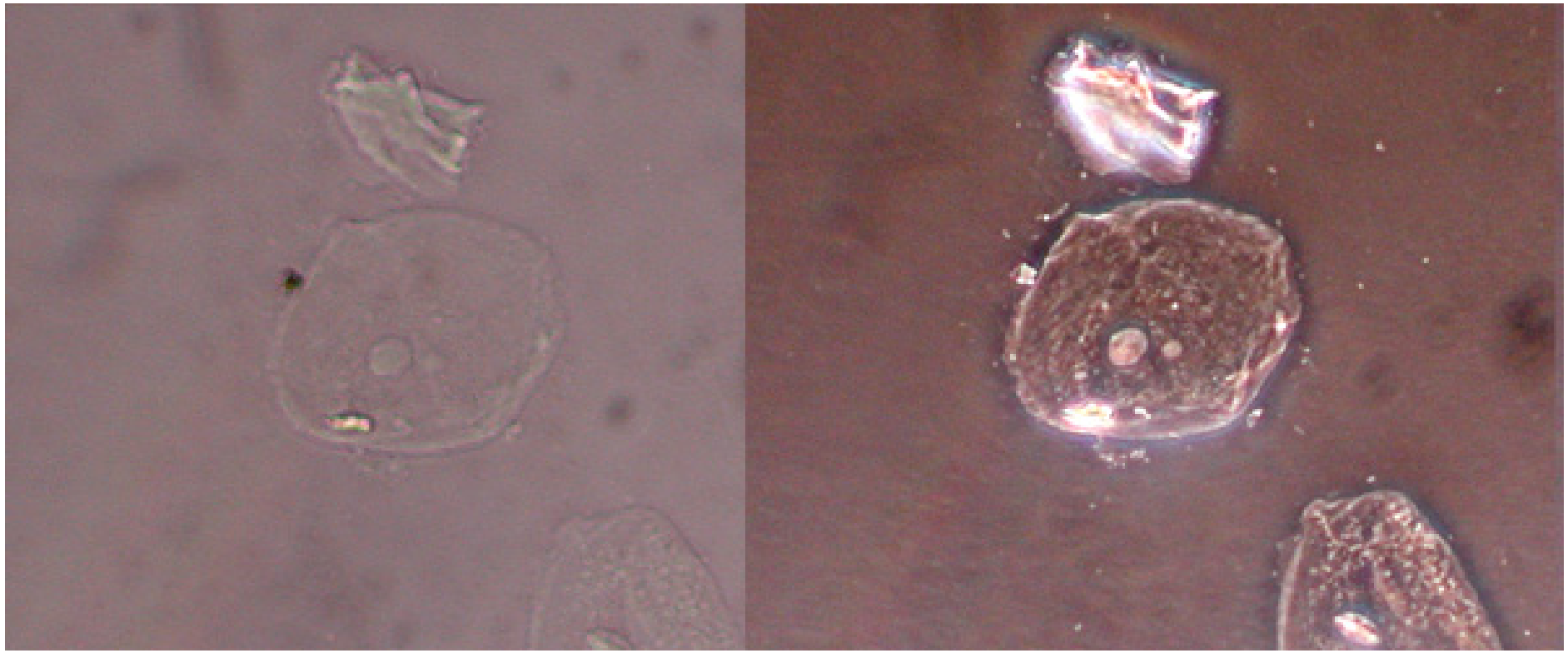
- Thus, this technique has been particularly useful for observing organisms such as ***Treponema pallidum*** , a spirochete that is smaller than 0.2 mm in diameter and therefore cannot be observed with a brightfield





Phase contrast microscopy

- Phase-contrast microscopy enables the internal details of microbes to be examined. In this form of microscopy, as parallel beams of light are passed through objects of different densities, the wavelength of one beam moves out of “phase” relative to the other beam of light (i.e., the beam moving through the more dense material is retarded more than the other beam)



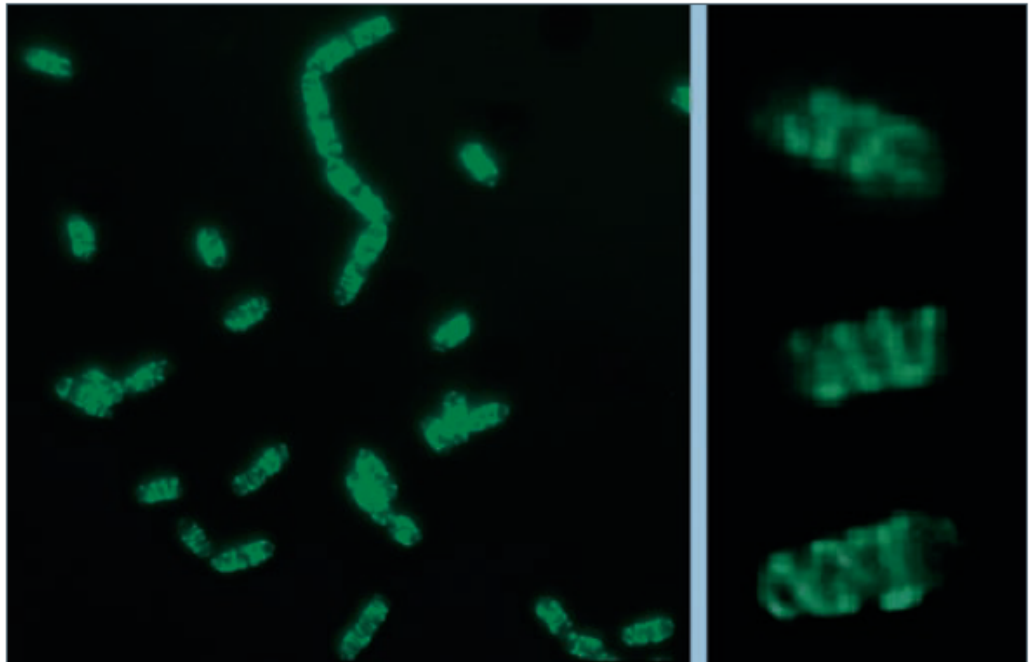
Brightfield Microscopy **Phase contrast microscope**

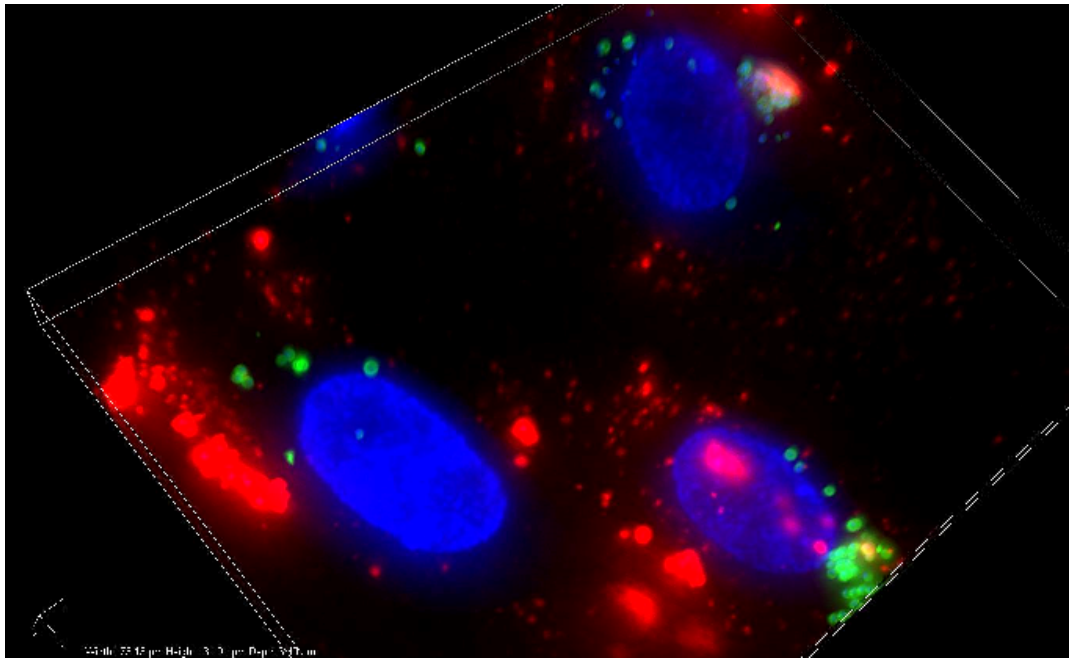
Fluorescent Microscopy

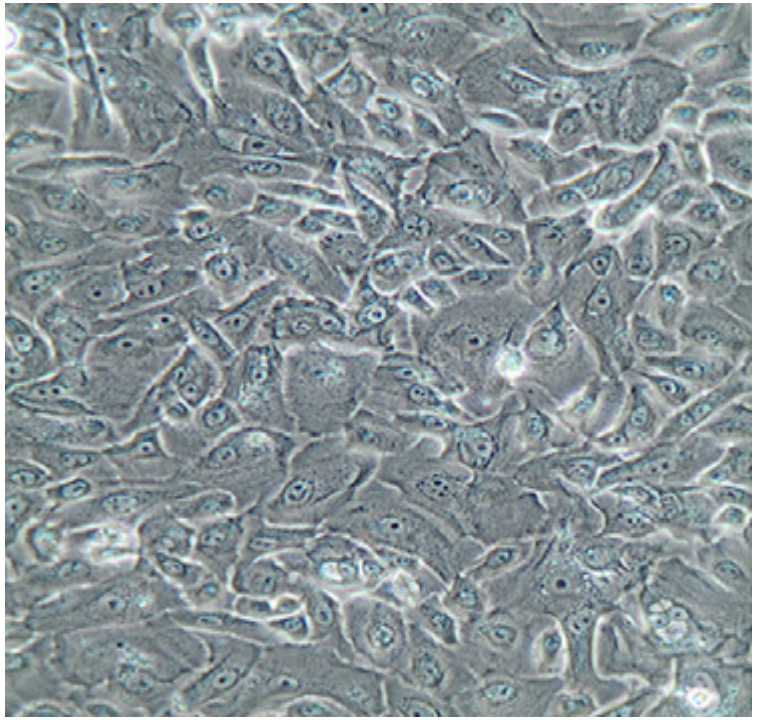
- **Fluorochromes** can absorb light at a certain wavelength and emit energy at a higher visible wavelength. Although some microorganisms show natural fluorescence **(autofluorescence).**

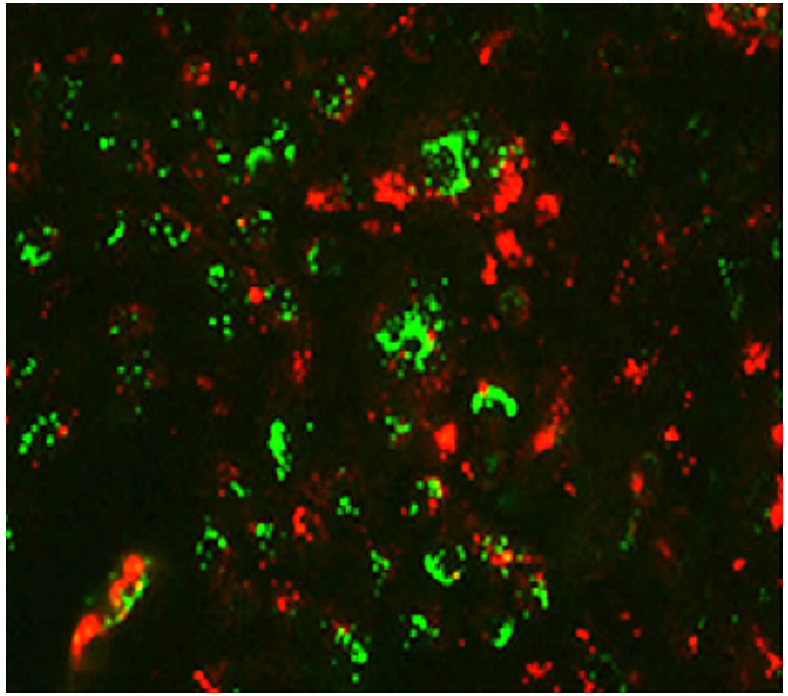
- Fluorescent microscopy typically involves examining organisms (or a substructure of an organism) with bound **fluorochromes (conjugated to antibodies, fluorescent dyes, expressed fluorochromes like GFP)** and then examining them with a specially designed fluorescent microscope.

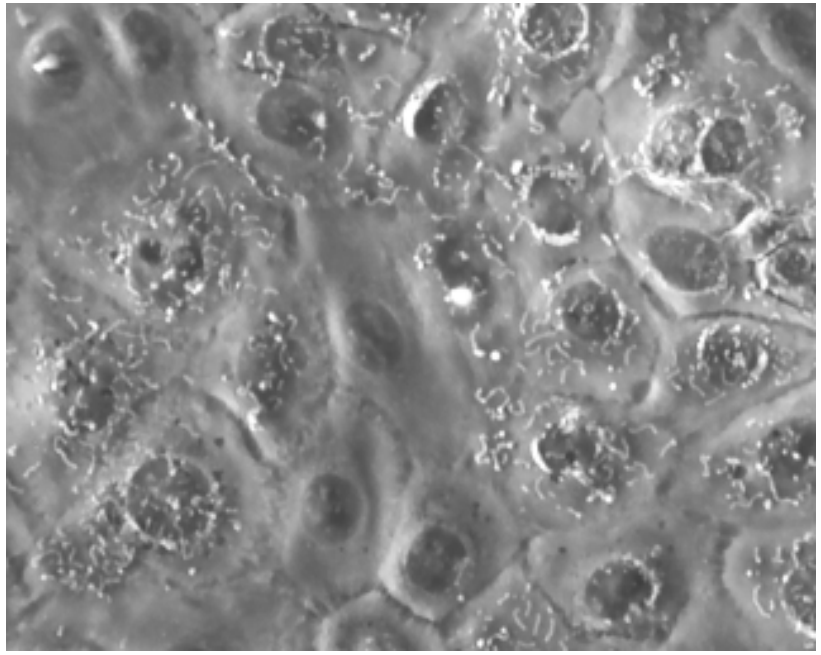
- Organisms and specimens stained with fluorochromes appear **brightly illuminated against a dark background,** although the **colors vary depending on the fluorochrome** selected.

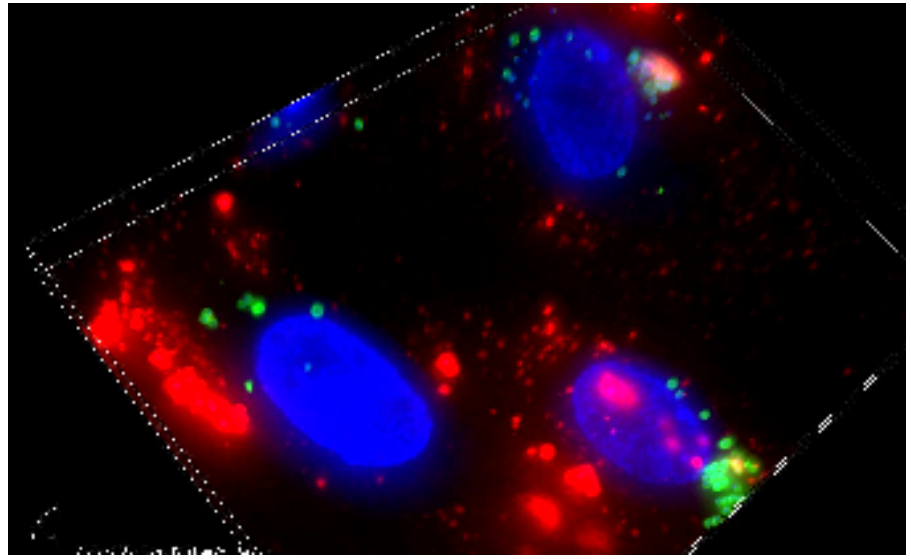


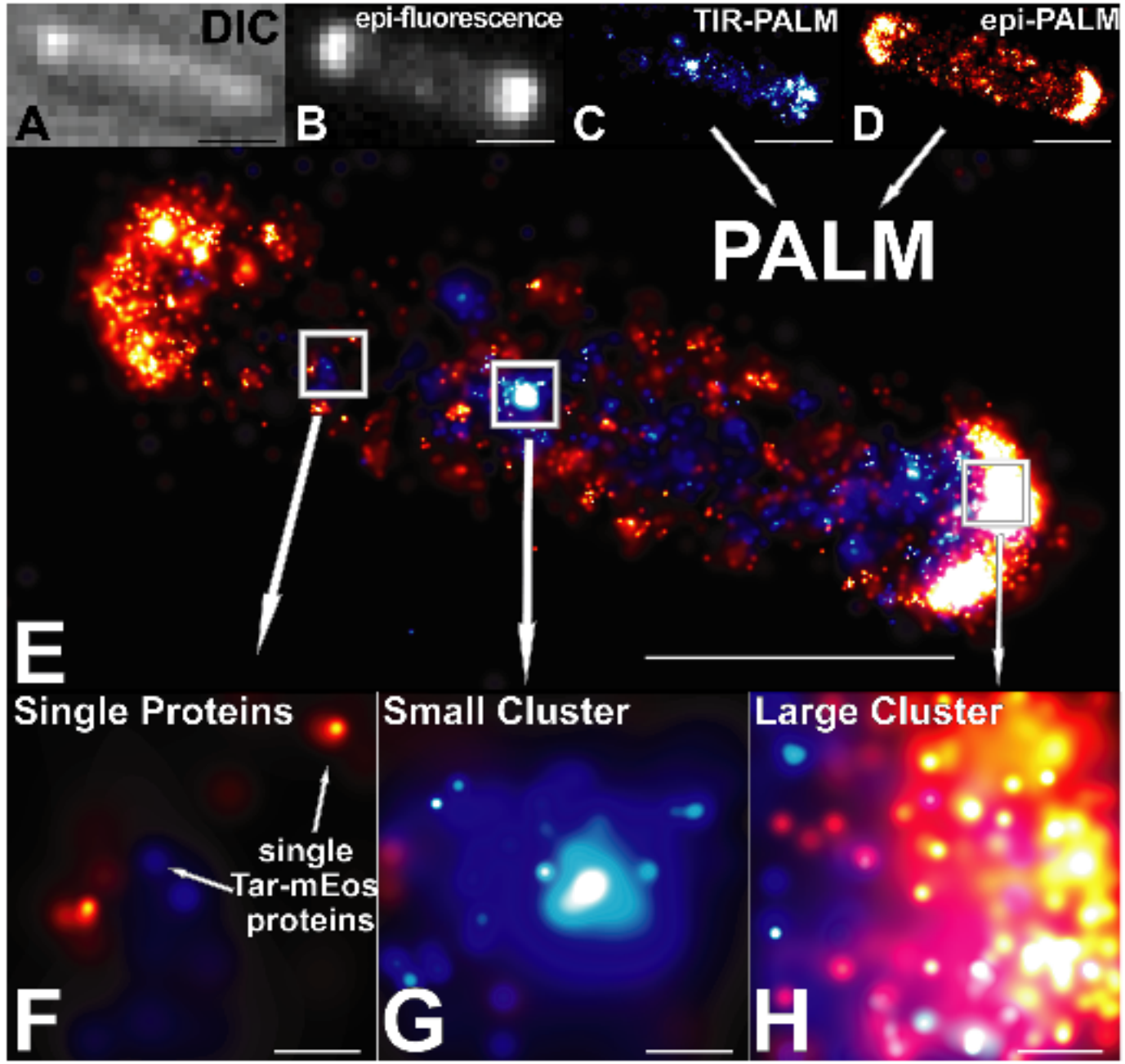










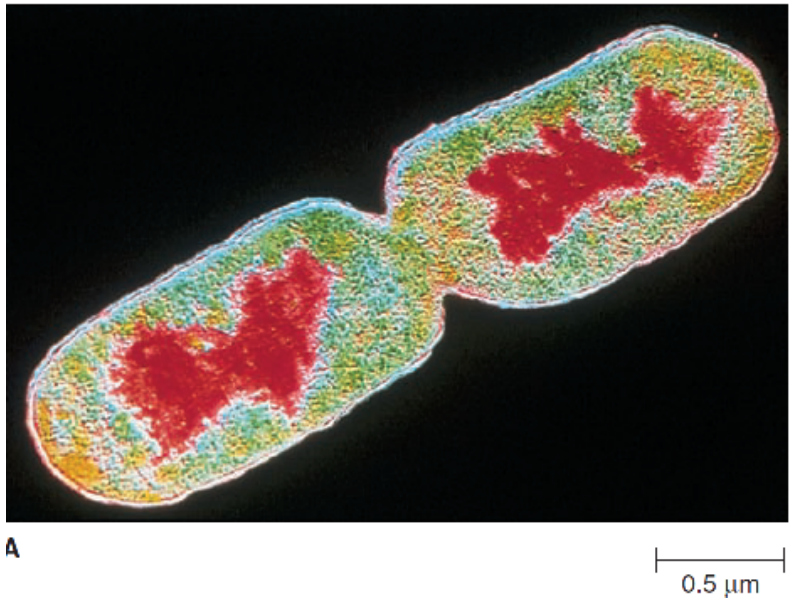

DIC
epi-fluorescence
TIR-PALM
epi-PALM
A
B
C
D
PALM
E
Single Proteins
single
Tar-mEos
proteins
Small Cluster
Large Cluster
F
G
H

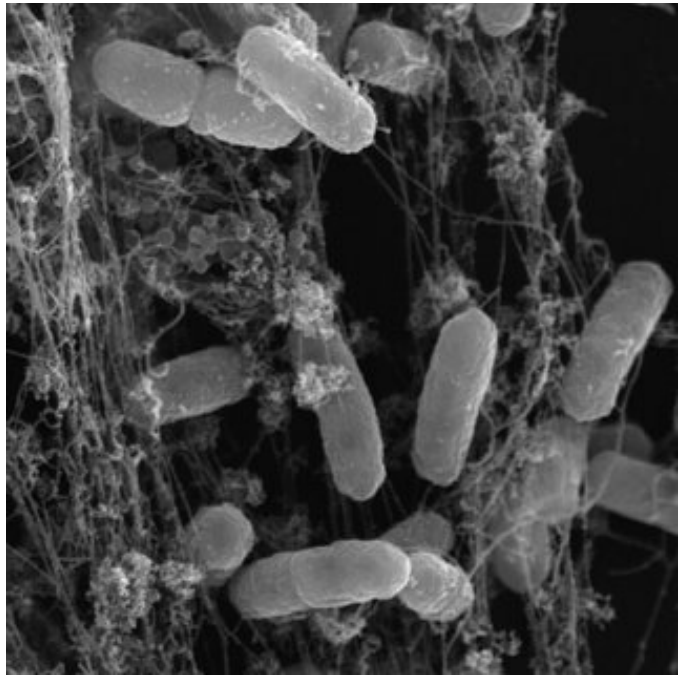
Electron Microscopy

- While a **light microscope** uses **light** to illuminate specimens and glass lenses to magnify images, an **electron microscope** uses a beam of **electrons** to illuminate specimens and magnetic lenses to magnify images.

- The **electron microscope** is capable of much higher magnifications and has a greater resolving power than a light **microscope**, allowing it to see much smaller objects in finer detail.

- There are two types of electron microscopes: **transmission electron microscopes,** in which electrons such as light pass directly through the specimen, and **scanning electron microscopes,** in which electrons bounce off the surface of the specimen at an angle and a three-dimensional picture is produced.





MOLECULAR DIAGNOSIS

- Like the evidence left at the scene of a crime, the **DNA** (deoxyribonucleic acid), **RNA** (ribonucleic acid), or **proteins** of an infectious agent in a clinical sample can be used to help identify the agent.

- **DNA probes** can be used like antibodies as sensitive and specific tools to detect, locate, and quantitate specific nucleic acid sequences in clinical specimens, The DNA probes can detect specific genetic sequences in fixed permeabilized tissue biopsy specimens by **in situ hybridization.** When fluorescent detection is used, it is called **FISH: fluorescent in situ hybridization.**

- The **polymerase chain reaction (PCR)** amplifies single copies of viral DNA millions of times over and is one of the most useful genetic analysis techniques. In this technique, a sample is incubated with two short DNA oligomers, termed **primers,** that are complementary to the ends of a known genetic sequence within the total DNA, a heat-stable DNA polymerase (Taq or other polymerase obtained from thermophilic bacteria), nucleotides, and buffers.

- **DNA sequencing** has become sufficiently fast and inexpensive to allow laboratory determination of microbial sequences for identification of microbes. **Sequencing of the 16S ribosomal subunit** can be used to identify specific bacteria. Sequencing of viruses can be used to identify the virus and distinguish different strains (e.g., specific influenza strains).

- Matrix-assisted laser desorption/ionization time-of-flight (**MALDI-TOF**) mass spectrometry is a powerful new rapid approach to determine RNA, DNA, and **protein sequences**. The DNA or RNA is inserted into the instrument, ionized, and fragmented, the fragments are separated based on their charge-to-mass ratio, and the nucleotide sequence is determined by analyzing the mass of the ionized fragments.

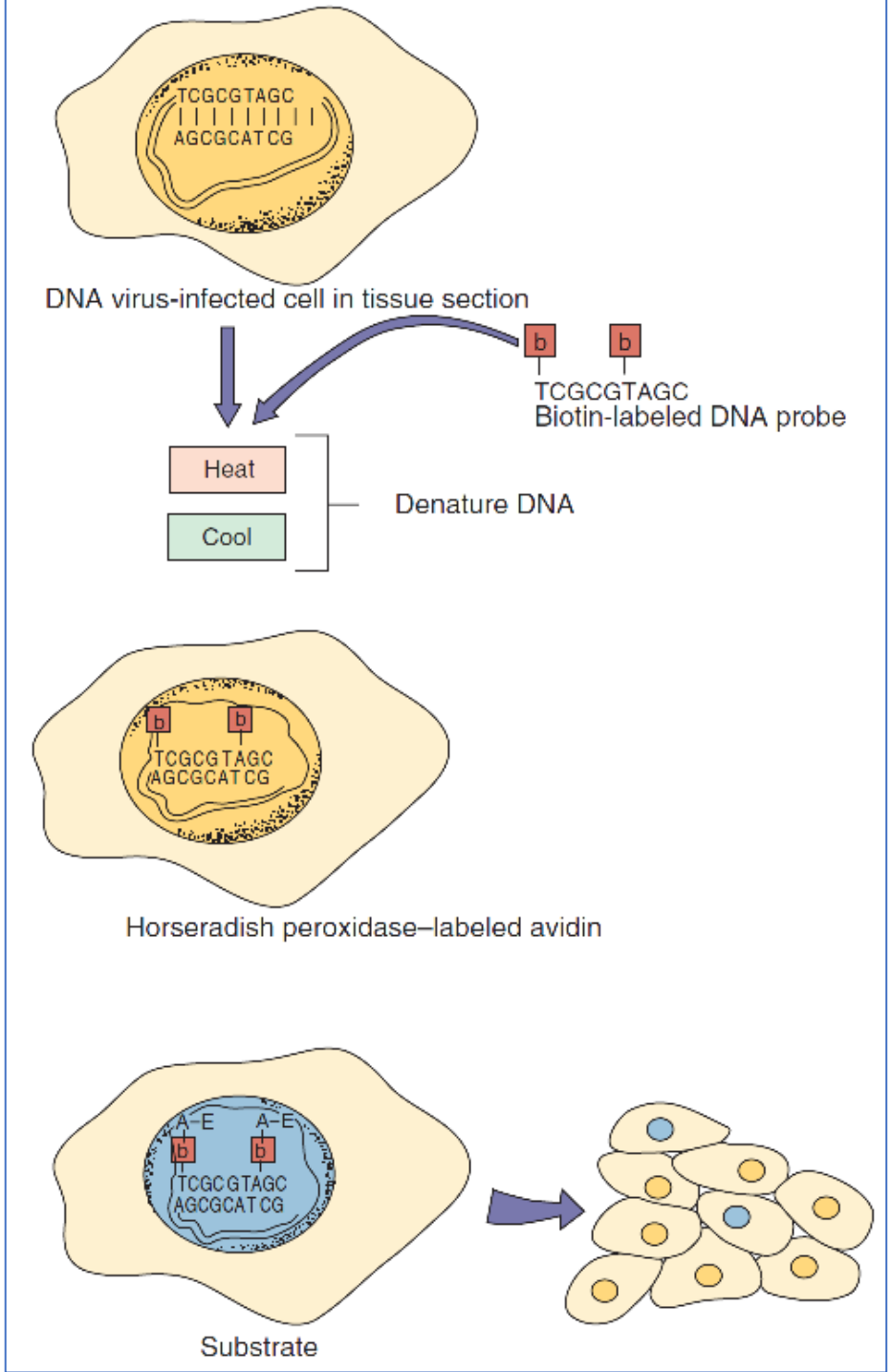
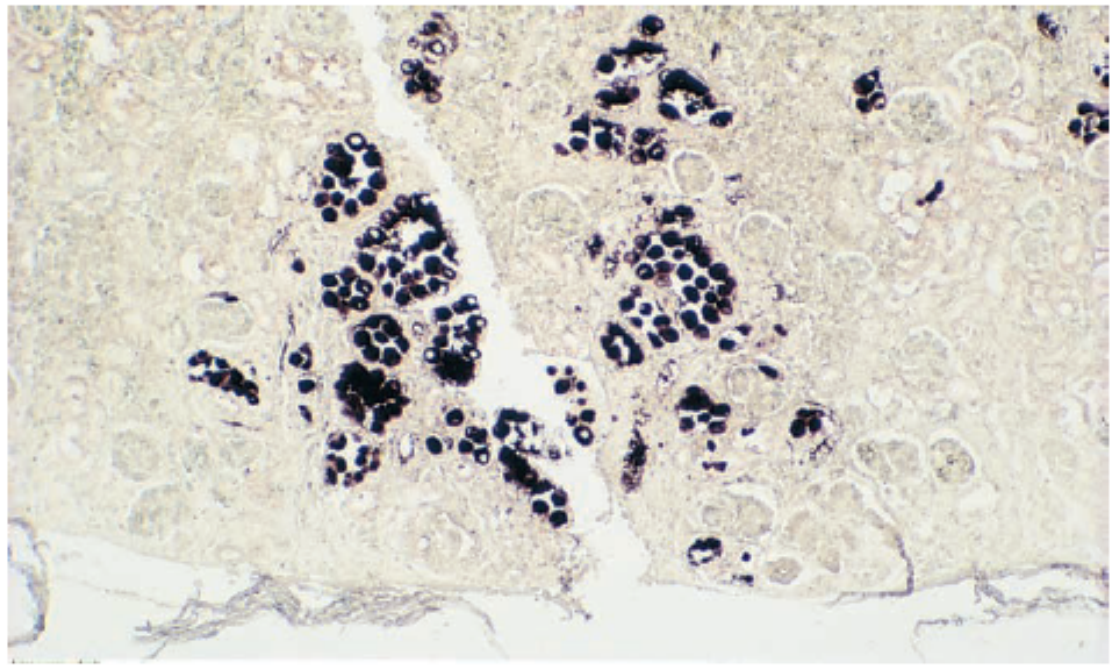
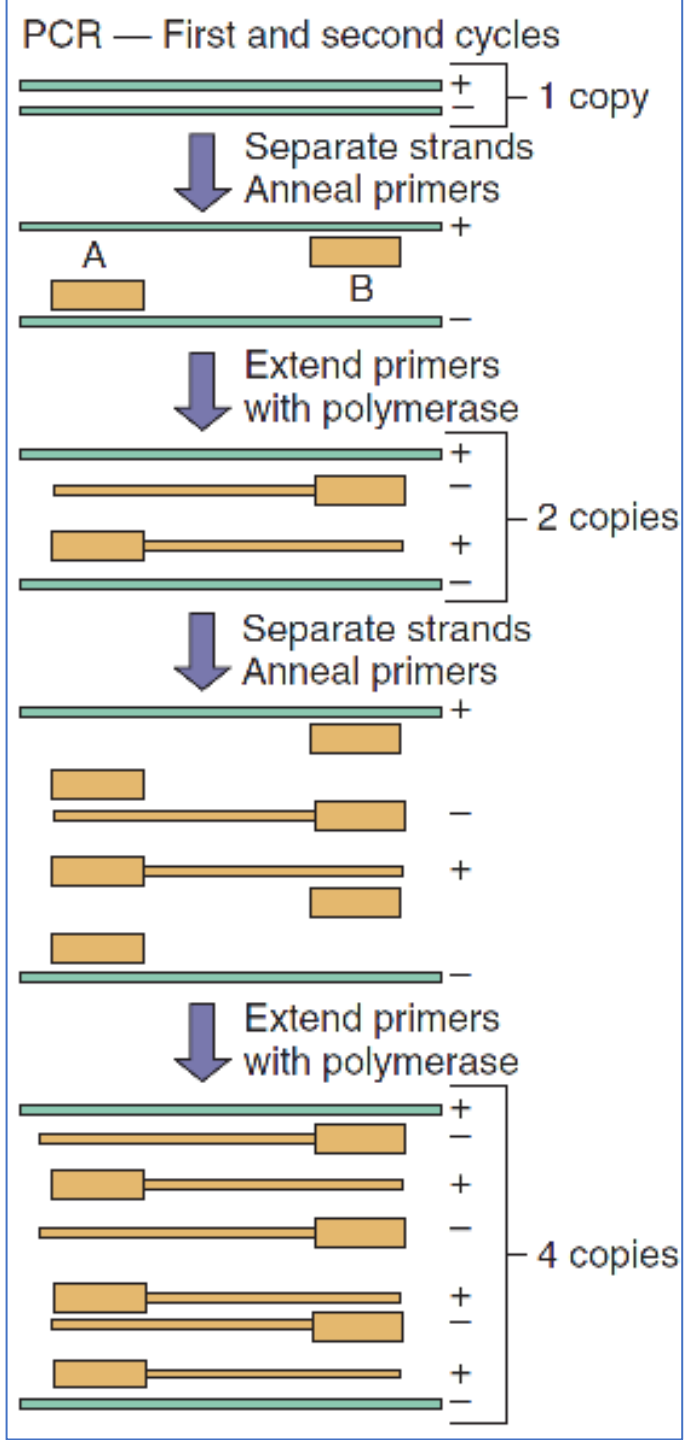


FIGURE 5-2 DNA probe analysis of virus-infected cells. Such cells can be localized in histologically prepared tissue sections using DNA probes consisting of as few as nine nucleotides or bacterial plasmids containing the viral genome. A tagged DNA probe is added to the sample. In this case, the DNA probe is labeled with biotin-modified thymidine, but radioactive agents can also be used. The sample is heated to denature the DNA and cooled to allow the probe to hybridize to the complementary sequence. Horseradish peroxidase–labeled avidin is added to bind to the biotin on the probe. The appropriate substrate is added to color the nuclei of virally infected cells. *A*, Adenine; *b*, biotin; *C*, cytosine; *G*, guanine; *T*, thymine.





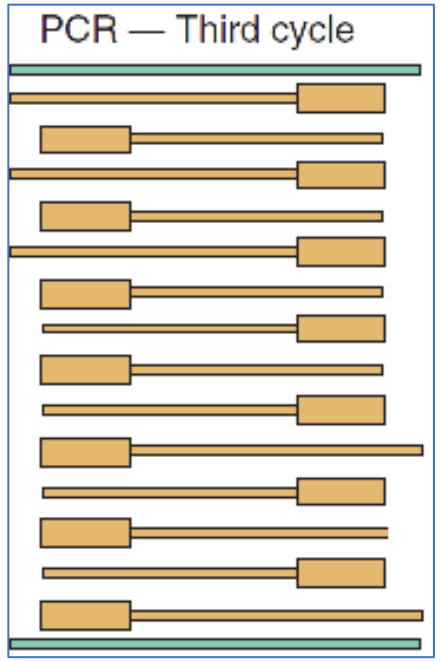


FIGURE 5-4 Polymerase chain reaction *(PCR).* This technique is a rapid means of amplifying a known sequence of DNA. A sample is mixed with a heat-stable DNA polymerase, excess deoxyribonucleotide triphosphates, and two DNA oligomers **(primers),** which complement the ends of the target sequence to be amplified. The mixture is heated to denature the DNA and then cooled to allow binding of the primers to the target DNA and extension of the primers by the polymerase. The cycle is repeated 20 to 40 times. After the first cycle, only the sequence bracketed by the primers is amplified. In the **reverse transcriptase PCR** technique, RNA can also be amplified after its conversion to DNA by reverse transcriptase. Labels *A* and *B,* DNA oligomers used as primers; + and –, DNA strands. (Modified from Blair GE, Zajdel MEB: The polymerase chain-reaction—already an established technique. *Biochem Educ* 20:87–91, 1992.)

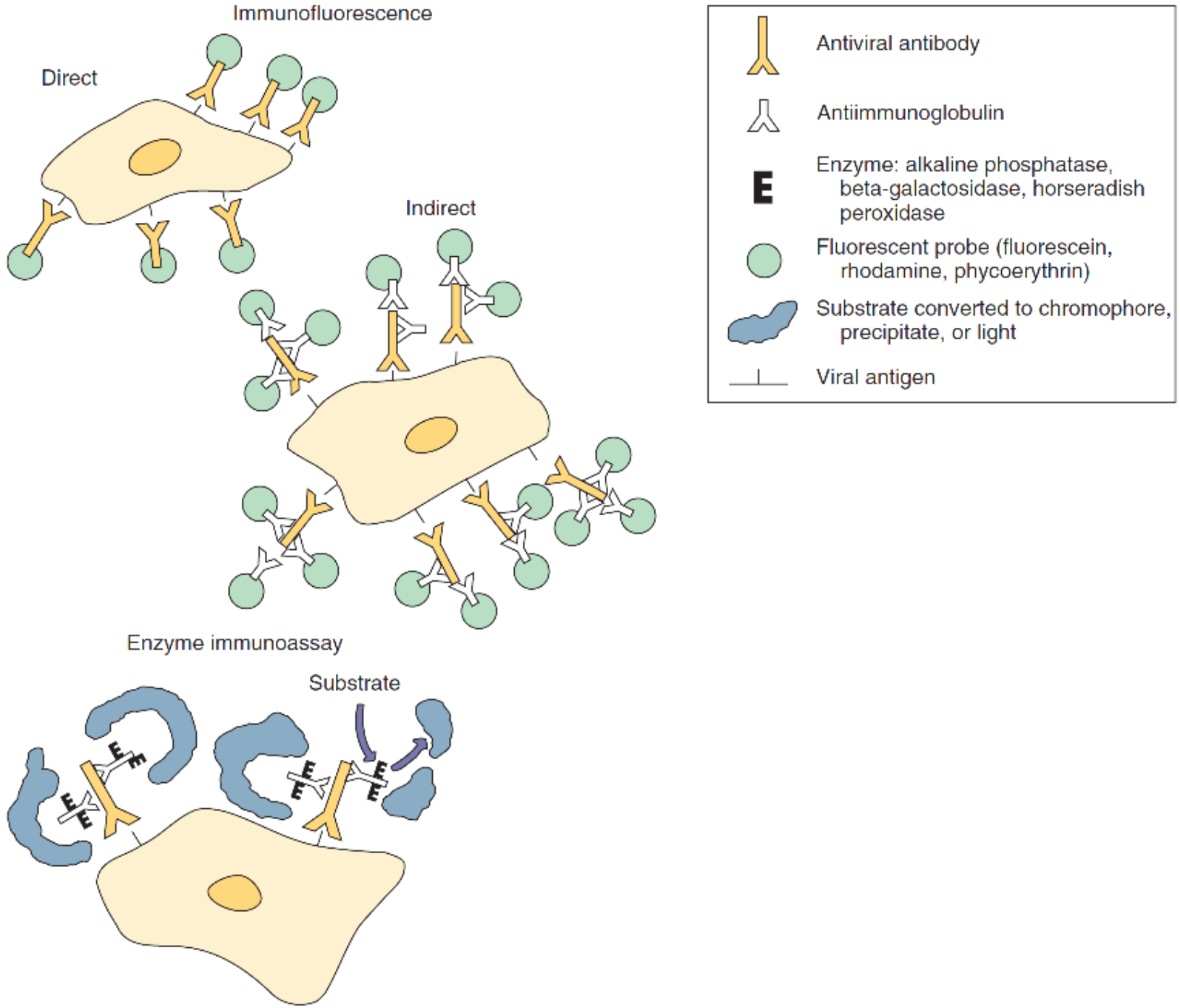
Serological diagnosis

- **Antibodies** can be used as sensitive and specific tools to detect, identify, and quantitate the antigens from a virus, bacterium, fungus, or parasite. The **specificity** of the antibody-antigen interaction and the sensitivity of many of the immunologic techniques make them powerful laboratory tools.

- These antibodies are **polyclonal;** that is, they are heterogeneous antibody preparations that can recognize many epitopes on a single antigen. **Monoclonal** antibodies recognize individual epitopes on an antigen.

- Antibody-antigen complexes can be detected directly, by **precipitation** techniques, or by **labeling** the antibody with a radioactive, fluorescent, or enzyme probe.

- The humoral immune response provides a **history of a patient's infections**. Serology can be used to identify the infecting agent, evaluate the course of an infection, or determine the nature of the infection—whether it is a primary infection or a reinfection, and whether it is acute or chronic.


Immunofluorescence
Direct
Indirect
Enzyme immunoassay
Substrate
Antiviral antibody
Antiimmunoglobulin
E
Enzyme: alkaline phosphatase, beta-galactosidase, horseradish peroxidase
Fluorescent probe (fluorescein, rhodamine, phycoerythrin)
Substrate converted to chromophore, precipitate, or light
Viral antigen

Further reading and material:

- Jawetz, Melnick & Adelberg's Medical Microbiology, 26th edition-
  Section 3: Bacteriology
    Chapter 9: Pathogenesis of bacterial infections